MODIFIABLE AND NON-MODIFIABLE RISK FACTORS FOR PELVIC ORGAN PROLAPSE

By

Ayush Giri

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Approved:

Todd L. Edwards, Ph.D., M.S.

Katherine E. Hartmann, M.D., Ph.D.

Digna R. Velez Edwards, Ph.D., M.S.

Melinda C. Aldrich, Ph.D., M.P.H.

Bingshan Li, Ph.D.

To my loving wife, Siddhida, for her unfaltering support
You exemplify the phrase “through thick and thin”

To my parents Rajendra and Neeta, and brother, Aniket Giri,
for their unconditional love and faith in me
It brings me immense joy to make you proud

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TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

ACTA1	actin, alpha 1, skeletal muscle
ACTA2	actin, alpha2 smooth muscle, aorta
ACTC1	actin, alpha cardiac muscle 1
ACTN2	actinin, alpha 2
ACTN3	actinin, alpha 3
ADAMTS10	ADAM metallopeptidase with thrombospodin type 1 motif, 10
ADAMTS2	ADAM metallopeptidase with thrombospodin type 1 motif, 2
ADAMTS9	ADAM metallopeptidase with thrombospodin type 1 motif, 9
ADAMTSL4	ADAMTS-like 4
ARID4B	AT rich interactive domain 4B
ASW	African ancestry in Southwest USA
ATP6V0A2	ATPase, H+ transporting, lysosomal V0 subunit a2
ATP7A	ATPase, Cu++ transporting, alpha polypeptide
B4GALT7	xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BMP1	bone morphogenetic protein 1
BTNL2	butyrophilin-like 2
CA	California
CADM2	cell adhesion molecule 2
CBS	cystathionine-beta-synthase
CEU	Utah residents with Northern and Western European ancestry from CEPH collection
CHD2	chromodomain helicase DNA binding protein 2
CI	confidence interval
COL11A1	collagen, type XI, alpha 1

COL11A2	collagen, type XI, alpha 2
COL1A1	collagen, type I, alpha 1
COL1A2	collagen, type I, alpha 2
COL2A1	collagen, type II, alpha 1
COL3A1	collagen, type III, alpha 1
COL5A1	collagen, type V, alpha 1
COL5A2	collagen, type V, alpha 2
COL9A1	collagen, type IX, alpha 1
CPEB4	cytoplasmic polyadenylation element binding protein 4
dbGaP	database of genotypes and phenotypes
DNA	deoxyribonucleic acid
DNAH14	dyenin, axonemal, heavy chain 14
DNM3	dynamin 3
EA	effect allele
EAF	effect allele frequency
ELN	elastin
eMERGE	electronic medical records and genomics
EMR	electronic medical records
ER-alpha	estrogen receptor alpha
ER-beta	estrogen receptor beta
ETV5	ets variant 5
FAIM2	fas apoptopic inhibitory molecule 2
FANCL	fanconi anemia, complementation group L
FBLN4	EGF containing fibulin-like extracellular matrix protein 2
FBLN5	fibulin 5
FBN1	fibrillin 1
FBN2	fibrillin 2
FGFR3	fibroblast growth factor receptor 3

FIN	Finnish in Finland
FTO	fat mass and obesity associated
GARNET	genome-wide association studies of treatment response in randomized clinical trials
GBR	British in England and Scotland
GCR	genotyping call rate
GNPDA2	glucosamine-6-phosphate deaminase 2
GPRC5B	G protein-coupled receptor, class C, group 5, member B
GRB14	growth factor receptor-bound protein 14
Het-p	p-value for heterogeneity among studies
HMM	hidden Markov models
HOXC13	homeobox C13
HR	hazard ratio
HRT	hormone replacement therapy
HT	hormone therapy
HWE	Hardy-Weinberg equilibrium
I2	percentage of heterogeneity due to factors other than chance
IBD	identity by descent
IBS	Iberian population in Spain
ICD	International Classification of Diseases
ITPR2	inositol 1,4,5-trisphosphate receptor, type 2
Kb	kilobases
KCTD15	potassium channel tetramerization domain containing 15
KREMEN1	kringle containing transmembrane protein 1
LAMC1	laminin, gamma 1
LAMP-ANC	local ancestry in admixed populations – ancestry option
LD	linkage disequilibrium
Ln	natural-logarithm
Log	logarithm

LOXL	lysl oxidase-like
LOXL1	lysl oxidase-like 1
LOXL3	lysl oxidase-like 3
LRP1B	low density lipoprotein receptor-related protein 1B
LRRN6C	leucine rich repeat and Ig domain containing 2
LTBP-2	latent transforming growth factor beta binding protein 2
LWK	Luhya in Webuye, Kenya
LY86	lymphocyte antigen 86
LYPLAL1	lysophospholipase-like 1
MAF	minor allele frequency
MAP2K5	mitogen-activated protein kinase kinase 5
Mb	mega bases
MCAR	metallocarboxypeptidase
MDS	multidimensional scaling
M_{eff}	effective number of independent tests
MKK	Maasai in Kinyawa, Kenya
MMP	matrix metallopeptidase
MMP-1	matrix metallopeptidase 1
MMP-3	matrix metallopeptidase 3
MMP-9	matrix metallopeptidase 9
MTCH2	mitochondrial carrier 2
MTIF3	mitochondrial translational initiation factor 3
MXL	Mexican Ancestry in Los Angeles, California
MYBPH	myosin binding protein H
MYH11	myosin, heavy polypeptide 11, smooth muscle
MYH3	myosin, heavy chain 3, skeletal muscle
MYO1E	myosin IE
MYOM2	myomesin 2

N	number
NHDS	National Hospital Discharge Survey
NEGR1	neuronal growth regulator 1
NFE2L3	nuclear factor, erythroid 2-like 3
NHANES	national health and nutrition examination survey
NISCH	nischarin
NOTCH1	notch 1
NRXN3	neurexin 3
NUDT3	nudix (nucleoside diphosphate linked moiety X)-type motif 3
OR	odds ratio
P5CS	pyrroline-5-carboxylate synthetase
PARP1	poly (ADP-ribose) polymerase 1
PGR	progesterone receptor
PIGC	phosphatidylinositol glycan anchor biosynthesis, class C
PLOD1	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1
PLOD3	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
POP	pelvic organ prolapse
POP-Q	pelvic organ prolapse quantification system
PRKD1	protein kinase D1
PTBP2	polypyrimidine tract binding protein 2
PYCR1	pyrroline-5-carboxylate reductase 1
Q	Cochran’s Q-statisitc
QC	quality control
QPCTL	glutaminyl-peptide cyclotransferase-like
RA	reference allele
RBJ	DnaJ (Hsp40) homolog, subfamily C, member 27
RGMA	repulsive guidance molecule family member a
RIN2	Ras and Rab interactor 2

RPL27A	ribosomal protein L27a
RR	relative risk
RRISK	reproductive risks for incontinence study at Kaiser
SD	standard deviation
SE	standard error
SEC16B	SEC16 homolog B, endoplasmic reticulum export factor
SH2B1	SH2B adaptor protein 1
SHARe	SNP Health Association Resource
SLC2A10	solute carrier family 2 (facilitated glucose transporter), member 10
SLC39A13	solute carrier family 39 (zinc transporter), member 13
SLC39A8	solute carrier family 39 (zinc transporter), member 8
SNP	single nucleotide polymorphism
SSPN	sarcospan
STAB1	stabilin 1
TBCE	tubulin folding cofactor E
TBX15	T-box 15
TFAP2B	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)
TGFB1	transforming growth factor, beta 1
TGFBR1	transforming growth factor, beta receptor 1
TGFBR2	transforming growth factor, beta receptor 2
THBS1	thrombospondin 1
TIMP-1	TIMP metallopeptidase inhibitor 1
TIMP-3	TIMP metallopeptidase inhibitor 3
TMEM160	transmembrane protein 160
TMEM18	transmembrane protein 18
TNNI3K	TNNI3 interacting kinase
TNXB	tenascin XB
TSI	Tosconi in Italy

TX	Texas
UCHL1	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
VEGFA	vascular endothelial growth factor A
WARS2	tryptophanyl tRNA synthetase 2, mitochondrial
WHI	Women's Health Initiative
WHI-HT	Women's Health Initiative Hormone Therapy
WHI-MS	Women's Health Initiative Memory Study
WHO	World Health Organization
YRI	Yoruba in Ibadan, Nigeria
ZDHHC24	zinc finger, DHHC-type containing 24
ZEB1	zinc finger E-box binding homeobox 1
ZNF608	zinc finger protein 608
ZNRF3	zinc and ring finger 3

CHAPTER I

INTRODUCTION AND SPECIFIC AIMS

Pelvic organ prolapse (POP), a common condition specific to women, is marked by descent of intra-pelvic organs including the uterus, bladder, rectum and the urethra, due to deficiencies in the pelvic support system [1;2]. The prevalence of POP ranges from 10% among all women ≥ 18 years of age, rising to 50% among post-menopausal women [3-5]. Women with POP may experience a range of debilitating symptoms including but not limited to the feeling of pressure or bulge in the pelvic area, pain, impaired sexual function, and urinary and fecal incontinence [1-6]. Costs associated with surgical correction for POP are over one billion dollars annually [7]; and POP recurrence rates after surgery are as high as 30% [8;9]. Causes of POP are likely multifactorial including a combination of genetic predisposition, inciting events that initiate POP and factors that promote POP progression [1;2;10]. Literature on POP identifies a number of risk factors associated with this condition, including increasing age, menopause, parity and higher total number of births, obesity, white race/ethnicity and genetic variation [1;11].

The mechanisms behind these relationships are poorly understood, and even less so in terms of how factors interact to cause POP. Postulated mechanisms for POP include damage to the pelvic floor muscle or nerves due to child birth, birth of macrosomic infants or damage due to surgical procedures. These events are considered to be factors that initiate the progression of POP and have thus been described as inciting factors. Deficiencies in the composition or repair of connective tissue matrix, which comprises a major portion of the pelvic floor support system

has also been proposed to influence POP. Finally, factors that exert excessive strain or pressure on the pelvic floor support system such as increased obesity, constipation, and chronic coughing have also been hypothesized to promote POP development over time, and have thus been described as promoting factors for POP.

With the exception of aging and parity [12-20], which have been most consistently associated with increased risk for POP, the relationships between obesity and POP [3;12;14;16;19;21-24], and race/ethnicity and POP [3;19;20;22;23] are not well understood, as reflected by heterogeneous effect estimates presented for these relationships across studies. Current literature reports effect estimates for POP for obese women (body mass index [BMI] $\geq$ 30 kg/m^2) range from null to a 2.5 fold increase in risk, when compared with women of normal weight. Even though several studies have examined the relationship between obesity, most often reported as BMI, and POP, we are not aware of any study that systematically assesses the strength and consistency of associations between obesity and POP across studies. A systematic review and meta-analysis of obesity and its relationship to POP will serve to bring the scientific community closer to a consensus on this issue and to highlight methodological issues that make the data challenging to aggregate. Similarly, some but not all studies suggest that African American women have a lower risk of having POP (reported associations range from no association to 50% reduced odds) than European American women [19;20;22;23]. However, it is not clear whether this difference reflects genetic heterogeneity, or cultural differences in seeking health care, or differences in ability to access care. Given the lack of understanding of the mechanisms influencing the relationship between race/ethnicity and POP, an investigation of whether the genetic determinants of geographic ancestry are associated with POP is warranted.

Additionally, the majority of studies examining the relationship between risk factors and POP have focused on exploring the main effects of these risk factors on POP occurrence. However, in the event genetic characteristics modify the relationship between inciting/promoting risk factors (such as number of births, or obesity) and POP, the main effects of these risk factors simply represent the weighted average of heterogeneous stratum-specific genetic effects that should not have been aggregated in the first place. Any parity, increasing number of births and especially increasing number of vaginal births are the strongest risk factors known for POP, and obesity is one of the few practically modifiable risk factors known for POP [1;2;25]. Understanding the reality that main-effect candidate gene studies have only resulted in modest evidence for supporting the notion of genetic predisposition to POP [26], a logical step for better understanding POP etiology is to investigate the interaction of genetic traits and individual characteristics. Specifically, an evaluation of interaction between parity and genetic variants and between obesity and genetic variants is warranted.

The following aims were devised to clarify the relationship between obesity and POP, if factors such as obesity and parity interact with genetic predispositions to contribute to POP and to understand if race/ethnicity-specific disparity in POP prevalence is at least in part explained by genetic differences.

Specific Aim 1: To evaluate the relationship between measures of obesity and POP through a systematic review and meta-analysis of observational studies and to probe for study attributes which may at least in part explain heterogeneous findings. A systematic review of published literature on obesity and POP was conducted. Random-effects models were then used to perform meta-analyses of cohort, case-control and observational studies reporting summary risk ratios, hazard ratios and odds ratios for the relationship between BMI and POP.

Hypothesis: We will observe a positive association between BMI and POP. Specifically, compared with normal weight women, the odds of having POP will be higher for overweight and obese women.

Specific Aim 2: To evaluate whether genetic variants modify the relationships between measures of BMI and POP, and parity and POP in European American, African American and Hispanic women. Genetic variants with plausible biological associations with POP or with plausible biological associations with connective tissue disorders in general, and genetic variants from gene regions which have previously been associated with measures of obesity were chosen for interaction analyses. Data from the Women's Health Initiative Hormone Therapy (WHI-HT) trial was used to evaluate two-way interactions between BMI and genetic variants, waist-to-hip ratio and genetic variants, and parity and genetic variants in relation to POP for all three race/ethnicity categories separately. I then performed a meta-analysis of the interaction terms generated by race/genotyping platform specific analyses. All analyses were performed using logistic regression models while adjusting for key factors chosen, *a priori*, that may confound the associations.

Hypothesis: We will find several genetic variants which modify the association between 1) BMI and POP and 2) parity and POP.

Specific Aim 3: To evaluate the relationship between individual ancestry proportion (global ancestry) and local ancestry (admixture mapping) in relation to POP in African American women from the WHI-HT study. Local ancestry and global ancestry estimates were inferred using randomly selected independent (linkage disequilibrium [LD]-pruned) markers throughout the genome using LAMP-ANC. The association between individual ancestry proportion (per 10% increase in European ancestry) and POP was assessed using logistic regression models

while adjusting for age, BMI and parity. The association between local ancestry and POP was assessed using: 1) a case-only design to compute Z-scores and 2) a case-control design in a logistic regression framework where POP was modeled against 0, 1 or 2 copies of European ancestry at the SNP level while adjusting for age, BMI, parity and genetic ancestry variables.

Hypothesis: Increasing proportion of European ancestry will be associated with increased odds of having POP. At the local level one or more ancestry-specific loci will be associated with increased or decreased risk for POP.

CHAPTER II

SIGNIFICANCE

Public Health and Economic Impact

POP is a major component of a broader constellation of conditions described as pelvic floor disorders [2;10]. POP is characterized by the descent of the pelvic organs, the uterus, bladder, and/or rectum into the vaginal space, and in extreme cases outside the vagina, due to lack of adequate anatomical support of the pelvic floor [1;2;27]. Women with prolapse may struggle with a variety of debilitating co-morbidities which diminish quality of life, especially as they age, including pelvic pain, sexual dysfunction, and urinary incontinence [1].

Although precise estimates of POP prevalence are not known, estimates from various studies [studies that report hospital rates of surgery for POP such as the National Hospital Discharge Survey (NHDS) [28], large clinical trials such as the WHI-HT [22], and symptom-assessment studies such as the NHANES [5]] suggest POP (any severity) to be common. Prevalence increases with advancing age and is highest in post-menopausal women [28]. The WHI-HT trial estimated POP prevalence among post-menopausal women to be approximately 41% at baseline when considering rectocele, cystocele and uterine prolapse of all grades of severity [22]. Not all severities of POP are symptomatic or require surgical intervention, and most adult women have some degree of prolapse that may or may not be symptomatic [1;29]. Although it is not clear to what degree of prolapse should be considered actionable, prolapse close to or below the hymen is often considered to be the level of clinical significance [1;29;30]. Another small cross-sectional study (n = 270 women) of the WHI-HT showed that

approximately 25.6% of post-menopausal women evaluated with the Pelvic Organ Prolapse Quantification (POP-Q) system (a validated measure of POP) had prolapse at or below the hymen [17]. Additionally, POP represents one of the most common indications for gynecologic surgery [31-33]. The lifetime risk for undergoing POP surgery has been estimated between 6 and 19% [9;34]. Data from the 2003 National Hospital Discharge Survey (NHDS) reported surgical rates for POP to be highest in post-menopausal women (31 per 10,000 women) and that 18.6% of these surgical procedures had complications [28]. Furthermore, corrective re-operation rates after surgery for prolapse is as high as 30% within 5 years of surgery. Direct costs associated with surgery for POP was estimated to be over 1.1 billion dollars in the year 1997 [7]. A trend analysis of the NHDS for the years 1979 to 2006 suggested that while POP surgery rates were decreasing among women < 52 years of age, surgery rates for POP remained stable for women 52 years of age or older [31]. By the year 2050, a projected 20% of the US population will be comprised persons over 65 years of age [35]. Extrapolating on US census population growth projection rates and on estimates of bothersome symptoms related to prolapse from the NHANES study, a recent study projected a 46% increase in POP surgeries from 2010 through 2050 [36]. Given the aging US population, the rising cost of health-care delivery, and the concurrently high prevalence of POP in post-menopausal women, POP poses a serious public health and economic concern.

Understanding the etiology behind POP

Current established remedies related to POP are mostly concentrated around treatment for POP once it has occurred and is symptomatic. While the literature identifies several potential modifiable and non-modifiable risk factors for POP, research on developing strategies that may potentially prevent, delay or lower risk for POP has been less successful. Identification of successful strategies that prevent or lower the risk for developing POP would first require a thorough understanding of how genetic and non-genetic factors contribute to POP etiology.

Advancing age and child birth are the only factors consistently associated with elevated risk for POP [1;10]. One study estimated 40% relative increase in prolapse risk for every decade increase in age [3]. It is generally agreed upon that aging contributes to increased risk for POP, especially between the ages of 30 to 80 years old [9;37]. Parity is the most strongly associated risk factor noted in the literature; estimates of risk associated with vaginal mode of delivery range from a 2-fold increase to a 10-fold increase in POP risk compared with nulliparous women [5;13;16;18-24;38-45]. Among parous women, cesarean section appears to significantly reduce the risk of prolapse [14;15;46]; however, the benefits of promoting this procedure as a preventative strategy for POP have not been deemed practical. The cost and benefit of promoting one surgical procedure to reduce risk of a subsequent condition that may or may not occur several decades later, and even if it occurs, may or may not require surgical intervention is difficult to quantify unless the ability to predict future POP occurrence at the individual level is extremely accurate. Additionally, elective caesarean section may have other unintended complications including increased rates of maternal and/or fetal mortality, hysterectomy, vesicle and ureteral injury, and uterine rupture in future pregnancies [47].

The knowledge that prolapse does happen in nulliparous women and that it is also closely associated with family history of prolapse [38;42] supports a role for genetic factors. Along these lines, several studies have identified single nucleotide polymorphisms (SNPs) in genes associated with collagen [48;49], and matrix metalloproteinases [50] to be associated with POP. It is also of interest that POP has been described to be less prevalent among African American women than European American women [19;20;22;23]. The reasons for this discrepancy are not clear and under-studied. The possibility of genetic differences between race/ethnicity as an explanation for POP disparity has not been investigated and cannot be ruled out.

Obesity as a modifiable risk factor for POP

Current literature also identifies BMI as a potential risk factor [1;25]. A cross-sectional analysis of over 20,000 women from the WHI study showed approximately 50% increased odds of POP in overweight and obese women, when compared to women with normal weight [22]. However, the range of associations reported in current literature varies widely, from no associations and statistically non-significant associations [5;13;18;19;24;38;41;45;46;51] up to a 2.5 fold increase in risk associated with obesity [3;12;14-16;20;20-23;39;40;42-44;52]. The inconsistency in findings across the literature is likely reflective of varying population characteristics, study design and inconsistent measures for POP (self-reported symptomatic POP, validated pelvic exam for POP such as POP-Q or Baden-Walker half way system, and definitions of POP – any severity of POP, only moderate to high grade POP). Among the various risk factors identified for POP, obesity appears to be the only risk factor that may be practically influenced on a population level to reduce the burden of POP, both from a public health and economic viewpoint. Targeting obesity as a modifiable risk factor to reduce burden of POP would have two broad implications: 1) whether POP occurrence can be lowered in future

populations by lowering obesity rates before women are at risk for having prolapse; and 2) whether POP progression can be delayed or reversed by reducing obesity among individuals who already have POP. However, before we can begin to address these key questions, an understanding as to whether obesity is associated with increased risk of having POP and of the mechanisms by which this occurs must be investigated. Given the wide range of effect estimates reported in the literature, a comprehensive qualitative and quantitative summary of studies examining the various degrees of obesity in relation to POP would greatly aid in generating evidence which can form the basis for more detailed epidemiologic and mechanistic investigations. Thus, we plan to conduct a systematic review and meta-analysis of the literature with the following goals:

1) Obtain overall effect estimates for POP in relation to various degrees of obesity, as measured by various indices of obesity including BMI and waist-to-hip ratio; and

2) Evaluate study characteristics such as study design, definition of POP, and method of POP measurement, which may in part explain the heterogeneous findings between studies examining obesity and POP.

Understanding POP as a combination of genetic, inciting and promoting factors

It is frequently mentioned that genetic factors, inciting factors such as parity and promoting factors such as obesity may contribute to POP [1;2;10]. However, there is a dearth of studies that evaluate the joint contributions of genetic predisposition and inciting or promoting risk factors in relation to POP. Studies that explore epidemiologic, individual and obstetric risk factors in relation POP have done so without considering potential genetic factors that may be in play. Similarly, studies evaluating genetic variants in relationship to POP, while they adjust for key risk factors, they have not attempted to evaluate if genetic variants interact with promoting

or inciting factors to contribute to POP. Effect modification/interaction analyses require large sample sizes. The lack of studies examining interactions between genetic variants and epidemiologic risk factors such as parity and obesity may partly be due to the lack of simultaneous availability of information regarding both genetic and epidemiologic risk factors with large sample sizes, and lack of reliable information about POP.

Evidence for POP heritability comes from studies evaluating family history for POP and from familial aggregation studies. A meta-analysis of eight studies found a 2.5-fold increased odds of having POP in women who had a family history of POP compared to women who did not have a family history of POP [53]. This is further supported by evidence from sibling studies, one of which suggests siblings of patients undergoing POP surgery are 5 times more likely to have POP than the general population [54]. A larger twin study showed correlations for POP as measured by review of surgical records to be 0.64 and 0.35 for monozygotic twins and dizygotic twins, respectively [55]. While these studies suggest genetic predisposition plays an important role in POP development, genetic studies conducted to date have had limited success in pinpointing genetic variants that may explain this heritability.

Molecular and genetic studies evaluating POP have mostly focused on variations in genes encoding proteins and enzymes that maintain the health of the pelvic floor, including components of the muscles and the extracellular matrix of the pelvic floor [11;26]. Focusing on these components, candidate gene studies in humans have found correlations between polymorphisms of matrix metalloproteinase (MMP) genes [50] and collagen type 3A-1 (*COL3A-1*) gene [48;49;56] and POP. Only one genome-wide association study (GWAS) has been conducted so far and it identified six potential loci in the genome that may be related to POP; however, results from this study are yet to be replicated [57]. Case-control studies have shown

decreased expression of the lysl-oxidase like (LOXL) family genes [58;59] and fibulin-5 gene in tissues [58;60] of POP cases compared to expression in tissues of controls. One study found decreased expression of the bone morphogenetic protein-1 (*BMP-1*) gene – a gene that regulates collagen deposition and activates LOXL genes – in severe POP cases compared to controls [61]. Interestingly, mouse knockout models have identified *LOXL-1* and fibulin-5 (*FBLN5*) as genes that contribute to POP, especially as it relates to parity [62;63]. Mice deficient in these genes developed POP after pregnancy [64]. Although results from mouse knockout studies cannot be directly generalized to humans, this is an example of how genetic and inciting risk factors such as birth events may work together to contribute to POP.

To better understand the etiology of POP, it is crucial to examine how genetic factors interact with factors that incite or promote POP development. Obesity is most likely the only practically modifiable risk factor, whereas parity is the strongest risk factor known for POP. Identification of genetic variants as factors that modify the risk for POP associated with obesity and parity should bring the field closer to the goal of eventually being able to predict who will or will not develop POP.

We evaluated gene environment interactions between genetic variants and measures of obesity, and genetic variants and parity using data from the WHI-HT trial. The WHI-HT trial is a large multi-centered clinical trial that was originally designed to evaluate the safety of hormone therapy in post-menopausal women [65]. The study collected baseline data on 27,400 women between the ages of 50 and 79, who were then followed up while collecting information on various outcomes. As routine procedure, this study performed pelvic exams on all women participating at baseline and subsequently at selected follow-up visits. Information on POP was measured as rectocele, cystocele and uterine prolapse using a grading system specific to the WHI

which also measures the various severities of POP through standardized pelvic exams. The WHI-HT also collected detailed information on several demographic factors, life-style factors and reproductive history. Women participating in the WHI-HT also provided blood samples at baseline. As a part of multiple initiatives post study recruitment, blood samples from over 12,000 WHI-HT participants were used to generate genome-wide association study (GWAS) data. The WHI-HT study therefore is a rich source of data with rigorous ascertainment for POP, with information on important risk factors for POP, and with GWAS data available for the European American, African American and Hispanic populations in the US.

With a sample size of over 12,000 individuals with validated measures of POP, genetic data for over 900,000 variants and crucial information available on key risk factors that affect POP, our study improves on previous studies by having greater power to evaluate interactions between genetic and non-genetic factors for a large number of SNPs. Sample size limitations have prohibited such investigations in the past. Additionally, most genetic studies for POP have been limited to either European American women or East-Asian women. We propose to examine these interactions in European American, African American, and Hispanic women, who represent the major race/ethnicities in the current US population.

Understanding cause of race/ethnicity specific POP disparity – is it genetic?

Epidemiologic studies also find differences in POP prevalence across racial/ethnic categories, although evidence is not consistent. For example, Whitcomb and colleagues reported a 5-fold higher risk of self-reported symptomatic prolapse in Hispanic and white women, compared with African American women [20]. However, in the same study, they did not find any difference in objective prolapse as measured by the POP-Q system. Swift and colleagues reported 4-fold increased odds of prolapse in Hispanic women, in comparison with white

women, but no such differences between white and African American women after adjustment for other risk factors [3]. Rortveirt and colleagues reported 60% reduced odds of prolapse in African-American women compared with European American women [19]. It is not clear, if these disparities are due to biological differences or due to differential reporting of POP by race/ethnicity status.

Whether disparity in POP by race/ethnicity status is a result of genetic differences has not been explored. If the association is biological, then an evaluation of the genetic components of race/ethnicity would provide etiological insight into POP development. Therefore, with the goal of exploring the plausibility that race/ethnicity specific difference in genetics contributes to POP, we plan to conduct the first admixture mapping study relating to POP in African Americans, using the WHI-HT data.

CHAPTER III

BACKGROUND

What is pelvic organ prolapse?

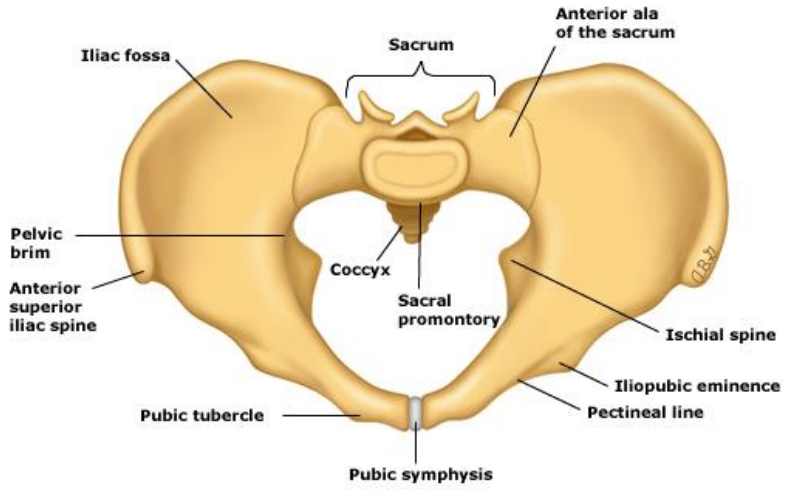
Pelvic organ prolapse is a condition in which one or more intra-pelvic structures in a female body is displaced from their normal anatomical positions and traverse downwards towards the vaginal space. In extreme cases structures may protrude outside the vagina [1]. The pelvic floor acts as a support system and normally holds the intra-pelvic structures in place including the bladder, uterus and the bowels. Structural degradation of the pelvic support system due to one or more defects is the most immediate basis for POP [1;66;67]. The following sub-sections describe the female pelvic floor anatomy, organ-based and structure-based classifications of POP, and systems devised to measure the degree of POP.

Female pelvic floor anatomy

The pelvic floor support system is comprised of the pelvic bones, the pelvic muscles and the connective tissue complexes that envelope the muscles. The bony pelvis is the bilaterally symmetric scaffolding that provides the framework for attachment of pelvic support structures. This cup-shaped structure consists of two identical innominate bones, each of which is composed of the ilium, pubis, and ischium [66]. These two bones are fused anteriorly at the pubic symphysis and posteriorly at the sacrum to form two basins: the major pelvis and the minor pelvis; the latter extends inferiorly to the major basin and harbors the pelvic musculature and connective tissue matrices that comprise the pelvic floor [66]. Attached to the inner surface of the minor pelvis, the coccygeus muscles and the levator ani muscle complex together form the

pelvic diaphragm [66]. Superior view of the pelvic bone and its components are shown in Figure 3-1.

Figure 3-1. Superior view of the pelvic bone



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The levator ani muscle complex is comprised of two major muscle groups named the iliococcygeus and the pubococcygeus muscles [66;68]. The pubococcygeus muscle is the bulkiest muscle structure and lies in the medial portion of the pelvic floor. The pubococcygeus muscles can also be subdivided to reflect its attachment to various organs, including rectum (puborectalis), anus (puboanalis), urethra (pubourethralis) and vagina (pubovaginalis) [68]. The levator ani complex is connected to the scaffolding directly from the back of the pubic bone and via the arcus tendinous at the anterior of the scaffolding. The acrus tendinous is a dense connective tissue matrix that runs all the way through the pubis and ischial spine connecting the left side of the pelvis and the right side of the pelvis with the coccygeus and levator ani which extend laterally from the medial portion of the pelvic floor [68-70]. The pubococcygeus muscle is further posteriorly attached to the coccyx, a structure that extends inferiorly to the sacrum. The

fibers of the pubococcygeus- and the illiococcygeus muscle further fuse posteriorly at the coccyx. This forms the levator plate, which in a standing body sits horizontally. It not only serves as the plate on which the pelvic organs rest but also contributes support to the rectum and the upper two thirds to the vagina. A small gap is created between the levator muscle and the pubic symphysis, named the levator hiatus, through which the urethra, vagina and anorecturm traverse [69;71]. The levator ani muscles are usually tonically contracted which serves to keep the integrity of the pelvic floor intact and to close the levator hiatus. Weakness in the levator ani muscle complex may cause the muscles to lose its tonicity and as a result may cause a widening of the levator hiatus [71;72]. The weakness could arise because of de-enervation of the muscles or due to damage to the musculature structure itself. Clinical examination of women with and without POP suggests that women with POP have a wider opening of the levator hiatus [71]. Superior view of the pelvic bone and orientation of the muscles of the pelvic floor are shown in Figure 3-2.

In addition to the pelvic diaphragm, the urogenital diaphragm (also called the perineal membrane) and the perineal body provides additional support [66]. Located below the pelvic diaphragm, the membrane is composed of muscular and connective tissue elements. It provides structural support for the distal vagina and the distal urethra, serves to close the levator hiatus, and bridges the gap between the pubic bone and the perineal body [66;68-70]. The perineal body is a fibromuscular structure which contains smooth muscles, nerve endings and elastic fibers. It is attached to the perineal muscles, the anal sphincter, and the vaginal slips from the pubococcygeus muscles. To provide a sense of anatomical orientation, the vagina and uterus are above the pelvic diaphragm, below which is the urogenital diaphragm, and the perineal body extends to the pelvic diaphragm vertical to the urogenital diaphragm.

Figure 3-2. Superior view the pelvic bone and muscles in the pelvic diaphragm

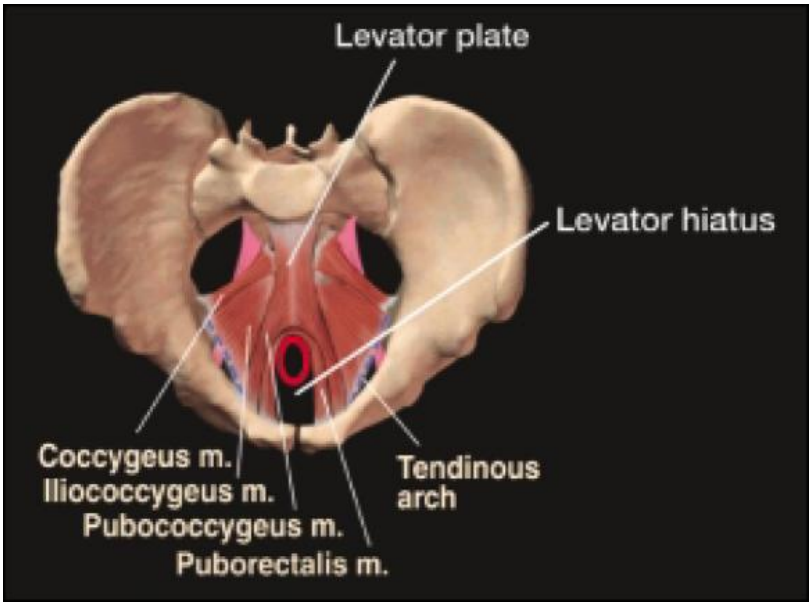


Figure reprinted with permission from Herschorn S., Female Pelvic Floor Anatomy: The Pelvic Floor, Supporting Structures, and Pelvic Organs, Rev Urol. 2004;6(suppl 5):S2-S10 [66]

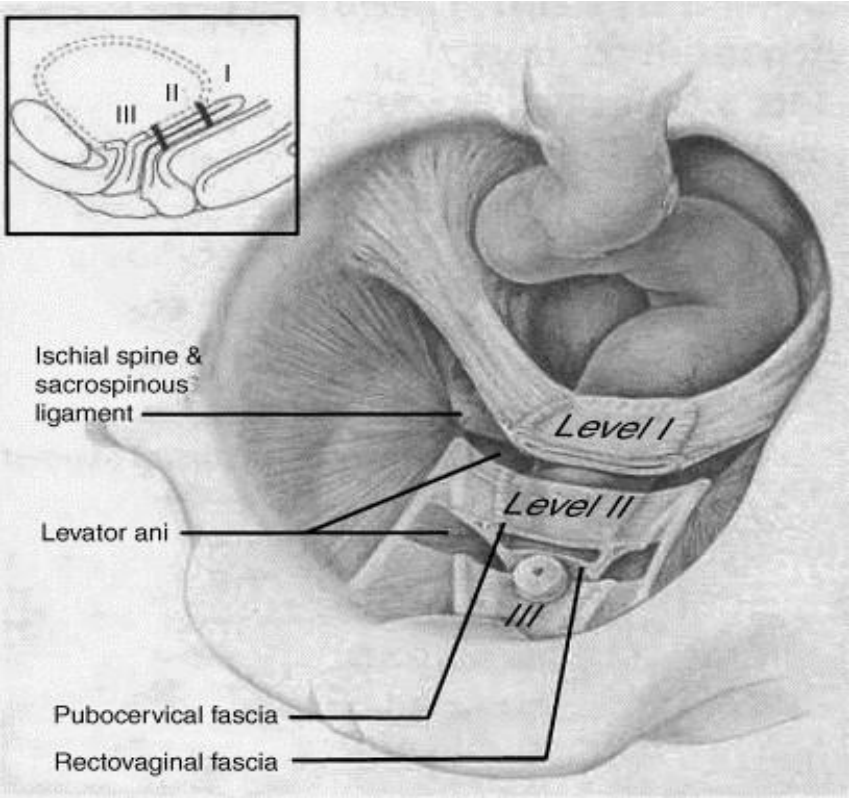
In addition to the muscular layers that provide support to the pelvic organs, various fasciae, which are tough fibrous tissues that envelope muscles and organs, connect the pelvic floor to the various organs [73]. The pelvic fascia envelopes the anterior, and posterior sides of the pelvic musculature structure [66;73]. The visceral fasciae envelope the pelvic organs and other underlying structures. In addition to providing protection these fascia also allow for changes in volumes of these organs when necessary [66]. The visceral fascia is connected to the pelvic fascia through an interconnected mesh-like connective tissue called the endopelvic fascia. The endopelvic fascia is continuous both to the visceral fascia and the pelvic fascia. The endopelvic fascia contains various vascular structures, smooth muscle cells and fibroblasts and along with various specialized ligaments that extend from the pelvic fascia connect the organs to the muscular and bony components of the pelvic floor [66;73].

The connective tissue matrices that support the urethra, bladder and the anterior wall of the vagina, extend all the way to the arcus tendinous region of the pelvic fascia [70;74]. At the

medial superior portion of the vagina and the cervix, the cardinal ligaments extend to the pelvic walls in addition to the continuous fasciae that extend from the organs to the pelvic walls [75]. The uterosacral ligaments extend from the cervix and the vaginal fornices to attach to the fascia surrounding the sacrum, in addition to the continuous connective tissue. It is thought that the cardinal and uterosacral ligaments together hold the upper vagina and the uterus in its place [66]. The posterior walls of the vagina are connected by the vaginal fascia to the endopelvic fascia. The rectovaginal fascia, the connective tissue between the posterior wall of the vagina and the rectum is the thickest at the lateral regions of the posterior vaginal and thinnest in the mid-posterior vaginal wall [75].

The literature identifies another way of grouping the levels of support provided by the various structures described above [67;69]. Level 1 support relates to the proximal (deep) region of the vagina and is provided by the cardinal and uterosacral ligaments which attach to the sacrum and the pelvic diaphragm. These provide support to the vaginal apex, cervix, and the uterus. Level 2 support concerns the pubocervical attachments to the levator ani fascia and the rectovaginal attachments to the arcus tendineus. These attachments provide support to the lateral walls of the vagina. Level 3 support has been referred to the distal end attachments to the perineal membrane and urogenital diaphragm anteriorly, to the leavotor ani muscles laterally, and to the perineal body posteriorly. The three levels of support as proposed by DeLancey are shown in Figures 3-3a.

Figure 3-3. Three levels of support for the pelvic organs as proposed by DeLancey et al.



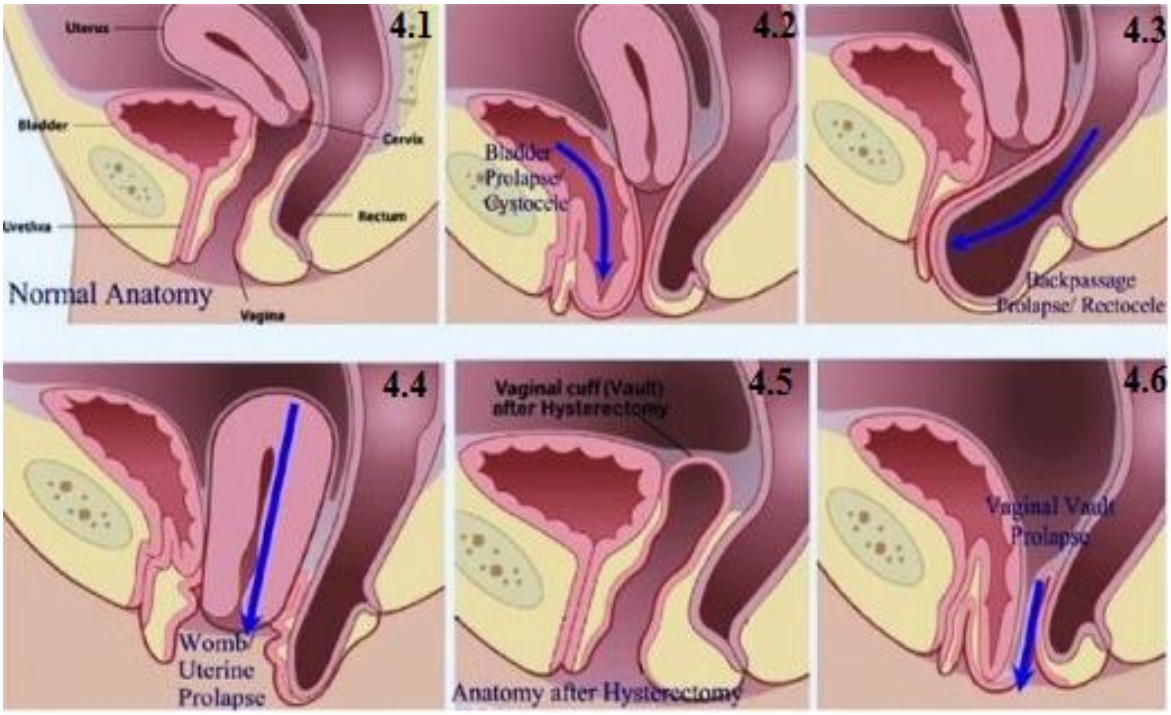
Level I consists of the cardinal and uterosacral ligaments, and suspends the vaginal apex. Level II consists of the endopelvic fascia connections to the arcus tendineus fascia pelvis, which attaches the vagina to the aponeurosis of the levator ani. Level III consists of the perineal body and includes interlacing muscle fibers of the bulbospongiosus, transverse perinei, and external anal sphincter. Figure 2.3a reprinted with permission from DeLancey, John OL. "Anatomie aspects of vaginal eversion after hysterectomy." American journal of obstetrics and gynecology 166.6 (1992): 1717-1728. [75]

Classification of pelvic organ prolapse

POP may be classified into various types; the type of prolapse is defined according to the origin of descent of the pelvic organ/structure [76]. Anterior wall prolapse, also called cystocele, occurs when the anterior wall of the vagina loses support and the bladder drops towards the vaginal opening [1;76]. This most likely occurs due to defect in the Level 2 support described above [67;69]. Posterior wall prolapse, also called rectocele, occurs when the posterior wall of the vagina loses support and the rectum drops towards the vaginal opening [1;76]. This most

likely occurs due to defect in the Level 3 support described above [67;69]. Cystocele and rectocele may occur in women with or without an intact uterus. Uterine prolapse occurs when the uterus drops from its anatomical position into the vaginal space [1;76]. Vaginal vault prolapse occurs in women without a uterus when the vaginal cuff pushes into the lower vagina [67;76]. Both vaginal vault prolapse and uterine prolapse most likely occur due to loss of the level 1 support described above [67;69]. Vaginal vault prolapse often occurs in combination with enterocele -- the herniation and dropping of the small intestine from its normal anatomical position towards the vagina. Enterocele prolapse most likely occurs due to loss of the level 3 support described above. These conditions may occur alone or as often is the case in combination [67;69]. Depictions of the normal pelvic anatomy and the various types of prolapse are shown in Figure 3-4

Figure 3-4. Depictions of the pelvic anatomy without POP and with different types of POP



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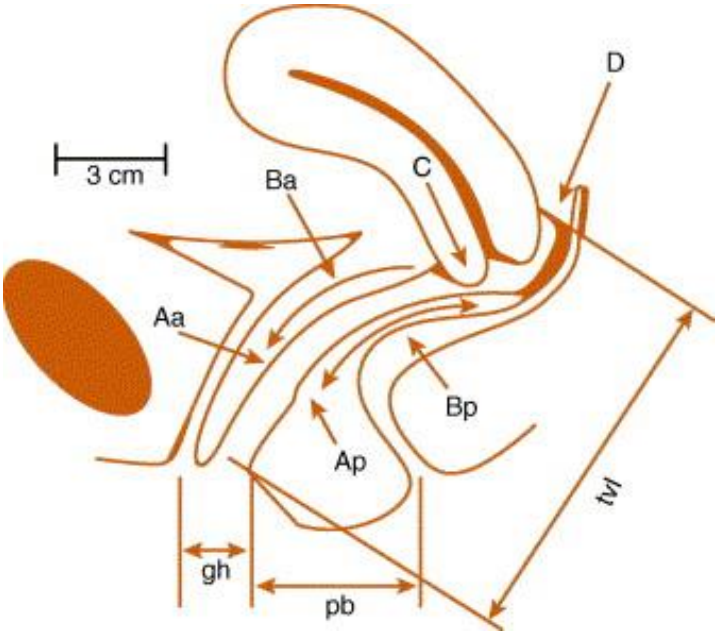
How is pelvic organ prolapse measured?

POP is usually diagnosed with a pelvic exam performed by a trained health professional. There are two major measurement systems that are widely accepted and frequently used by clinicians to diagnose and grade the severity of prolapse: the POP-Q system [77] and the Baden-Walker Halfway system [76;78]. The POP-Q system is the most widely accepted measurement system as it is a standardized quantification system that has high intra and inter-rater reliability [76].

In the POP-Q system a total of six points are measured at the vagina with the hymen serving as the reference point [76;77]. Point Aa and Ba refer to the proximal and distal positions (in reference to the hymen) of the anterior vaginal wall, respectively (Figure 3-5). Points Ap and Bp similarly refer to the proximal and distal positions (in reference to the hymen) of the posterior vaginal wall, respectively. Point C refers to the distal edge of the cervix and point D refers to the posterior fornix. The total vaginal length (tvl) refers to the depth of the vagina; more specifically the distance from points C or D if these were in their normal positions till the hymen. These points are measured in the centimeter units. Since the hymen is the point of reference, the location of any point at the hymen is marked as 0, the location of any point beyond the hymen is marked as a positive number, and the location of any point above the hymen is marked by a negative number.

Figure 3-5. Pelvic organ prolapse Quantification (POP-Q) system of measurement for POP



Aa: Point A in anterior wall; Ba: Point B in anterior wall; C: Point refers to the cervix or vaginal cuff; D: Point refers to the posterior fornix; Ap: Point A in posterior wall; Bp: Point B in posterior wall; gh: genital hiatus; pb: perineal body; tvl: total vaginal length. *Reprinted with permission* from Bump, Richard C., et al. "The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction." American journal of obstetrics and gynecology 175.1 (1996): 10-17. [77]

Table 3-1. Defining stages of POP as described by the POP-Q system

Stage	Leading edge of POP (at hymen score = 0)
Stage 0	<-3 cm
Stage I	< -1 cm
Stage II	≤ +1 cm and ≥ -1 cm
Stage III	> +1 cm
Stage IV	≥ total vaginal length -2 cm

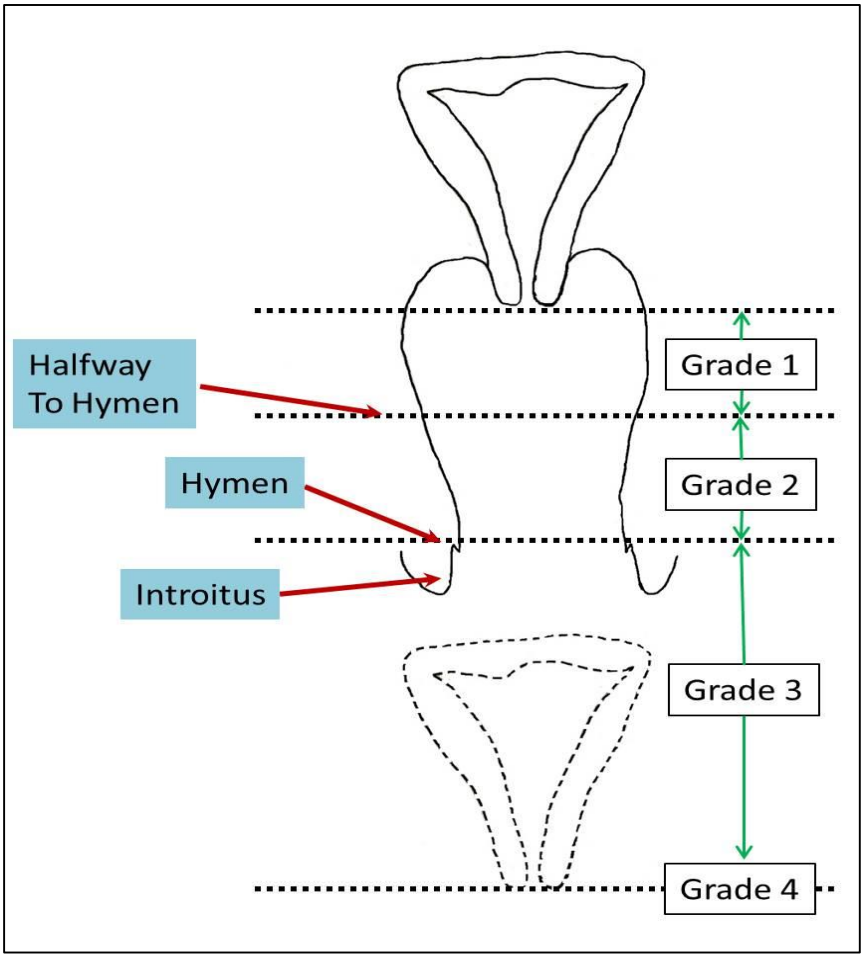
Zero denotes the leading edge of POP is at the hymen;
Negative integer denotes leading edge of POP is above the hymen;
Positive integer denotes leading edge of POP is past the hymen

An individual is considered to have no prolapse/Stage 0 if the Aa, Ap, Ba and Bp points have a score of -3 cm and if C or D is less than or equal to –(tvl -2), indicating that all of the pelvic organs and structures are intact in their respective places (Table 3-1). If one or more of the stage 0 criteria are violated and the leading edge of the prolapse is less than -1 cm then this is considered to be Stage I prolapse. Stage II prolapse is when the leading edge is greater than or equal to -1 cm but less than or equal to +1 cm. Stage III prolapse is when the leading edge of the

prolapse is greater than +1 cm but less than + (tvl -2) cm. Finally, stage IV prolapse is when the leading edge is greater than or equal to + (tvl -2) cm.

The Baden-Walker Halfway system was widely used prior to the standardization of measurement by the POP-Q system proposed by Bump and colleagues in 1996 [76]. The system of measurement is semi-quantitative and thus is thought to have lower intra- and inter-rater reliability than the POP-Q system [76]. Nonetheless, the intuition behind the system is simple, easily applicable and informative. Like the POP-Q system, the Baden-Walker Halfway system also classifies POP into four different grades and uses the hymen as the point of reference. Grade 0 is when there is no prolapse and all anatomical locations are in their respective places. Grade 1 prolapse is considered when the leading edge of the prolapse is halfway to the hymen. Grade 2 prolapse is considered when the leading edge of the prolapse is towards the hymen but not past it. Grade 3 prolapse is considered when the leading edge of the prolapse is past the hymen but not that the descent is only halfway past the hymen. Finally, grade 4 prolapse is considered as the maximum possible descent past the hymen for one or more of the different types of prolapse (Figure 3-6).

Figure 3-6. Baden-Walker half way classification system for POP



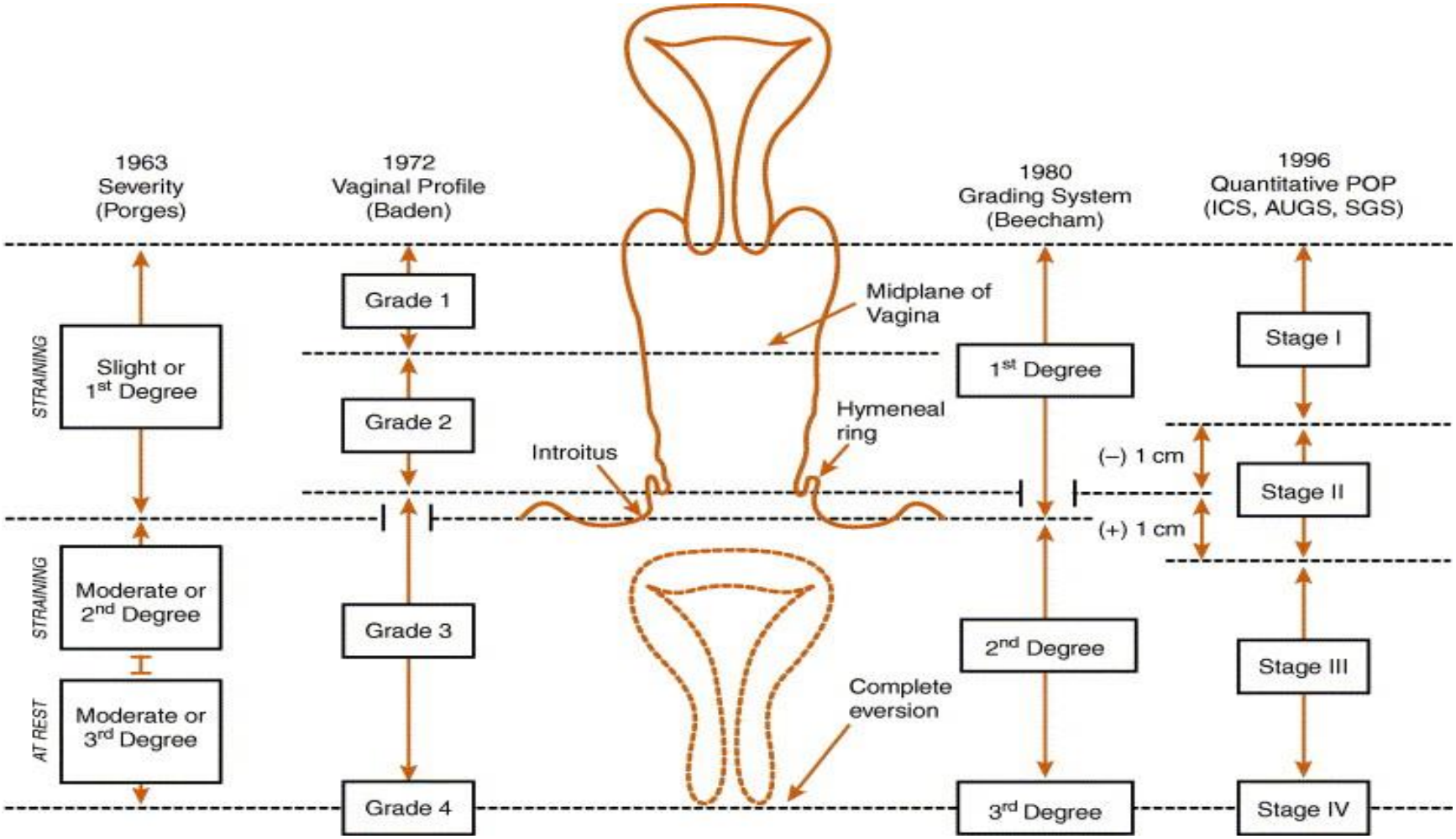
The area marked as 1, 2, 3, and 4 represent prolapse of grade 1, prolapse of grade 2, prolapse of grade 3 and prolapse of grade 4, respectively.

The Baden-Walker Halfway system is not a standardized system, and its gradations of measurement – halfway in reference to the hymen, introduces subjectivity to the measurement process if one were to consider the exact extent of prolapse. From a research perspective, the POP-Q system provides a continuous measure of prolapse that may be used as it is, while also providing the flexibility of using the staging category. The Baden-Walker Halfway system fails in this respect as it does not provide quantitative measurements of the prolapse. However, if the goal of a research team were to examine the relationship between risk factors and any prolapse,

or even only clinically significant prolapse, one could consider both of the measurement systems to be fairly comparable. Both systems utilize the hymen as the reference point, partly because it has been suggested that prolapse that is close to the hymen or beyond the hymen is clinically meaningful and correlates more so with symptoms/comorbidities associated with prolapse. In comparing the two systems of measurement starting from the most severe forms of prolapse, Baden-Walker Halfway grades 3 or more effectively covers all of Stage IV POP-Q prolapse and all of the Stage III POP-Q prolapse that have points ≥ 0. Since the POP-Q Stage III criteria includes some prolapse that lie above the hymen, some of the POP-Q Stage III would be classified as Baden-Walker Halfway grade 2 prolapse. So, grade 2 prolapse is likely to cover Stage II and Stage III of the POP-Q system. Because of the subjectivity in the Baden-Walker Halfway grading system, it is difficult to say if grade 2 may include some of POP-Q stage I prolapse. However, one could speculate that since grade 2 covers some of POP-Q Stage III prolapse that majority of grade 2 prolapse is more likely to include POP-Q Stage II and POP-Q Stage III, but less likely to include POP-Q Stage I prolapse. Similarly, one could speculate that majority of grade 1 prolapse may correspond to POP-Q Stage I prolapse, but it cannot be ruled out that some of grade I prolapse may correspond to the POP-Q Stage II prolapse or vice versa. A graphical representation comparing the Baden-Walker grading system and the POP-Q staging system is shown in Figure 3-7.

In addition to clinical assessment of the pelvis, three-dimensional ultrasound or magnetic resonance imaging could also be used for post-clinical assessment for severity of POP, functional assessments of the pelvic floor or post-surgical assessment of the pelvic floor [76;79;80]. However, these assessment techniques are not currently incorporated into routine procedures and currently only remain as investigational research tools.

Figure 3-7. Comparing measurement systems for POP



Baden = Baden-Walker Halfway system; ICS, AUGS SGS Quantitative POP = Pelvic Organ Prolapse Quantification System; Figure reprinted with permission from Mouritsen, Lone. "Classification and evaluation of prolapse." Best Practice & Research Clinical Obstetrics & Gynaecology 19.6 (2005): 895-911.[81]

Risk factors for POP

POP has been recognized as a medical condition for more than 100 years. In a book entitled "Clinical Memoirs on the Diseases of Women, Bernutz and colleagues in 1867 described the historical mention of uterine prolapse in times of ancient Greece and ancient Egypt, more than 3000 years ago [82]. Yet, the etiology behind POP is still poorly understood. We know that structural defects relating to the pelvic floor support system leads to prolapse [66;67;69;71;73], but we do not know the causes behind these defects, or why they manifest in some women but not in others. Identification and proper evaluation of risk factors and how they interact is key to understanding the etiology of POP. Research has identified a number of risk factors for POP. Some of these factors include non-modifiable risk factors such as genetic predisposition, or modifiable risk factors such as obesity. Bump and Norton classified risk factors for pelvic floor dysfunction in general into more informative categories [10], instead of simply categorizing them as modifiable and non-modifiable. The categories include predisposing factors, inciting factors, promoting factors and decompensating factors. The first three categories of risk factors are most relevant to POP and are thus discussed below.

Evidence for predisposing factors

Family history as a risk factor for POP

The first line of evidence that suggests genetic factors may predispose women to develop POP in humans comes from studies that evaluate the relationship of family history of POP in women with and without POP. A systematic review in 2012 reported that there are 16 studies that evaluated family history for POP with the risk for POP [53]. However, only 8 of the 16 studies had valid control groups (controls had no history of prior POP surgery) and provided information about family history of POP in women with and without POP. Of the eight studies

that were eligible for a meta-analysis, three studies had a cohort design [51;83;84], and five studies were case-control studies [38;43;85-87], of which two were matched case-control designs with age and parity as the matching variables [85;87]. Across these eight studies the measurement of family history was also variable, two of which provided no-specification as to what family history meant [84;87], two asked about maternal history of POP [38;51], one asked if mother or sister had POP [43], one asked if mother or grandmother had history of pelvic floor disorder [43;85], one asked if up to first degree relatives had POP [86], and one asked if there was any history of POP or hernia in family [83]. In addition to concerns with variable definitions that make comparisons difficult, recall bias poses a greater concern for case-control studies, as cases might be more likely to scrutinize family history of POP in comparison to controls. The authors of the meta-analysis clearly state this and report an odds ratio of 2.58 (95% CI: 2.12, 3.15) [53]. It is of note that although the effect estimates varied widely from 1.51 (95% CI: 0.52, 4.38) to 8.84 (95% CI: 2.62, 29.79), and even though studies adjusted for different confounders, all of the effect estimates were in the same positive direction. Although these results are suggestive of the possibility that hereditary factors may influence POP, issues related to study design such as recall bias, or that confounding by unmeasured environmental factors or residual confounding that correlate with genetic factors within families could provide alternate explanations as well.

Sibling studies

A small study of ten women who had severe prolapse under the age of 55 suggested that siblings of patients who had prolapse were approximately five times as likely to have POP in comparison to risk in the general population [54]. The authors performed analysis of familial inheritance pattern for the ten patients and reported an autosomal dominant mode of transmission

from both maternal and paternal contributions and suggested a high degree of penetrance. Another study examined 101 sibling pairs (1:1 parous to non-parous ratio) to evaluate the relationship between parity and prolapse and familial concordance for prolapse [88]. While the majority of the 101 sibling pairs did not have prolapse, the concordance of compartment-specific prolapse ranged from 74% to 91%. At the same time they also reported that in discordant sister pairs, parous sisters were 88% more likely to have advanced form of prolapse than nulliparous sisters, suggesting a role of parity in addition to potential genetic contribution [88]. In another larger twin study conducted in Sweden using data from 3,376 monozygotic twins and 5,067 dizygotic twins, Altman and colleagues estimated correlations of 0.64 and 0.35, respectively with regard to POP [55]. Additionally, they estimated that genetic components explained approximately 43%, shared non-genetic environmental components explained 17% and non-shared non-genetic environmental components explained 40% of the variability with respect to POP [55].

Genetic studies relating to POP

Although the different types of studies mentioned above collectively suggest that genetic predisposition may in part explain influence POP risk, it is equally important to discover and pinpoint the specific sources of genetic predisposition towards POP. To this end, several studies have evaluated the relationship between SNPs, which are the most abundant type of genetic variation, and POP. Most of the studies conducted to date have been case-control candidate gene studies that focus on components that maintain the health of the pelvic floor support system. A recent systematic review [26] of studies that evaluated the genetic determinants of POP by Ward and colleagues reported 21 studies in total, of which 18 were candidate gene studies [42;48-50;56;89-101], only one study was a GWAS [57] and two were linkage studies [102;103]. A

majority of the candidate gene studies that have evaluated the relationship between polymorphism of genes and POP have focused on genes that contribute towards support for the pelvic floor including collagen genes, proteases that act on collagens, and other regulatory genes that are essential for connective tissue development and POP. Summaries of these genetic studies conducted in humans are listed in Table 3-2. Meta-analysis results of select SNPs evaluated by two or more studies are presented in Table 3-3.

Table 3-2. Systematic review of genetic variants evaluated for association with POP

First Author	Race and ethnicity (study country of origin)	Cases (with POP)	Controls (no POP)	Phenotype-Cases	Phenotype-Controls	Gene	SNPs evaluated
GWAS							
Allen-Brady et al. [57]	Cases: white (USA) Controls: white	115	Illumina iControlDB 2,976	Treated for POP with a family history of prolapse or other pelvic floor disorders	Pelvic floor information not known. Excluded duplicate and closely related samples.		rs1455311,4q21.21; rs1036819,8q24.22; rs430794,9q22.2; rs8027714,15q11.2; rs1810636,20p13; rs2236479,21q22.3
Linkage analysis							
Allen-Brady et al. [102]	European descent (USA)	70 cases (Familial study: 32 families)	N/A	Treated for moderate-severe POP, usually POP-Q stage III-IV (41/66)	N/A		LOD score 3.41; Chr9: 80.35Mb-88.81Mb with HLOD ≥1.86
Nikolova et al. [103]	NR (USA)	6		Prolapse evaluated by POP-Q		*LAMC1* sequence variant 1q31	rs10911193
Case-control, candidate gene analysis							
Chen et al. [89]	White and African American; results reported by race (USA)	165 (102 White 63 African American)	246 (163 White 83 African American)	POP-Q stage III, IV	POP-Q stage 0-1; Cases and controls were matched on age, race, menopausal status, smoking history, BMI and parity.	*LAMC1*	rs10911193; rs20563
Chen et al. [91]	NR (Taiwan)	69	141	POP-Q stage II-IV	POP-Q stage 0-I	*ER-Beta*	rs2987983 (-13950 T/C) Promoter; rs1271572 (-12214 G/T) Promoter; rs9444599 (-1213 T/C) Promoter; rs1256049 (25652 A/G) Exon 6; rs1255998 (110943 G/C) 3'-UTR

First Author	Race and ethnicity (study country of origin)	Cases (with POP)	Controls (no POP)	Phenotype-Cases	Phenotype-Controls	Gene	SNPs evaluated
Chen et al. [92]	NR (Taiwan)	88	153	POP-Q stage II-IV	POP-Q stage 0-I	*ER-Alpha*	rs17847075 (exon 1 C/T); rs2207647 (exon 1 G/A); rs2234693 (intron 1 T/C); rs3798577 (exon 8 C/T); rs2228480 (exon 8 G/A)
Chen et al. [48]	NR (Taiwan)	84	147	POP-Q stage II-IV	POP-Q stage 0-I	*COL3A1*	rs1800255 (Exon 30 G>A); rs1801184 (exon 32 T>C)
Chen et al. [90]	NR (Taiwan)	87	150	POP-Q stage II-IV	POP-Q stage 0-I	*PGR*	rs500760 (exon 8 A/G); rs484389 (3'-untranslated region C/T)
Chen et al. [50]	NR (Taiwan)	92	152	POP-Q stage II-IV	POP-Q stage 0-I	*MMP-9*	rs3918242; rs17576; rs2250889
Cho et al. [93]	Korean (Korea)	15	15	POP-Q stage III-IV (women undergoing hysterectomy)	POP-Q stage 0 (hysterectomy for uterine myoma)	*COL1A1 Sp-1* binding site	No polymorphism seen at *Sp-1* binding site in *COL1A1* (all G/G, cases and controls)
Feiner et al. [94]	White or Ashkenazi-Jewish (Israel)	36	36	POP-Q stage III-IV POP	POP-Q stage 0-I	*COLIA1 Sp-1* binding site	*SP\p-1* binding site (no rs#)
Ferrari et al. [95]	NR (Italy)	137	96	POP-Q stage II-IV	POP-Q stage 0-I	*COL1A1, MMP1,3,9*	SP1 site of *COL1A1* point mutation (G-T) in 1st intron; neg 1562 /T of *MMP9*; neg 1171 5A/6A of *MMP3*; neg 1607 1G/2G of *MMP1*
Ferrell et al. [56;96]	African American and White (USA)	137	141	POP-Q stage II-IV	POP-Q stage 0-I, matched to cases on age, race, menopausal status, smoking history, BMI and parity	*LOXL1*	No rs # (labeled -659 in promoter)

First Author	Race and ethnicity (study country of origin)	Cases (with POP)	Controls (no POP)	Phenotype-Cases	Phenotype-Controls	Gene	SNPs evaluated
Jeon et al. [56]	Korean (South Korea)	36	36	POP-Q stage II-IV, Postmenopausal and parous	POP-Q stage 0-I, no stress urinary incontinence, postmenopausal and parous	*COL3A1*	5'-AAGTATACAAATTTCTAGATTG-3' (forward)/5'-ATAAATGATCAGAAGGAAATCA-3' (reverse)
Kluivers et al. [49]	European, Dutch (Netherlands)	202	102	POP present (not defined)	Vaginally parous, descent <1 cm above hymenal remnants, no prior POP surgery	*COL3A1*	rs1800255
Martins et al. [42]	White and Nonwhite (Brazil)	107	209	POP-Q stage III-IV, postmenopausal, no HRT	POP-Q stage 0-I, no documented vaginal surgery or stress incontinence, postmenopausal, no HRT	*COL3A1*	No rs# (labeled exon 31 G allele)
Rodrigues et al. [97]	White and Nonwhite (Brazil)	107	209	POP-Q stage III-IV	POP-Q stage 0-I	*COL1A1* Sp1 binding site	*COL1A1* Sp-1 binding site polymorphism (no rs#)
Skorupski et al. [98]	NR (Poland)	37	40	POP-Q stage III-IV	POP-Q stage 0-I	*COL1A1*	position 1240 in 1st intron; G -> T substitution; transcription factor Sp1 binding site of COL1A1
Skorupski et al. [99]	NR (Poland)	133	132	POP-Q "grade' II-IV, undergoing surgery	POP-Q "grade' 0-I,dysfunctional uterine bleeding or undergoing TAH/SCH	*MMP1, 3*	*MMP1* polymorphism (position -1607/-1608); *MMP3* polymorphism (position -1612/-1617)

First Author	Race and ethnicity (study country of origin)	Cases (with POP)	Controls (no POP)	Phenotype-Cases	Phenotype-Controls	Gene	SNPs evaluated
Wu et al. [100]	White, non-Hispanic (USA)	239	197	POP-Q stage III-IV, not pregnant; no age cutoff but preferentially recruited younger women	POP-Q stage 0-I, no history of POP surgery, preferentially recruited older women	*LAMC1*	rs10911193; rs1413390; rs20558; rs20563; rs10911206; rs2296291; rs12041030; rs12739316; rs3768617; rs2483675; rs10911211; rs41475048; rs1058177; rs12073936
Wu et al. [101]	White, non-Hispanic (USA)	239	197	POP-Q stage III-IV, no age cutoff but preferentially recruited younger women	POP-Q stage 0-I, no history of POP surgery, preferentially recruited older women	*MMP9*	rs3918253; rs3918256; rs3918278; rs17576; rs2274755; rs17577; rs2236416; rs3787268

POP=pelvic organ prolapse; POP=pelvic organ prolapse quantification system; SNP=single nucleotide polymorphism. *Table adapted and reprinted with permission from* Ward, *Renée M., et al. "Genetic epidemiology of pelvic organ prolapse: a systematic review." American journal of obstetrics and gynecology 211.4 (2014): 326-335. (Table 3-1)[26]*

Table 3-3. Meta-analysis of odds ratios for SNPs within *COL3A1*, *MMP1*, *MMP9*, and *LAMC1* evaluated in association with POP in the literature

Study	Effect	Ref	OR	95% CI	Weight	P value [a]	Het-P value [b]	I2 [c]
COL3A1 (rs1800255)								
Chen	AG	GG	0.74	(0.41, 1.26)	40.38%			
Kluivers	AG	GG	0.96	(0.59, 1.58)	59.62%			
Meta-analysis	AG	GG	0.87	(0.59, 1.27)	100%	0.46	0.52	0.00%
Chen	AA	GG	4.59	(1.17, 18.05)	44.98%			
Kluivers	AA	GG	4.95	(1.44, 17.06)	55.02%			
Metaanalysis	AA	GG	4.79	(1.91, 11.98)	100%	0.001	0.94	0.00%
MMP1 (1607/1608)								
Ferrari	1G/2G	1G/1G	2.24	(1.16, 4.30)	42.05%			
Skorupski	1G/2G	1G/1G	0.96	(0.55, 1.67)	57.95%			
Meta-analysis	1G/2G	1G/1G	1.39	(0.90, 2.09)	100%	0.15	0.05	73.40%
Ferrari	2G/2G	1G/1G	2.81	(1.25, 6.33)	44.98%			
Skorupski	2G/2G	1G/1G	0.93	(0.49, 1.75)	55.02%			
Meta-analysis	2G/2G	1G/1G	1.41	(0.86, 2.33)	100%	0.18	0.04	77.50%
MMP9 (rs17576) [d]								
Chen et al	GG/AG	AA	5.67	(1.28, 25.12)	7.78%			
Wu et al	GG/AG	AA	0.74	(0.48, 1.14)	92.22%			
Meta-analysis	GG/AG	AA	0.87	(0.57, 1.31)	100%	0.5	0.01	84.90%
LAMC1 (rs10911193) [d]								
Chen et al African Americans	TT/TG	GG	1.83	(0.59, 5.65)	10.85%			
Chen et al Whites	TT/TG	GG	0.88	(0.48, 1.62)	37.68%			
Wu et al	TT/TG	GG	1.29	(0.77, 1.68)	51.47%			
Meta-analysis	TT/TG	GG	1.16	(0.80, 1.68)	100%	0.43	0.46	0.00%
LAMC1 (rs20563) [d]								
Chen et al African Americans	AA/AG	GG	1.43	(0.56, 3.65)	11.78%			
Chen et al Whites	AA/AG	GG	0.8	(0.45, 1.46)	29.19%			
Wu et al	AA/AG	GG	1.44	(0.95, 2.19)	59.03%			
Meta-analysis	AA/AG	GG	1.22	(0.88, 1.68)	100%	0.23	0.28	22.30%

[a] P value that tests null hypothesis that overall OR = 1; [b] tests if OR for the individual studies are heterogeneous; [c] I^2 explains the percentage of variation in the OR attributable to heterogeneity; [d] OR for SNPs are based on the dominant model. Table reprinted with permission from Ward. Genetic epidemiology of pelvic organ prolapse. Am J Obstet Gynecology 2014 (Table 3-2) [26]

Collagens

Collagens are fibrous proteins that contribute to the properties and strength of connective tissues depending on the type of collagen present and the degree of crosslinking present in these fibers [104]. There are several types of collagen in the body with type I and type III collagens providing the most amount of support in connective tissues [73;104]. The type I collagen molecules are the most abundant type of collagen in the body and are present in many tissues including cartilage, bone, and tendon [73;104]. Collagen type I, alpha I (*COL1A-1*) is the largest component of the type I collagen family [73;104]. Hypothesizing that a polymorphism might affect the expression of this gene, 5 independent studies evaluated polymorphisms in the intronic region of *COL1A-1* gene where the Sp-1 transcription factor binds in relation to POP. The study by Cho and colleagues conducted this study in a population of Korean women, with only 15 POP cases and 15 controls [93]. Skorupski and colleagues evaluated this relationship in a Polish population with only 37 cases and 40 controls [98]. Finally, Rodrigues and colleagues evaluated this relationship in 107 POP cases and 209 controls in a Brazilian population of white and non-white participants [97]. Feiner and colleagues evaluated this relationship in Caucasian or Ashkanazi-Jewish populations with 36 cases and controls [94]. Finally, Ferrari and colleagues evaluated this relationship in 137 cases and 96 controls in Italy [95]. Despite biological plausibility all five studies failed to find a relationship between the Sp1-binding site polymorphism of Guanine → Thymine and POP. However, the lack of a statistically significant association due to small sample sizes cannot be ruled out.

The collagen type III, alpha I (*COL3A-1*) gene codes for the pro-alpha1(III) chain; after enzymatic processing, three chains arrange to form thin fibrils, and each of these fibrils then cross-link together to form mature type III collagen fibrils. It is of interest that mutations in

several collagen genes including *COL3A-1* cause a vascular condition called Ehlers-Danlos syndrome. A study found women with Elhers-Danlos syndrome and Marfan's syndrome have elevated risk for POP [105]. Investigators have also evaluated the relationship between polymorphisms in the *COL3A-1* gene and POP. In a Taiwanese population with 84 POP cases and 147 controls, Chen and colleagues in 2008 found a positive association between SNP rs1800255 and POP [48]. This signal at this SNP was replicated by Kluivers and colleagues in a Dutch population using 202 cases and 102 controls [49]. A meta-analysis of the two studies by Ward and colleagues reported an odds ratio of 4.79 (1.91, 11.98) for the AA genotype compared with the GG genotype [26]. However, the association was not found when comparing the AG genotype with the GG reference genotype, suggesting a recessive model for this SNP [26]. Investigators have evaluated other SNPs at the *COL3A-1* locus (Exon 31, 2092 G → A). Jeon and colleagues [56] found a positive relationship between this SNP and POP in a small sample of Korean women (36 cases, 36 controls), however the signal was not replicated in a study conducted in a Brazilian population (107 cases, 209 controls) [42].

Matrix metalloproteinases

Members of the MMP family of genes have also been targets for investigation in relation to POP. The MMPs are a broad family of proteinases secreted by connective tissue cells that are important for degradation of various proteins [106;107]. The *MMP-1* gene is also called interstitial collagenase and it acts on substrates including collagen types I through IV. MMP types 3 and 9 are also called stromelysins and they act on proteoglycans, laminins and fibronectins present in the pelvic tissue. Expression studies have found increased expression of the MMP proteins in the uterosacral ligaments and vaginal tissues of women with POP compared with women without POP [108;109]. However, only examining the results from expression

studies it is impossible to discern whether changes in expression of these genes in POP cases compared to controls are the cause of POP or if they are the consequences of POP. Studies of polymorphisms have not been as successful in garnering evidence for the MMP genes in relation to POP. Chen and colleagues in a case-control study (92 cases, 152 controls) among Taiwanese women, published in 2010, reported an odds ratio of 5.57 (1.28, 25.12) for the SNP rs17576 (comparing GG/AG genotypes vs. the AA genotype) in the MMP-9 gene in relation to POP [50]. However, this signal was not replicated in a study by Wu and colleagues, who evaluated this relationship in a larger case-control study in white, non-Hispanic women (239 cases and 197 controls) [101]. But they showed a marginally significant association between SNP rs3918253 in the *MMP-9* gene in relation to POP (OR 0.64; 95% CI: 0.41, 1.00), modeled as a C nucleotide dominant framework [101]. Other SNPs evaluated in the *MMP-9* region by Chen and colleagues [50] and Wu and colleagues [101] have not been promising either. Ferrari and colleagues [95] and Skorupski and colleagues [99] evaluated the relationship between nucleotide insertion (l1607/1608 G → GG) in the *MMP-1* gene and POP. Ferrari and colleagues [95] reported a positive association (OR: 2.24; 95% CI: 1.16, 4.30), however Skorupski and colleagues [99] did not find a similar association (OR: 0.93; 95% CI: 0.49, 1.75). In the same article, Skorupski and colleagues evaluated an adenosine (A) insertion to a poly-A tail (1612/1617 5As → 6As) in the *MMP-3* gene; however, they failed to find an association in relation to POP [99].

Laminins

The laminins are a class of glycoproteins that are thought to be a major non-collagenous component in extracellular matrices [11;73]. Investigators have evaluated the relationship between several polymorphisms of laminin subunit gamma-1 (*LAMC-1*) gene and POP. A linkage study suggested an autosomal dominant mode of transmission of the rs10911193 SNP

located in the promoter region of the *LAMC-1* gene [89;103]. Chen and colleagues [89] and Wu and colleagues [100] attempted to replicate this signal in candidate gene studies in relation to POP. Although both studies were relatively large (165 cases in the Chen and colleagues study and 239 cases in the Wu and colleagues study), they failed to find a relationship between this SNP and POP. Similarly, both studies also evaluated rs20563 and rs20558 in relation to POP and did not find an association. It is of interest that Chen and colleagues evaluated these associations in a population of African Americans and European American participants, and reported differences in allele-frequencies for these SNPs across the two populations, but they were not associated with POP in either of the populations [89]. A closer examination of the allele frequencies across populations and evaluation in relation to POP suggests that the TT/TG SNP showed a statistically non-significant yet elevated OR for the rs10911193 SNP in the African American population (OR: 1.83; 95% CI: 0.59, 5.65); but that the effect estimate for the Caucasian population was in the opposite direction (OR: 0.88; 95% CI: 0.48, 1.62) [89]. It is possible that reduced stratum-specific sample sizes may have hindered the detection of a population specific signal for this SNP in the *LAMC-1* gene.

Lysl oxidase-like genes

LOXL-1 gene belongs to a family of genes that is essential for of the biogenesis of the connective tissue [11]. It works to form crosslinks in collagens and elastin proteins and may be of importance in the formation and maintenance of the pelvic floor connective tissue. Only one candidate gene study has evaluated the relationship between a polymorphism in the promoter of the *LOXL-1* gene (4500878A → C) and POP. Ferrell and colleagues used a case-control design with African American and Caucasian women (137 cases and 141 controls) to evaluate this association, but did not find a statistically significant relationship [96]. The impetus behind

examining the LOX-1 gene comes from expression studies in humans, which have shown decreased expression of *LOXL-1* gene [58] the *BMP-1* gene [61] – a gene that regulates collagen deposition and activates LOX genes, in POP cases compared with controls. Interestingly, expression studies in humans comparing POP cases with controls have also found decreased expression of the fibulin-5 gene in POP cases [59;60]. The *FBLN5* protein plays an essential role in the assembly of elastic fibers. Elastic fibers in turn are extremely important for the strength and elasticity of connective tissues, which may go through morphological changes due to pressure exerted by factors such as child birth or obesity. To our knowledge no study has evaluated the relationship between polymorphisms in or around the *FBLN5* gene in relation to POP. However, mouse knockout models have found that mice deficient in the *FBLN5* gene and the *LOXL-1* gene develop POP after pregnancy [62-64].

Endocrine pathway genes

Other candidate gene studies have focused on the estrogen and progesterone pathways in relation to POP. Chen and colleagues in 2008 reported the associations between POP and various SNPs in the estrogen receptor alpha (*ER-alpha*) and estrogen receptor beta (*ER-beta*) genes [91;92]. Their evaluation of the rs2229480 SNP in the *ER-alpha* gene showed a statistically significant association with the GA genotype (OR: 2.05; 95% CI: 1.05, 4.02) but not with the GG genotype (OR: 0.52; 95% CI: 0.05, 5.03) both in comparison with the AA reference genotype [92]. In 2009, Chen and colleagues reported the relationship between POP and SNP rs484389 in the progesterone receptor (PGR) gene [90]. Similar to the *ER-alpha* results they reported a positive association for the CT heterozygote (OR 4.77; 95% CI: 1.93-11.79), but not for the CC homozygote (OR: 1.06; 95% CI: 0.28, 5.07), both compared to the TT homozygote genotype [90]. These associations have not been investigated by other studies in relation to POP.

Other than these candidate gene studies, there have only been a few studies that have performed a genome-wide scan for genetic signals that may be associated with POP. Allen-Brady and colleagues performed a linkage study of 70 cases in 32 families of Caucasian descent that went through POP surgery (POP-Q stages III and IV) [102]. They identified a logarithm of odds (LOD) score of 3.41 for the chromosome 9: 80.35 megabases (Mb) to 88.81 Mb region (96). This signal has not been replicated. There has only been one GWAS conducted on this topic. Allen-Brady and colleagues studied 115 European American POP cases with 2,976 European American controls and reported statistically significant associations (p-value less than the GWAS threshold for significance: $< 5.0 \times 10^{-8}$) for 6 SNPs: rs145531, rs1036819, rs430794, rs8027714, rs1810636 and rs2236479 [57]. Although the authors had a large number of controls that were sampled from a general population, there was no way to ascertain if individuals were misclassified as controls, as information on POP status was not available for the control group. Despite this potential misclassification, which would tend to bias the results towards the null the authors found these signals. However, these signals have not been replicated.

Race as a predisposing factor for POP

Epidemiologic studies also find differences in POP prevalence rates across racial/ethnic categories, although evidence is not consistent. Whitcomb and colleagues reported five-fold higher risk of self-reported symptomatic prolapse in Latina and white women compared with African American women [20]. However, in the same study, they did not find any difference in objective prolapse as measured by the POP-Q system [20]. Swift and colleagues reported five-fold increased odds of prolapse, as measured by the POP-Q system in Hispanic women, in comparison with white women; but no such differences between white and black women after adjustment for other risk factors [3]. Rortveirt and colleagues reported 60% reduced odds of

symptomatic self-reported prolapse in African American women, and a 30% increased odds of prolapse in Latina women, both compared with white women [19]. These inconsistencies in results concerning self-reported vs. objective prolapse suggest a potential for bias introduced by cultural differences in self-reporting or by differences in care seeking behavior that varies with race/ethnicity.

However, cross-sectional [22] and longitudinal analyses [23] from the WHI-HT, in which participants from all races were objectively measured for POP, are in agreement with results by Rortveirt and colleagues. African Americans had reduced hazard ratios for any prolapse (hazard ratio [HR]: 0.70; 95% CI: 0.60, 0.81) and for severe prolapse (POP grades 2/3) (HR: 0.53; 95% CI: 0.40, 0.71), compared with white women. In the same study, analyses suggested similar rates of POP in whites and Hispanic women [23]. These data provide convincing evidence for a real association between race and POP that is not biased by reporting or ascertainment. Interestingly, a small study by Hoyte and colleagues reported increased muscle bulk in the levator ani muscle complex and smaller angle in the pelvic arch due to a closer puborectalis attachment in nulliparous African American women compared with nulliparous white women; thus providing anatomic clues to support observed differences in prevalence of POP by race/ethnicity [110]. However, we are not aware of any studies to date that evaluate the role of genetics in explaining the POP prevalence disparity between European American and African American women.

Inciting risk factors for POP

Bump and Norton used the term “inciting factors” to describe risk factors that could theoretically be modified, but are often not avoidable [10]. In relation to POP, factors such as parity and previous surgery for POP would come under this category. Parity by far is the most consistent factor, and strongest risk factor identified for POP. Over 20 studies have reported on

the association between parity and POP either as a primary or secondary exposure of interest [3;5;12-16;18-24;38-46;52]. Select noteworthy studies evaluating the relationship between parity and POP are described below, and a more comprehensive list of studies have been summarized in Table 3-4 (effect estimates for parity in column 7).

Table 3-4. Summary of key studies evaluating parity and BMI in relation to POP

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Hendrix et al. [22]	Pelvic organ POP in the Women's Health Initiative: Gravity and gravidity	Cross sectional analysis of WHI-HT clinical trial baseline data	Post-menopausal women from 50 to 79	POP identified through WHI POP grading system (pelvic exam), WHI-grading system; types of POP included uterine POP, rectocele and cystocele	27,342 / 10,868	Adjusted for age, ethnicity, waist circumference, BMI, smoking status, alcohol use, HRT use, and parity	OR (95% CI) from logistic regression models: ref group nulliparous. Uterine POP: adjusted-1birth: 2.13 (1.67-2.72); Rectocele: adjusted- 1birth: 2.22 (1.84-2.68); Cystocele: adjusted-1 birth: 1.91 (1.67-2.19)	OR (95% CI from logistic regression models: ref group Normal weight vs. obese. Adjusted - Uterine POP: 1.40 (1.24-1.59); Rectocele: 1.75 (1.54-1.99); Cystocele: 1.57 (1.41-1.74)
Progetto Menopausa Italia Study Group [44]	Risk factors for genital POP in non-hysterectomized women around menopause: Results from a large cross-sectional study in menopausal clinics in Italy	Multi-center cross-sectional evaluation of menopausal women (intact uterus) attending clinics in Italy	Mean age 53 years of age, SD not provided	POP measured through Baden-Walker half way system systematically across all 25 centers; only considered uterine POP	21,449 /1,182	Measured variables: age, education, BMI, smoking status, parity, method of delivery, infant's birth weight, age at menarche and age at menopause	OR (95% CI) from logistic regression model: ref group nulliparous. Any POP adjusted - 2 births: 2.7 (1.9-3.8); ≥3 births: 3.0 (2.1-4.3). Grade 2 or higher POP adjusted - 2 births: 3.4 (1.7-6.7); ≥3 births: 4.6 (2.3-9.1)	OR (95% CI) from logistic regression models: ref group BMI < 23.8 vs. >27.2. Adjusted - Any POP: 1.6 (1.3-1.9); Grade 2 or higher POP: 1.8 (1.3-2.4)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Mant et al. [16]	Epidemiology of genital POP: observations from the Oxford Family Planning Association Study	Cohort study; National Health Service; women attending family planning clinics in England and Scotland between 1968 to 1974; POP assessed until 1994	Age range at study entry: 25 to 39 years	Hospital diagnosis of genital POP; no specification of types of POP or grade of POP	17,032 /597	Measured variables: age, parity, calendar period, social class, smoking status oral contraceptive use, obesity and contraceptive method. Adjusted for age and calendar period	RR (95% CI) from Poisson regression model: ref group nulliparous. Adjusted- ≥4 births: 10.85 (4.65-33.81)	RR (95% CI) from Poisson regression model: ref group BMI <20 vs. BMI ≥ 28. Adjusted: 1.31 (0.90-1.81)
Forsman et al. [21]	Diabetes and obesity-related risks for pelvic reconstructive surgery in a cohort of Swedish twins	Twin cohort study using Swedish Twin Register born from 1926 to 1958	Mean age (SD): 64.1 (9.2)	POP identified through surgical procedure records	16,886 /1,099	Adjusted for age, BMI, and child birth	OR (95% CI) from logistic regression models: ref group nulliparous. Adjusted - at least 1 birth: 6.1 (3.3-11.4)	OR (95% CI) from logistic regression models: ref group normal weight vs. obese. Adjusted - 1.4 (0.7-2.8)
Kudish et al. [23]	Risk factors for prolapse development in white, black, and Hispanic women	Prospective Cohort; Cox-regression analysis of WHI-HT data; only women who did not have POP at baseline	Post-menopausal women from 50 to 79	POP identified through WHI POP grading system (pelvic exam), similar system as the Baden-Walker half way system; types of POP included uterine POP, rectocele and cystocele	White: 5,442/ 881; Black: 436/41 ; Hispanic: 323/65	Adjusted for ethnicity, age, parity, smoking status, constipation, asthma emphysema HRT use, Incontinence, waist circumference, BMI, and physical activity	HR (95% CI) from Cox models: ref group nulliparous. Any POP. Adjusted - 2 births: 1.43 (1.26-1.61); ≥5 births: 1.70 (1.49-1.93). WHI POP Grade 2 or higher. Adjusted - 2 births: 3.49 (2.51-4.87); ≥5 births 5.87 (4.24-8.14)	HR (95% CI) from Cox models: ref group Normal weight vs. obese. Adjusted - Any POP: 1.16 (1.02-1.30); WHI POP Grade 2 or higher: 1.27 (1.05-1.54)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Gyhagen et al. [15]	Prevalence and risk factors for pelvic organ prolapse 20 years after childbirth: a national cohort study in singleton primiparae after vaginal or caesarean delivery	Longitudinal national survey in Sweden among primipare women; POP measured 20 years after delivery	Age at delivery <23 to ≥ 35	symptomatic POP was assessed through a standardized questionnaire	5,199/ 663	Measured variables: mode of delivery, Age at delivery, infant birth weight, infant head circumference, gestational length, BMI at questionnaire, hysterectomy, and estrogen therapy	Odds ratio (95% CI): ref group caesarean section: adjusted- vaginal delivery: 2.36 (1.76-3.17)	Odds ratio (95% CI): ref group normal weight vs. obese. Adjusted - In women with C-section only: 1.60 (0.86-2.96); In women with Vaginal delivery: 1.74 (1.38-2.18)
Fritel X et al. [40]	Symptomatic pelvic organ POP at midlife, quality of life and risk factors	Cross sectional examination of the GAZEL cohort	50 to 61 years	symptomatic POP was measured through questionnaire Symptoms used: feeling of bulge	2,285/ 158	Measured variables: age, BMI, occupation, education, menopausal status, parity/mode of delivery; multivariable model includes only BMI, and parity/mode of delivery	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted - 2 vaginal births: 2.49 (1.23-5.04); ≥3 vaginal births: 3.55 (1.65-7.62)	Odds ratio (95% CI): ref normal weight vs. overweight or obese. Adjusted: 1.41 (1.01-1.97)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Whitcomb et al. [20]	Racial differences in pelvic organ prolapse	Population based cohort; Kaiser Permanente Medical Care Program of Northern California; cross sectional analysis	Mean age (SD): 55 (9)	Measured Symptomatic POP through a five-point scale (based on questionnaire). Also measured using POP-Q on a subset of women; categorized POP in two ways: 1) ≥ Stage II, 2) leading edge at or below hymen	Sympt omatic POP: 2,270/ 74; POP-Q: 1,136/ 762	Adjusted for age, race, education. BMI and parity	OR (95% CI) from logistic regression models: ref group nulliparous. Symptomatic POP, adjusted- ≥1 vaginal births: 2.10 (0.88-5.02); POP-Q ≥ Stage II, adjusted- ≥1 vaginal births: 1.14 (1.08-1.20); Leading edge at/below hymen, adjusted - ≥1 vaginal births: 2.44 (1.56-3.81)	OR (95% CI) from logistic regression models: ref group normal weight vs. obese. Symptomatic POP, adjusted: 1.43 (0.76-2.68); Stage II or higher: 1.09 (1.01-1.14); Leading edge at/below hymen: 1.67 (1.22-2.29);
Rortveit et al. [19]	Symptomatic pelvic organ prolapse	Population based cohort; Kaiser Permanente Medical Care Program of Northern California; cross sectional analysis	Mean age (SD): 55 (8.6)	Symptomatic POP was assessed through a standardized questionnaire; POP present in the previous year	2,001/ 118	Adjusted for age, race, education, health status, constipation, irritable bowel syndrome, current smoking status, hysterectomy, parity, and delivery type	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted - 2 vaginal births: 4.1 (1.8-9.5); ≥3 vaginal births: 5.3 (2.3-12.3)	OR (95% CI) from logistic regression model: ref group normal weight vs. obse. Unadjusted: 0.9 (0.6-2.2)
Nygaard et al. [5]	Prevalence of symptomatic pelvic floor disorders in US Women	Multicenter cross-sectional analysis of NHANES data	20 to >80	Interview - self report - symptom based	1,961/ 58	Measured variables: age, race/ethnicity, parity, education, family poverty income ratio, and BMI	Prevalence rate (95% CI) by parity status; Parity 0: 0.6 (0.0-1.5); 1: 2.5 (0.1-4.9); 2: 3.7 (1.7-5.6); ≥3: 3.8 (2.1-5.4)	Prevalence rate (95% CI) by BMI status; Normal weight: 1.7 (0.6-2.9); Overweight: 3.4 (1.2-5.5); Obese: 3.6 (2.0-5.2)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Dolan et al. [12]	Obstetric risk factors and pelvic floor dysfunction 20 years after first delivery	Cross sectional; consecutive women who gave first birth at Princess Mary Maternity Hospital, UK	Mean age (SD) at delivery: 26.2 (4.8); Mean age (SD) during questionnaire: 45.7 (4.8)	Sheffield Pelvic Floor Assessment Questionnaire	1,831/ 248	Measured variables: Age, BMI, Social Class, Parity, Gestation, birth weight, and mode of delivery among others	Odds ratio (95% CI) ref group: parity = 1. unadjusted (≥3 births): 1.28 (0.97-1.69); adjusted (≥3 births): 1.24 (0.92-1.67)	Odds ratio (95% CI) ref: normal weight vs. obese. Adjusted- In primiparie women: 3.08 (1.32-7.16); In all women: 1.30 (0.89-1.88)
Fornell et al. [24]	Factors associated with pelvic floor dysfunction with emphasis on urinary and fecal incontinence and genital POP: an epidemiological study	Population based cross-sectional survey; Sweden	40 year old and 60 year old women from the community	POP identified through standardized questionnaire that asked about symptoms of POP including pelvic heaviness, sensation of bulging and digitation of the perineum or vagina by defecation	1,330/ pelvic heaviness: 226/bulge: 53/digitation by defecation: 160	Only presented results from univariate analysis	OR (95% CI) from logistic regression model: ref group nulliparous. OR for Vaginal delivery, three outcomes. Unadjusted models only. Pelvic heaviness: 1.8 (1.0-3.1); Genital bulge: 7.4 (1.0-53.9); digitation by defecation: 1.2 (0.0-7.4)	OR (95% CI) from logistic regression models: ref group normal weight vs. obese. Three outcomes. Unadjusted models only. Pelvic heaviness 1.4 (0.9-2.2); genital bulge: 1.2 (0.5-3.2); digitation by defecation: 1.3 (0.7-2.1)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Scherf et al. [45]	Epidemiology of pelvic organ POP in rural Gambia, West Africa	Community-based reproductive health survey - cross-sectional analysis of 1,348 women in 20 villages in Gambia	Mean age: 32.6 years (SD) not provided	Gynecologic health questionnaire follwed by vulval inspection by speculum in select women	1,067 examined for genital POP/ 448 with any POP	Measured variables: age, marital status, ethnicity, parity, current pregnancy, history of problems with pregnancy, and deficient perineum, Adjusted for "significant demographic/fertility/gynecological variables	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted- 1-3 births: 6.39 (2.24-18.22); 4-7 births: 11.69 (4.0-34.13); ≥8 births: 14.95 (4.94-45.24)	OR (95% CI) from logistic regression model: ref group normal weight vs. overweight and obese. Unadjusted OR only: 1.33 (0.86-2.04)
Swift et al. [3]	Pelvic Organ Support Study (POSST): The distribution, clinical definition, and epidemiologic condition of pelvic organ support defects	Multicenter cross-sectional study; gynecology annual visits	Range: 18 to 83	POP-Q measurement; POP defined as leading edge of POP ≥ -0.5	1,004/ 218	Measured variables: age, BMI, race, parity, gravidity, vaginal deliveries, weight of delivered infant, HRT ever, Income, Smoking history, and chronic illness	Odds ratio (95% CI) per birth (continuous measure): unadjusted- 1.39 (1.27-1.52); adjusted - 1.11 (0.71-1.73)	OR (95% CI) from logistic regression model: ref group normal vs. obese. Adjusted: 2.56 (1.23-5.35)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Glazener et al. [14]	Childbirth and POP: long-term associations with symptoms and objective measurement of pelvic organ POP	Retrospective longitudinal study - medical databases from 3 maternity units who gave birth during 1993-1994 in UK and NZ; POP follow-up after 12 years	Mean age (SD) at birth: 26.5 (4.9)	POP measured using POP-Q system: POP at or below the hymen was considered cases	762/182	Measured variables: age at first birth, parity, delivery mode history, and BMI	Odds ratio (95% CI) ref group: parity = 1. adjusted- 2-3 births: 3.30 (1.49-7.32); ≥4 births: 5.23 (2.04-13.39)	OR (95% CI from logistic regression models: ref group Normal weight vs. obese. Adjusted: 1.48 (0.91-2.40)
Miedel et al. [43;46]	Nonobstetric Risk Factors for Symptomatic Pelvic Organ POP	population-based cross-sectional study from Sweden	30 to 79; Controls mean age (SD): 49.1 (13.5); Cases mean age (SD): 53.3 (12.3)	First measured through a validated 5-item questionnaire. Then greater than 80% of the respondents identified as symptomatic POP caseswere evaluated for POP through the POP-Q exam. Also valided 206 random controls with POP-Q exam	558/273 Analysis used 443 subjects; N-cases: NR	Adjusted for age, parity, and family history of POP	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted - 2 births: 4.71 (2.23-9.95); 3 births: 4.40 (1.85-10.51); 4-6 births: 6.31 (1.75-22.73)	OR (95% CI) from logistic regression model: ref group normal weight vs. obese. Adjusted: 2.07 (0.95-4.50)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Tegerstedt et al. [46]	Obstetric risk factors for symptomatic POP: A population-based approach	Population-based case-control study conducted through a survey among participants in the Swedish Population Register	30 to 79	POP measured through valided 5-item questionnaire that has 94.2% specificity and 66.5% sensitivity with POPQ exam.	554/ 332	Adjusted for age, parity and obstetric variables including caesarean delivery, instrument delivery, vaginal rupture, anal sphincter tear, and episiotomy	OR (95% CI) from logistic regression model: ref group 1 birth. Adjusted - 2 births: 1.6 (1.1-2.5); 3 births: 1.7 (1.0-2.9); ≥4 births: 3.1 (1.3-7.1)	OR (95% CI) from logistic regression model: ref group BMI <20 vs. BMI ≥20. Adjusted: 1.3 (0.5-3.7)
Erata et al. [13]	Risk factors for pelvic surgery	Hospital based retrospective case-control study	30 to 88; mean age (SD): 51.0 (8.6)	Cases - Surgical procedure codes; Controls: no operations related to pelvic floor disorders; and routine visits	379/ 184	Measured variables: Age, BMI, age at delivery, parity, smoking, and route of delivery. Adjusted variables: NR	Odds ratio (95% CI) ref group: parity = 0. 2 births: 3.61 (1.14-12.64); ≥4 births: 9.00 (2.64-33.80); ≥4 births by vaginal delivery: 11.75 (3.84-38.48)	OR (95% CI) from logistic regression model) BMI modeled as a continuous variable. Adjusted: 0.94 (0.82-0.95)
Ghetti et al. [41]	Risk factors for surgically managed pelvic organ prolpase and urinary incontinence	Case-control study from Kaiser Permanente Northwest population	NR	Cases were women who went through primary surgical treatment for POP and Urinary incontinence; Controls; age matched women with no history of POP as indicated by medical records	532/ 245	Measured variables: age, self-reported race, height weight, parity, route of delivery, estrogen status, smoking history, medical and surgical history. Authors only specified that they used multivariate logistic regression. Adjusted variables: NR	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted - vaginal deliveries: 4.1 (1.7-9.4)	OR (95% CI) from logistic regression model: BMI modeled as a continuous variable. Adjusted: 1.00 (0.96-1.01)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
de Araujo et al. [39]	Pelvic floor disorders among indigenous women living in Xingu Indian park, Brazil	Cross-sectional evaluation of indigenous women living in Xingu Indian Park	12 to 77; mean age (SD): 31 (15)	Measured POP using POP-Q system. Denoted as a POP case in two different ways: 1) if had POP stage II or higher. 2) if Ba point ≥ 0	377/ 241	Measured variables: age, vaginal parity, BMI, resting pressure, maximum pressure; Adjusted variables: NR	OR (95% CI) from logistic regression model: ref group nulliparous. ≥ Stage II POP adjusted- vaginal delivery: 11.26 (5.69-22.29); Ba ≥ 0 Adjusted - vaginal delivery: 12.10 (2.81-31.42)	OR (95% CI) from logistic regression model. Ref group BMI ≤25 vs. >25. ≥ Stage II POP adjusted- ≥Stage II POP: 1.05 (0.60-1.82); Ba ≥ 0 adjusted: 1.33 (0.79-2.24)
Quiroz et al. [18]	Vaginal parity and pelvic organ prolapse	Multicenter cross-sectional study; Johns Hopkins affiliated clinics	>40	POP measured using POP-Q system	290/72	Measured variables: age, parity, BMI, race/ethnicity, weight of largest child delivered vaginally and history of hysterectomy. Adjusted only for age	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted- First Vaginal Birth: 9.73 (2.68-35.35)	Only present BMI (SD) by Stage of POP: Stage 0: 28.6 (7.3); Stage I: 28.2 (6.6); Stage II: 29.6 (6.5); Stage III: 28.4 (5.1)
Moalli et al. [52]	Risk Factors Associated With Pelvic Floor Disorders in Women Undergoing Surgical Repair	Hospital based case control study; USA	Cases mean age (SD): 50.3 (11.6); Controls mean age (SD): 49.0 (7.0)	Cases were women who had pelvic surgical repairs: 25% for urinary incontinence only, remaining for a combination of POP related and urinary incontinence. Controls: general gynecology visits	256/80	Adjusted for obstetric and gynecology variables including mode of delivery, age at first delivery, BMI, history of gynecology surgery, menopausal status	OR (95% CI) from logistic regression model: ref group Cesarean. Adjusted - Spontaneous Vaginal delivery: 2.9 (0.9-10.0)	OR (95 % CI) from logistic regression model: ref group BMI ≤ 26 vs. BMI > 26. Adjusted: 3.0 (1.6-5.7)

First Author	Title	Study design	Age (years)	POP assessment	N/N-POP cases	Variables included in models	Parity effect estimates	BMI effect estimates
Chiaffarino et al. [38]	Reproductive factors, family history, occupation and risk of urogenital POP	Hospital based case control study	Cases: mean age: 58.5; Controls mean age: 59.8	POP measured using Baden-Walker half way classification system. Controls were patients admitted to the same hospital and did not have POP	208/108	Measured age, education, menopausal status, age at menopause, smoking status, occupation, HRT, BMI, family history of POP. Did not specify what they adjusted for.	OR (95% CI) from logistic regression model: ref group nulliparous. Adjusted ≥2 births: 2.8 (0.9-8.5); ≥2 vaginal births: 4.5 (1.6-13.1)	OR (95% CI) from logistic regression model: ref group BMI ≤ 23 vs. BMI > 26. Adjusted: 0.9 (0.5-1.7)

NR = Not recorded because authors did not clearly provide information

The Oxford Family Planning Association Study examined 17,032 women aged 25 to 39 years who attended 17 large family planning clinics in England and Scotland between 1968 and 1974 [16]. They were followed up until July 1994. In this study of relatively young women, compared with nulliparous women, women who had four or more children had an age- and calendar-period-adjusted relative risk of 10.85 (4.65, 33.81) for diagnosis of POP. Test for trend suggested that each additional birth was significantly associated with POP, but that the trend in risk increase attenuated after the second birth. While identification of POP was limited to inpatient diagnoses and the criteria for diagnosis and factors such as severity of prolapse were not noted, this is one of the largest studies that reported a quantitative association between parity and POP. Additionally, they reported that among women who had a hysterectomy, the risk of having subsequent diagnosis of genital prolapse was 5.5 times higher in women whose initial reason for hysterectomy was due to genital prolapse, compared with women who had other reasons for hysterectomy.

In 2000, the Progetto Menopausa Italia Study Group published results from a large multi-centered cross-sectional study evaluating the risk factors for genital prolapse in women without hysterectomy at around menopause [44]. In this large study of 21,449 women, the authors reported a gradual increase in odds for any POP in women who had 1, 2 and 3 or more children, with corresponding odds ratios of 2.6 (95% CI: 1.8, 3.8), 2.7 (1.9, 3.8) and 3.0 (2.1. 4.3), respectively, all compared with nulliparous women. Despite the fact that there were 258 recruitment centers, the authors uniformly used the Baden-Walker system for grading severity of POP. They also reported odds ratios for individuals who had grade 1 POP, and those who had POP of grade 2 or higher. Compared with nulliparous women, women with 3 or more child births had an odds ratio of 2.5 (1.7, 3.7) for grade 1 POP, and an odds ratio of 4.6 (2.3, 9.1) for

POP with grade 2 or higher. In an analysis limited to parous women, the authors also reported decreased odds of POP associated with caesarean section (OR: 0.6; 95% CI: 0.5, 0.8).

In 2002, Hendrix and colleagues reported a cross-sectional analysis of baseline data from the WHI-HT trial [22]. All participants in the study underwent a pelvic exam conducted by trained professionals, who recorded the occurrence of POP as uterine prolapse, cystocele or rectocele. The severity of prolapse was measured using a system similar to the Baden-Walker system. In this baseline analysis, the authors evaluated the relationship between parity and uterine prolapse, cystocele and rectocele (grade 1 prolapse or higher) in addition to other risk factors. Compared with women who had no term births, women with one child birth an had odds ratio of 2.13 (95% CI: 1.67, 2.72) for uterine prolapse, 2.22 (95% CI: 1.84, 2.68) for rectocele, and 1.91 (95% CI: 1.67, 2.19) for cystocele. Each additional birth was associated with 1.1 to 1.21 fold increase in POP. The WHI-HT did not collect information on modes of delivery for parous women. However, if caesarean section is protective for prolapse and given that some women likely had a C-section, the composite odds ratio for parity reported by this article is likely attenuated. Additionally, the WHI-HT did not collect information about reason for hysterectomy for those women who had gone through the procedure. Interestingly, hysterectomy was associated with decreased odds of prolapse for rectocele and cystocele in this population, which could either mean women who went through hysterectomies also had corrective surgery for POP or that majority of the surgeries for hysterectomy were not at all related to POP. Despite these drawbacks, this is the largest study (n = 27,342) that has evaluated the relationship between risk factors for prolapse, including parity. Additionally, the study examined the risk factors with a higher degree of granularity in reporting similar odds ratios for parity in relation to different

types of prolapse in a diverse study population of European American, African American and Hispanic women.

In a more refined analysis, Kudish and colleagues re-evaluated the WHI-HT data using follow-up data for women without hysterectomy who did not have POP at baseline [23]. Compared with nulliparous women, the hazard ratio for women who had five or more births was 1.70 (95% CI: 1.49, 1.93) for any prolapse, and 5.87 (95% CI: 4.24, 8.14) for moderate/severe prolapse (grade 2 or higher). The authors additionally examined the relationship between parity and moderate/severe POP by strata of self-reported race/ethnicity. In a sample size of 11,185 white women, the authors reported increasing hazard ratio for each additional birth; the largest hazard ratio was reported for women with five or more births (HR: 5.29; 95% CI: 3.79, 7.38). In 800 African American women, the authors again reported increasing hazard ratio with each additional birth; the largest hazard ratio reported for African American women was for five or more births (HR: 10.41; 95% CI: 1.38, 78.77). Hazard ratios for parity for Hispanic women were not reported, most likely due to small sample sizes, however, they reported that the trend test was statistically significant ($p = 0.004$).

In a population based cohort investigation study called the Reproductive Risks for Incontinence Study at Kaiser (RRISK), Rortveit and colleagues assessed the risk factors for symptomatic POP in 2,001 women through a structured questionnaire [19]. Compared with nulliparous women, the odds ratio for POP was 2.8 (95% CI: 1.1, 7.2) in women who had one vaginal birth and 5.3 (95% CI: 2.3, 12.3) in women who had 3 or more vaginal births. In 2009, Whitcomb and colleagues reported the relationship between parity and POP in a second Kaiser cohort named RRISK2 [20]. This time they not only reported on symptomatic self-reported prolapse but also performed objective measurements of POP on approximately 50% of the

participants. They once again reported increased odds for POP associated with vaginal parity. A more comprehensive list of other studies that reported on the relationship between parity and POP, together with their characteristics, are presented in Table 3-4.

Studies have also evaluated the relationship between obstetric factors and POP. Caesarian section has been suggested to be protective for POP [14;15;46]. Gyhagen and colleagues conducted a national Swedish cohort study to evaluate this [15]. They collected records from 5,236 singleton primiparae women who had given birth approximately 20 years previously, and evaluated symptomatic POP using a validated standardized questionnaire. The study was likely immune to confounding due to the homogeneity of their population, at least in terms of parity. They reported women with vaginal parity had a 2.55 (95% CI: 1.98, 3.28) fold increased odds of prolapse compared with women who went through a caesarian section. They also reported that compared to women who's infant's birth weight was less than 3,000 grams, women whose infant's birth weight was 4,500 or more grams had a 2.09 (95% CI: 1.26, 3.47) increased odds of prolapse. This association was only true in women with vaginal parity but not among women who elected to have caesarean section. Studies comparing mode of delivery illuminate the mechanisms by which parity might be related to POP. It is possible that excessive strain on the vaginal walls during labor and delivery might be the causally contributing factors for POP rather than pregnancy itself.

Promoting factors for POP

Factors such as obesity, smoking, chronic coughing, constipation (chronic straining) and heavy physical exercises that exert unduly pressure on the pelvic floor have been categorized as factors that promote POP [1;2]. The mechanisms behind these risk factors have mostly been hypothesized to be related to exerting pressure on the pelvic floor. For example, excess

deposition of abdominal fat in women may lead to a higher level of pressure exerted constantly on the pelvic floor, which could lead to gradual weakening of the pelvic floor muscles. Other than this hypothesized mechanism, it is not clear if there are other molecular mechanisms related to obesity that may affect POP. If there are, then these remain largely unknown and not discussed in the literature.

During coughing, air is forced out of the lungs. The relaxation of the diaphragm during coughing is accentuated by the contraction of the abdominal and other expiratory muscles which exerts pressure on the pelvic floor muscles. Chronic coughing may thus lead to weakening of the muscles and connective tissue over time.

Finally, excessive straining due to constipation also causes an increase in pressure in the pelvic region. The strain related to constipation may be emulated by performing the Valsalva maneuver, in which a patient is told to breathe out with their mouth shut and their nostrils shut tight with their hands. In spite of these proposed mechanisms, epidemiological studies have not always reported consistent effect measures relating to these risk factors.

Obesity as a risk factor for POP

Evidence for obesity as a risk factor for POP has been evaluated by many studies [3;5;12-16;18-24;38-46]. Hendrix and colleagues used baseline data from the WHI-HT study to conduct the largest investigation of the association between categories of BMI [BMI <25 kg/m^2 (normal-weight), BMI 25-30 kg/m^2 (over-weight), and BMI ≥ 30 kg/m^2 (obese)] and POP thus far, in 27,342 post-menopausal women [22]. Compared with women with normal-weight, obese-women had odds ratios of 1.40 (95% CI: 1.24, 1.59) for uterine prolapse, 1.75 (95% CI: 1.54, 1.99) for rectocele/anterior wall prolapse, and 1.57 (95% CI: 1.41, 1.74) for cystocele/anterior wall prolapse. They also reported positive odds ratio for both rectocele (OR: 1.17; 95% CI: 1.06,

1.29) and cystocele (OR: 1.17; 95% CI: 1.08, 1.27) among those who had a waist circumference of >88 cm. However, this was not the case for uterine prolapse.

Using the same WHI-HT data, Kudish and colleagues examined the relationship between BMI and POP (treating uterine prolapse, rectocele or cystocele as any prolapse) in women without hysterectomy by strata of race/ethnicity [23]. In their baseline examination, compared with normal weight women, European American obese women (OR: 1.87; 95% CI: 1.44, 2.43) and Hispanic obese women (OR: 2.22; 95% CI: 0.67, 7.33) were more likely to have POP, but African American obese women were less likely to have POP (OR: 0.32; 95% CI: 0.10, 1.02). The reason for this discrepancy is not clear. However, in the same manuscript, the authors also performed Cox-proportional hazards analysis among women without hysterectomy who did not have POP at baseline. In these analyses, compared with normal weight women, the multivariable adjusted hazard ratios for grade 2/3 POP for obese white women, African American women and Hispanics were 1.23 (95% CI: 1.00, 1.50), 2.12 (95% CI: 0.73, 6.19) and 1.82 (95% CI: 0.70, 4.73), respectively. In this analysis of incident POP compared with baseline BMI, although the effect estimates were not statistically significant for all races, they are at least in the same direction.

A large study of 21,449 Italian women without hysterectomy, Parrazini and colleagues conducted a cross-sectional evaluation to report odds ratios for any prolapse, Baden-Walker grade 1 prolapse and Baden Walker grade 2 or higher prolapse [44]. Compared with women with BMI < 23.8 kg/m^2, odds ratios for women who had BMI >27.2 kg/m^2 were 1.6 (95% CI: 1.3, 1.9) for any prolapse, 1.5 (95% CI: 1.2, 1.8) for grade 1 prolapse, and 1.8 (95% CI: 1.3, 2.4) for grade 2 or higher prolapse. Another large-cohort evaluation of British Caucasian women reported a statistically significant association between increasing BMI and genital prolapse as

measured by the Breslow Day test for trend; however, comparison of extreme categories (≥ 28 kg/m^2 vs. <20 kg/m^2) did not reveal statistically significant results. The resulting adjusted relative risk from Poisson regression model was 1.31 (95% CI: 0.90, 1.81) [16]. A Swedish study of 4,066 women who had one singleton birth in their reproductive lifetime approximately 20 years previously showed elevated odds of prolapse for obese women compared with normal weight women [15]. The effect estimate was elevated for obese women who had had a vaginal delivery (OR: 1.74; 95% CI: 1.38, 2.18), and for obese women who had had a caesarean section (OR: 1.60; 95% CI 0.86, 2.96).

The RISSK cohorts provide further conflicting evidence. In the first RISSK cohort of 2,001 women where they evaluated the relationship between BMI and symptomatic prolapse based on self-report showed mostly null associations [19]. Compared with women whose BMI < 25 kg/m^2, the odds ratios for women with BMI 25 – 30 kg/m^2, 30-35 kg/m^2, and 35-40 kg/m^2 were 0.8, 0.9, and 1.1; all of which included unity. However, in a second publication using data from the RISSK2 cohort, investigators reported statistically non-significant but elevated odds for symptomatic prolapse in obese women (OR: 1.43 (95% CI: 0.76, 2.68)), but a statistically significant result for objectively measured prolapse at or below the hymen in obese women (OR: 1.67 (95% CI 1.22, 2.29) [20].

There are several other studies that either reported elevated effect estimates or null associations when evaluating the relationship between obesity and POP. A more comprehensive (but not exhaustive) list of studies is presented in Table 3- 4 (Column 8). These studies along with other studies from a systematic literature search are evaluated in the systematic review/meta-analysis (Specific Aim 1). The reasons behind these discrepant results for BMI and POP are not clear. Possible factors contributing to heterogeneity could include factors such as

measurement of POP (self-report vs. objectively measured POP), or study design (population based cohort, cross-sectional, or hospital based study) – which could lead to different selection of comparison groups potentially biasing the associations.

Other promoting risk factors for POP

The relationship between other promoting factors and POP has not been studied as well as BMI, and studies do not always show consistent results. Using the RISSK cohort Rortviert and colleagues reported 2.5 fold increased odds of prolapse in women who reported to be constipated once a month or more, compared with women who reported to have constipation less frequently [19].

Compared with women who did not have chronic obstructive pulmonary disorder (COPD), the unadjusted odds ratio for POP was 1.4 (95% CI: 0.7, 2.8) for women who had COPD. In the RISSK2 cohort, Whitcomb and colleagues performed chi-squared tests for comparing the frequency of COPD and constipation among POP cases versus the rest of the cohort and reported a p-value of 0.01 for COPD and a p-value of 0.11 for constipation [20].

Reports from the WHI-HT datasets also have not been consistent, with the baseline analysis showing no association to a 10% increased odds for POP in relation to constipation [22]. The associations between smoking and POP are also unclear. Smoking may increase risk for POP due to coughing associated with smoking. Alternatively, smoking is associated with reduced rates of constipation and would therefore be predicted to reduce risk for POP. The WHI-HT baseline analysis and cohort analysis suggest statistically significant inverse associations between smoking and POP [22;23]. Other studies report odds ratios for smoking and POP which are mostly statistically non-significant and range from inversely associated [16;22;38;41;43], null [3;44] or positively associated [13;19].

CHAPTER IV

RESEARCH GAP

As detailed in the sections above, several studies have evaluated the association between obesity and POP, mostly measured as BMI. However, a first glance at the effect estimates reported across studies suggests a considerable amount of heterogeneity (ranging from no association to a two fold increase in odds for POP). To our knowledge, there has been no effort to systematically and quantitatively assess the weight of evidence in the literature concerning obesity and POP. Additionally, we do not know of any other study that has tried to systematically explore the reasons behind the heterogeneity across studies. A systematic qualitative and quantitative evaluation of the relationship between measures of obesity reported in the literature and POP will greatly benefit the field in deciding whether or not obesity is a modifiable risk factor for POP.

Furthermore, although the literature classifies risk factors for POP into categories of predisposing factors, inciting factors and promoting factors, we are not aware of any study that evaluates how these factors interact with each other to cause POP. Parity, an inciting factor, is a strong risk factor for POP, but not all women that give birth vaginally develop POP, and nulliparous some women have POP. The possibility that predisposing genetic factors may modify the relationship between parity and POP has not been explored. Obesity is the only promoting factor that seems to be practically modifiable in relation to POP. Obesity as a risk factor for POP has been more extensively studied than other promoting factors for POP, however there is considerable heterogeneity in the literature regarding its effect estimate. Amongst

varying study characteristics, one source of heterogeneity could be due to effect modification by genetic factors.

Finally, a few studies suggest POP prevalence and incidence may vary by categories of self-reported race/ethnicity. African Americans have been reported to have much lower risk for POP than European Americans. The causes of this disparity could be manifold, including differential reporting of POP by race due to cultural differences. At the same time, the possibility that the disparity could be reflective of underlying biological causes has not yet been ruled out. Small but important genetic differences due to contributions from varying continental ancestral populations in African Americans compared with European Americans could potentially contribute to this POP disparity. To our knowledge, no study has attempted to evaluate if genetic differences attributed to ancestry at least in part explain the disparity in POP.

The following Specific Aims were proposed to address the gaps in research relating to POP presented in this section.

Specific Aim 1: To conduct a systematic review and meta-analysis of the relationship between obesity measures and POP in analytic observational studies.

Specific Aim 2: To evaluate whether SNPs within/around select- pre-specified gene regions modify the association between 1) BMI and POP and 2) parity and POP, in European American, African American and Hispanic women from the WHI-HT study.

Specific Aim 3: To evaluate whether genetically-inferred global or local ancestry (European or African) is associated with increased risk for POP in African American women from the WHI-HT study.

CHAPTER V

METHODS

Methods for Specific Aim 1: To evaluate the relationship between measures of obesity and POP through a systematic review and meta-analysis of observational studies

Search Strategy and Manuscript Review

To conduct this review, the MEDLINE database was systematically queried using appropriate search terms relating to POP. A number of combinations of search terms were tried in order to ensure that the search strategy was sensitive enough to capture studies that contain information regarding obesity and POP, but also at the same time was specific enough to exclude studies that are not related to the query topic. The final combination of search terminologies is presented in Appendix 1.

The final search terminologies were applied in PUBMED to generate titles and abstracts of studies published from inception of the database until June 18, 2015. Title level listings were scrutinized to eliminate studies that were clearly not related to the topic of interest. Titles of the articles which referred to prolapse of the heart valves (e.g. mitral valve prolapse), umbilical cord prolapse, iris prolapse, or those titles which were clearly non-relevant to POP were removed from consideration for further review. Abstracts of remaining articles were then reviewed to identify articles which evaluated the association between risk-factors for POP. Articles which were relevant to description of surgical procedures for POP, comparative studies evaluating different surgical procedures for pelvic floor surgery, letters, commentaries, and editorial notes were excluded. Studies published in a language other than English were removed. Upon review

at the abstract level, if it was unclear whether a given study evaluated risk factors for POP, then the study was retained for full text review in addition to abstracts which clearly indicated evaluating risk factors for POP. A full-text review of these articles was then conducted to keep only those articles which evaluated the relationship between BMI and POP for a qualitative summary of the literature and for further eligibility evaluation for meta-analysis.

Eligibility criteria for meta-analysis

Population: Studies that reported effect estimates on the relationship between BMI and POP in women of any age were eligible to be included in the meta-analysis. Studies involving women with or without hysterectomy were included. Women with previous hysterectomy are still at risk of developing other forms of prolapse including anterior and posterior vaginal wall prolapse. Studies specifically evaluating prolapse recurrence following surgery for urinary incontinence or POP were not eligible for analysis.

The eligibility criteria for this meta-analysis were permissive because firstly, we want to be able to generalize the findings of this meta-analysis to a broad population of women, and secondly restricting the age limit to studies that only examine elderly women would severely limit the number of studies available for analysis.

Study design: Analytic observational studies of all types including cross-sectional, case-control and cohort designs with at least 40 cases of POP were eligible to be included into the meta-analysis. A minimum of 40 cases was chosen as criteria to only include estimates from studies which provide relatively reliable estimates of the association between categorical BMI and POP. Additionally, to be eligible for meta-analysis studies needed to report a risk ratio (odds ratio (OR), relative risk (RR) or hazard ratio) or must have provided sufficient information to allow calculation of a relevant risk ratio. For the primary analysis, all of these three risk ratios

were aggregated together to present a meta-analysis risk ratio, regardless of study design. Case-control studies which specifically matched on BMI status were not considered eligible for analysis.

Outcomes: The primary outcome for this meta-analysis is POP as a dichotomous variable (yes, no). All forms of prolapse reported as POP, uterine prolapse, genital prolapse, enterocele, cystocele/anterior wall prolapse, or rectocele/posterior wall prolapse are counted as an outcome. For our primary aim, we include self-reported symptomatic prolapse, prolapse indicated by ICD codes, surgical procedure codes, as well as prolapse measured through pelvic exams by trained professionals for all severities of prolapse. We do not make a distinction between staging criterion described by the Baden-Walker Halfway grading system or the POP-Q staging system. To allow ease of data aggregation, reports of Baden-Walker Halfway grading system of grade 2 or more or POP-Q Stage II or more are considered as clinically significant POP.

Assessment of BMI: Studies which presented risk ratios by categories of BMI were considered eligible for meta-analysis. Ideally studies must have reported risk ratios for the following BMI categories: BMI <25 kg/m^2 (reference group), BMI 25-<30 kg/m^2, and BMI ≥30 kg/m^2. These cut offs for BMI were chosen *a priori* because these are standard measures of categorization of BMI as prescribed by the World Health Organization (WHO), and are also widely used in the current medical literature. In the event studies report risk ratios for categories of BMI that were not conventional, a judgment call was made to group effect estimate to the nearest conventional BMI category. For example, studies reporting risk ratios for BMI categorized by tertile cut points, the first tertile was used as the reference category, the second tertile was aggregated with the overweight analysis and the third tertile was aggregated with the obese group. If studies presented risk ratio for BMI <25 kg/m^2 (ref) versus BMI ≥25 kg/m^2, then

these studies were put into the overweight category. Despite these inconsistent, yet, overlapping categories, analysis categories are referred to as normal weight, over-weight and obese for the sake of simplicity. As long as the non-conventional reference category did not include women with BMI greater than 25 kg/m^2, the study was considered eligible for analysis. Studies which combined overweight and/or obese individuals into their lowest category (reference category) were not considered comparable and are therefore only described qualitatively. If the study did not provide a risk ratio adjusted for key covariates, the unadjusted risk ratio was used for analysis. If a given study did not report any risk ratio, but provided counts which allowed calculation of risk ratios, then the study was considered eligible. Studies which only provided mean or median BMI measures by case-control status were not considered eligible for meta-analysis. Similarly, studies which calculated risk ratios using BMI as a continuous measure were not considered eligible for meta-analysis with categorical representation of BMI.

Data duplication: In the event two or more studies used the same or over-lapping study populations, only one of the studies was chosen for the meta-analysis. Preference was given to the study with the larger sample size when both provided adjusted risk ratios or both provided unadjusted risk ratios. A study which provided an adjusted risk ratio was given preference over the study which provided raw numbers or unadjusted risk ratios. A prospective-cohort analysis was given higher preference over a cross-sectional/case-control analysis regardless of sample size. When a given study provided two or more risk ratios for varying definitions of POP for the same population (symptomatic POP, objective POP with any grade of POP, or objective POP with moderate/severe POP) then all risk ratios were recorded but not to be considered in the same meta-analysis set (This is described in more detail in the statistical analysis section).

Data abstraction: Once studies providing effect estimates by categories of BMI were identified, the following fields were abstracted from each article: study title, first author, year of publication, study design (cross-sectional, case-control, cohort), mean age (SD)/range or median (interquartile range) if provided, percent of post-menopausal women represented in the study if provided (or could be estimated if the study provided adequate information for estimation), racial/ethnicity composition of study if provided (or could be estimated based on country of study), method of POP assessment (symptomatic prolapse through self-report, or objectively measured prolapse), categories of BMI utilized by authors, risk ratios provided (OR, RR, or HR) by each category of BMI, raw numbers for risk ratio calculation by categories of BMI and POP status if adjusted risk ratios or unadjusted risk ratios were not provided, information on whether study adjusted for key covariates (yes, no), and the list of covariates which were adjusted for in regression models. When a study provided two or more risk ratios for each category of BMI (overweight or obese), for example for symptomatic POP and objectively measured POP, then both reported risk ratios were abstracted as separate entries and marked as duplicate to avoid aggregating correlated data in a given meta-analysis set.

Statistical Analysis

For primary analyses, all studies with non-overlapping study populations reporting risk ratios were meta-analyzed together using inverse variance weighted random effects models. Meta-analysis summary effect estimates and corresponding 95% confidence intervals for POP are presented for two main analyses: 1) effect estimates and 95% CIs for overweight women, and 2) effect estimates and 95% CIs for obese women; both compared to women with BMI < 25 kg/m^2. Some studies provided more than one risk ratio, for example: symptomatic prolapse and/or varying degrees of objectively measured prolapse. To accommodate these effect estimates

using non-overlapping populations, we performed two sets of analyses (for both the overweight and obese categories): one utilizing the smallest of the two or more effect estimates (referred from here on as minimum scenario) and another utilizing the largest of the two or more effect estimates (referred from here on as maximum scenario).

Assessing heterogeneity

In addition to obtaining overall effect estimates for the relationship between obesity measures and POP, another goal of this study was to evaluate potential sources of heterogeneity in effect estimates across studies. Heterogeneity in effect estimates across studies was formally assessed using the I-squared statistic. The I^2 statistic is described by the following formula:

$$I^2 = \left(\frac{Q-df}{Q}\right) \times 100\%,$$

where Q is the chi-squared statistic and df is the degrees of freedom in the model. The I^2 statistic quantifies the percent of variability in effect estimates across studies that is attributed to sources other than random error. The larger the statistic the more likely it is that the heterogeneity is attributable to factors other than random error. Conversely, the smaller the statistic the less likely it is that there is no or little evidence of systematic sources of error in the effect estimates across studies. Heterogeneity was first assessed in the primary analyses models described above. The I^2 statistic was examined for the primary models to see if it was closer to 0 or 100%. The rules for considering the level of heterogeneity across studies are arbitrary. Nonetheless, the Cochrane handbook provides some guidelines for evaluating heterogeneity [111]. Roughly speaking, a 0 to 30% value for I^2 is considered negligible, >30% to 60% is considered moderate heterogeneity, and >60% is considered to be substantial heterogeneity in effect estimates across studies.

After assessing the I^2 statistic in the model with all studies, variables representing study level characteristics were computed as categorical variables. Study-level characteristics which were of interest in evaluating sources of heterogeneity included: method of POP assessment (self-reported symptomatic POP, objective POP of any grade, objective POP of severe/moderate grade [defined as Baden-Walker grade ≥2, POP-Q Stage >II, POP at or below the hymen, or POP which warranted surgical correction]), whether the study provided effect estimated adjusted for key covariates (yes, no), percent of post-menopausal women in study (<50%, ≥50%), percent of post-menopausal women in study (<33%, ≥33%- ≤66% and ≥67%), whether study presented effect estimates by WHO categories of BMI (yes, no), choice of effect estimate (odds ratio, relative risk, hazard ratio) and study design (case-control, cross-sectional, or cohort). These categories were then utilized to perform sub-group analyses by strata of defined study attribute to present sub-group-specific meta-analysis effect estimates, 95% confidence intervals and within-group heterogeneity statistics (I^2). The aggregated sub-group-specific effect estimates were formally compared for between-group heterogeneity (p-value) when it was statistically appropriate to do so, that is, when each of the subgroups had two or more studies, and the subgroups being compared did not have overlapping populations (as is possible for the method of POP assessment categories since some studies provided two or more effect estimates for symptomatic prolapse, and various severity of prolapse). It is important to note that the sub-group-specific analyses should not be interpreted at the individual level as these are study level characteristics. To clarify with an example, if studies with a higher level of characteristic A showed a higher effect estimate than studies with lower level of characteristic A, it should not be interpreted to mean that the effect of BMI on individuals with characteristic A are higher than those individuals with lower level of characteristic A. The comparison between sub-group

analyses simply shows that studies with a higher level of characteristic A showed higher effect estimates in evaluating BMI and POP than studies with lower level of characteristic A.

Assessing publication bias

Evidence for publication bias was first evaluated by visual inspection of funnel plots. Funnel plots are graphical tools that assist in evaluating the potential for publication bias by assessing the symmetry around the mean effect estimate calculated for all studies. If the effect estimates are fairly equally distributed on both sides of the mean meta-analysis effect estimate, this is suggestive of a lack of publication bias. For example, if we found an overall null association between BMI and POP and there was very little publication bias, then we would expect majority of the larger studies to converge towards the mean effect estimate and the effect estimates from smaller studies would be equally distributed on both sides of the mean. In another scenario, if majority of the larger studies converge toward the null but most of the smaller studies systematically present larger effect estimates in one direction, this asymmetry would be evidence for publication bias. It should be noted that this evaluation is subjective and may not be reliable when only a few studies are available for meta-analysis.

Another method of testing publication bias is the Egger's test, which is a more formal statistical evaluation. The test essentially examines if there is a linear relationship between size of the studies (estimated by the inverse of the variance for each effect estimate) and the magnitude of the effect estimate. If there is no evidence for relationship between the inverse of the variance and the effect estimate, then it is thought that there is insufficient evidence to suggest publication bias. However, like most frequentist tests, the power of this test is driven by the number of studies evaluated.

Parent Study for Specific Aims 2 and 3

The Women's Health Initiative

The following section and subsections describe the Women's Health Initiative study, which is the source population for Specific Aims 2 and 3 of this dissertation. In addition to providing relevant description of the WHI study and sub-studies relevant to Aims 2 and 3, I provide the criteria that were used to define cases and controls. The same definitions of cases and controls are used in Specific Aim 2 and Specific Aim 3. I also provide descriptions of variables pertinent to aims 2 and 3 and how they were measured by the WHI study investigators.

Women's Health Initiative (WHI) studies

The WHI studies were a compilation of studies, including one observational study and three clinical trials, which were originally designed to investigate the causes of morbidity and mortality associated with several diseases including cardiovascular diseases, cancers and osteoporosis in postmenopausal women [65]. Women were recruited to participate in one or more of the three clinical trials: the dietary modification (trial), the calcium and vitamin D (CaD) supplementation trial and the hormone replacement therapy (HRT), later simply called the hormone therapy (HT) trial. These three trials collectively enrolled over 161,000 post-menopausal women in the US.

WHI-HT trial

The HT component of the clinical trial was intended to study the potential protective effects of hormone therapy in relation to coronary heart disease and osteoporotic fractures [65]. Women who did not have a uterus were given estrogen alone or placebo. Women who had an intact uterus were given estrogen and progesterone or placebo with the same goal of testing

coronary heart disease and osteoporotic fractures. The progesterone was added to potentially diminish the risk of endometrial cancer in women with an intact uterus.

Eligibility, recruitment and follow-up

Participants for the WHI-HT trial were recruitment from 40 clinical centers throughout the US from the years 1994 through 1998. A total of 27,342 women between the ages of 50 to 79 years of age were recruited [22;65]. Women were considered eligible to participate in the HT trials if they were postmenopausal at the time of recruitment, had no plans of moving or were unlikely to die within three years, were currently not participating in any other clinical trials, and were not using hormone therapy (or if they were willing to stop using hormone therapy prior to randomization). Collectively, the WHI studies made an extended effort to represent major minority groups in the US. In the WHI-HT study, approximately 81.5% of the recruited women identified themselves as white/European American, 7.4% of the women identified themselves as African American and 6.6% of the women identified themselves as Hispanic [22].

Due to safety concerns, the estrogen + progesterone arm of the clinical trial was stopped in July 2002 after an average of 5.6 years of follow-up, and the estrogen alone study was stopped in February 2004 after an average of approximately seven years of follow-up. Although the trial was stopped, participants were still followed to monitor the health risks and benefits of stopping the trials.

For this sub-study, only women participating in the WHI-HT study for whom information on POP was available for at least one visit and additionally for whom GWAS data was available were considered. This sub-study was approved by the Women's Health Initiative Publication and Presentation committee, dbGaP, and the Institutional Review Board at Vanderbilt University.

Genotyping data were acquired through dbGaP accession numbers phs000200.v9.p3.c1 and phs000200.v9.p3.c2.

WHI-HT Measurement of POP

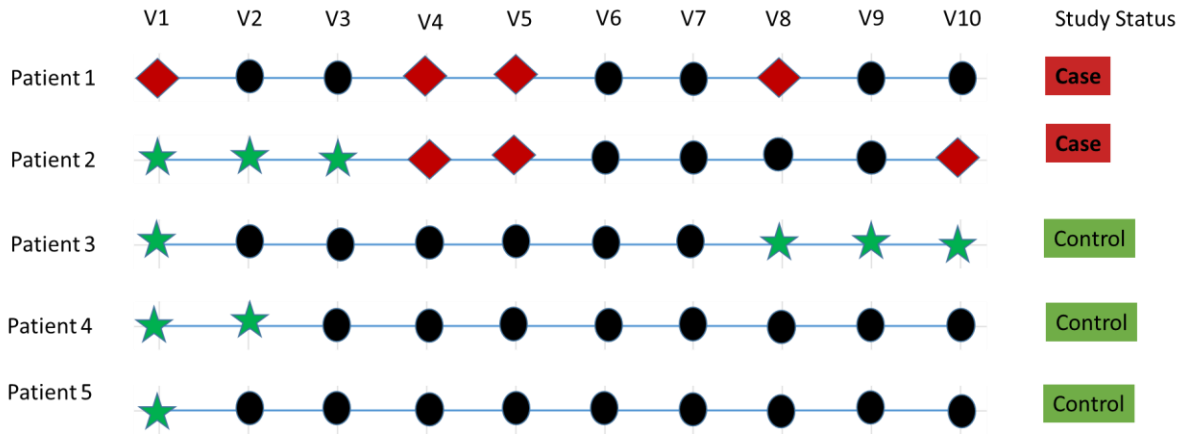
As a part of standard recruitment protocol, all women participating in the WHI-HT went through a pelvic exam at baseline in the supine lithotomy position. WHI trained gynecologist used standard procedures to measure degree of uterine prolapse, cystocele or rectocele with/without a Valsalva maneuver. The gynecologist performing the pelvic exam then recorded the degree of prolapse in standardized forms for each of the types of prolapse mentioned above (Appendix 2). The specific grading criteria adopted by WHI to evaluate the severity of prolapse are similar to the Baden-Walker Halfway system. Prolapse was divided into four specific categories: grade 0 indicated no prolapse; grade 1 indicated prolapse in vagina; grade 2 indicated prolapse in the vagina and more towards the vaginal introitus; and grade 3 represented prolapse past the introitus and outside the vagina. To ensure standardization of measurements, all gynecologists performing the pelvic exam and recording the degree of prolapse were provided a review of the examination procedure, and were certified by a central WHI clinic gynecologist. In addition to measurement of prolapse at baseline, women also underwent pelvic exams during at least one of ten annual follow-up visits planned by the WHI during the course of the study.

POP case and control selection

The WHI-HT trial conducted pelvic exams on participating women at baseline and during selected follow-up visits. Individuals who had grade 1 or higher POP (uterine prolapse, cystocele or rectocele) at baseline or during follow-up visits were considered as cases for Specific Aims 2 and 3. In addition to this primary definition of POP, individuals who had POP grade 2 or higher were categorized as moderate/severe POP. We utilized two definitions of controls for both of the

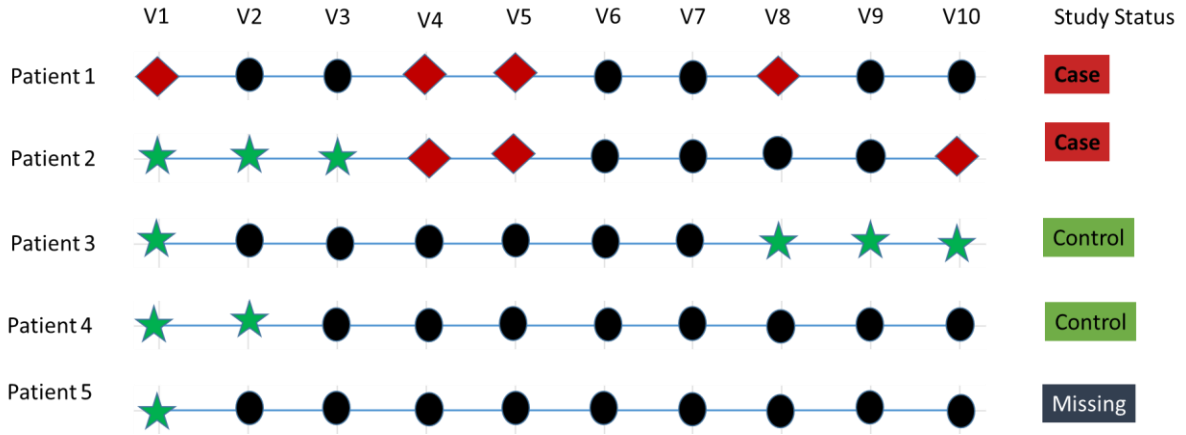
Specific Aims that follow. First, individuals who did not have POP at baseline and additionally did not develop POP during the follow-up pelvic examinations were considered as controls. If individuals had no POP at baseline but were lost to follow-up, then they were considered controls under this definition. A schema providing examples of this definition of control is provided in Figure 5-1. This definition of control was primarily constructed in order to maximize control sample size for both of our aims. However, for individuals who were lost to follow-up there was no way to decipher if these individuals remained controls or subsequently developed POP. The primary control definition is thus prone to outcome misclassification. Therefore, in order to potentially reduce outcome misclassification, we chose a second control eligibility criterion; controls selected using this criterion will be noted from here on as 'stringent controls'. Under this eligibility criterion, in order to qualify as a control, WHI participants needed to have undergone at least two pelvic exams undertaken by the WHI team and had to have been classified as having grade 0 prolapse for all three types of prolapse for at least two of these visits with no evidence of POP prior to being lost-to-follow-up. Although this approach does not guarantee complete specificity, it minimizes the potential for outcome misclassification. A schema providing examples of this definition of controls is provided in Figure 5-2. We identified 1,763 African American women, 931 Hispanic women and 9,920 European American women who were eligible to participate in our study either as cases or controls.

Figure 5-1. Examples of original case and control definition used in Specific Aims 2 & 3



Green Star = Control Status; Red Diamond = Case-status; Black circle = No information

Figure 5-2. Examples of original case and modified control definition used in Specific Aims 2 & 3



Green Star = Control Status; Red Diamond = Case-status; Black circle = No information

In a case-control study, a major potential for selection bias exists in a scenario when the control population is sampled from a population that does not originate from the same source population as the case-sample. Since cases and controls for this study are being drawn from a well-defined source population, the chance of incomparability of cases versus controls is minimized from this perspective.

Typically the use of prevalent and incident cases in a case-control design is suboptimal due to potential survival bias and potential for reverse causality [112]. The nature of our effect modifiers and phenotype provide a unique opportunity to circumvent problems associated with this approach, while simultaneously allowing us to increase sample size. Firstly, POP is not associated with increased mortality, thus eliminating concerns regarding survival bias. Secondly, our effect modifiers of interest are SNPs, and since genetic make-up precedes the outcome of interest, the concern for an association due to reverse causality is minimized. When these problems are minimized, inclusion of prevalent and incident cases provides the added benefit of increased sample size and reduced misclassification of cases and controls.

However, it should be noted that our study does potentially aggregate signals for early-onset POP (for individuals who already had POP at baseline, there is no way to ascertain incidence) and late-onset POP (for individuals who develop POP during the follow-up), if they are indeed different. With this caveat in mind, the added power we gain by increasing the number of cases by including prevalent and incident cases of POP may improve our chances of detecting genetic signals for POP.

Measurement of exposure variables in WHI-HT

Parity

At baseline the WHI-HT participants were asked to fill out a standardized reproductive history questionnaire. The questionnaire included a series of questions to determine their number of pregnancies, full-term pregnancies and births. Women were first asked "Have you ever been pregnant? It is very important that we know about all of your pregnancies, including live births, stillbirths, miscarriages, tubals (ectopics), and abortions." The women were prompted to choose "No" or "Yes". If they answered yes, they were further asked "How many times have you been

pregnant?" They were prompted to choose in the following categories, 1, 2, 3, 4, 5, 6, 7, or 8 or more. They were then asked "Did you ever have a pregnancy that lasted at least six months?" If they answered yes, they were asked to answer a series of questions that included asking about the number of these pregnancies that they had (using the same eight categories above to choose from), and they were asked to report the categories of ages at the end of the first of these pregnancies and at the end of the last of these pregnancies. Women were also asked to report the number of live births they had, the number of still births they had (from a pregnancy lasting six months or more), the number of miscarriages they and the number of tubal (ectopic) pregnancies they had, they were asked to choose from same eight categories of choices described above). From these, the WHI calculated the variables gravidity and parity. The gravidity variable measures the number of pregnancies a women had. Parity measures the number of term pregnancies the women had. Since child birth and not pregnancy seems to be more important for POP risk, we chose to use the parity variable in our analysis.

Although the WHI asked numerous questions regarding reproductive history, they did not ask about the method of delivery during child birth. As studies have shown, this is an important factor for POP occurrence, information for which is not available from the WHI and remains a drawback of this study. Women with caesarean section have been shown to have lower rates of POP than those women who vaginally delivered. Therefore, the increased risk reported for parity using the WHI data is likely to be an underestimate. However, it should also be noted that these women who were between the ages of 50 to 79, most likely had their reproductive years, during 1980s, if not before. The rates of caesarean section in the US were much lower then, than they are currently. The rates of caesarean section prior to the late 1990s were approximately 23% or

lower in the US. It is only recently, in the late 1990s and beyond, that the rates of caesarean section have dramatically increased to over 30% [113].

Measurement of obesity

Trained WHI-HT study staff took anthropometric measurements at baseline. Weight of dressed participants without shoes was measured using a balanced beam scale to the nearest 0.1 kg. Height was also measured without shoes on, with a wall-mounted stadiometer to the nearest 0.1 centimeter. BMI was computed as the weight in kg, divided by the square of the height measurement in meters. Trained staff also measured waist circumference and hip circumference with a measuring tape, to the nearest 0.5 cm. Waist to hip ratio was then computed as the ratio between the waist measurements in centimeters and the hip measurement in centimeters. BMI (kg/m^2) represents the indicator of overall adiposity, and waist-to-hip ratio represents the indicator of central/abdominal adiposity.

Assessment of other relevant covariates

The WHI collected information on race/ethnicity in their eligibility screening questionnaire. Participants were asked "How do you describe your racial or ethnic group? If you are of mixed blood, which group do you identify with most?" The participants were asked to choose from the following choices: 1) American Indian or Alaskan Native, 2) Asian or Pacific Islander (ancestry is Chinese, Indo-Chinese, Korean, Japanese, Pacific Islander, Vietnamese), 3) Black or African American (not of Hispanic origin), 4) Hispanic/Latino (ancestry is Mexican, Cuban, Puerto Rican, Central American, or South American), 5) White (not of Hispanic origin), or 6) Other, in which case, they were asked to specify.

Whether an individual had an intact uterus or if they had undergone a hysterectomy was assessed in the same pelvic exam that was used to measure degree of POP. Gynecologists trained by WHI assessed the presence of absence of the uterus in women undergoing the pelvic exam. A dichotomous variable was created by WHI indicating whether a participant had undergone a hysterectomy "Yes" or "No". If the participant refused the exam, then the value for hysterectomy was listed as missing.

Information on constipation was collected using a standard questionnaire. Participants were asked to report how bothersome constipation has been in the past four weeks, and were asked to choose from the four following choices: Symptom did not occur, symptom was mild, symptom was moderate, symptom was severe. The symptom was considered to be mild if it did not interfere with usual activities. It was considered to be moderate if it interfered somewhat with usual activities. It was considered severe if it was so bothersome that usual activities could not be performed.

Information regarding emphysema or chronic bronchitis (both factors that might lead to excessive coughing), was collected using a standardized medical history questionnaire. Participants were asked the following question "Has a doctor told you that you have any of the following conditions or have you had any of the following procedures? (Please mark all that apply)". Information regarding emphysema or chronic bronchitis was then coded as one dichotomous "Yes" or "No" variable.

Information regarding smoking was collected using a standardized questionnaire relating to personal habits. Participants were asked a series of questions to determine their smoking habits. They were first asked the following question: "During your entire life, have you smoked at least 100 cigarettes?" If they answered no, then they were considered to be life-time non-

smokers. If they answered yes, they were asked to report the age-category when they first started smoking cigarettes regularly. They were then asked if they currently smoke. If they said no, they were asked to detail when they quit smoking. For former smokers and current smokers, they were asked to report the average number of cigarettes they smoked each day when they smoked/smoke. They were then asked to report the number of years they had been a regular smoker or have remained a regular smoker. Information from these questions was used to calculate two important variables: smoking status and number of pack-years of smoking. For the smoking status variable, individuals were categorized as either never smokers, past smokers, or current smokers.

Genotyping Data

Our study includes individuals who have information on POP (only the WHI-HT measured POP) and additionally have information available on genetic data as measured by SNPs throughout the genome. Genetic data was not collected for all women participating in the WHI-HT study. Among the 27,342 individuals who participated in the WHI-HT study, GWAS data is available for 13,597 European American, African American and Hispanic individuals on various platforms.

Genetic data for African American and Hispanic women participating in the WHI-HT are available from the SNP Health Association Resource (SHARe) initiative, which was an effort funded by the National Health Lung and Blood Institute (NHLBI) to evaluate genetic determinants of disease in approximately 12,008 African American and Hispanic women participating in the WHI studies. Specifically, the SHARe initiative was designed to discover and/or replicate genes associated with traits such as blood pressure, blood lipids and BMI in the African American and Hispanic populations. These samples were genotyped with the Affymetrix

6.0 (Affymetrix, Santa Clara, CA) whole genome genotyping platform which includes a total of 934,940 oligos/SNPs. Of the 12,008 African American and Hispanic specimens, 1,861 specimens belonged to African American women and 1,126 belonged to Hispanic women participating in the WHI-HT study.

Genetic data for European American women participating in the WHI-HT is available from two additional WHI initiatives. The Genome-wide Association Studies of Treatment Response in Randomized Clinical Trials (GARNET) study is an ancillary study that was designed to evaluate the contribution of genetic differences in differential responses in the hormone therapy study. The GARNET sub-sample included a total of 4,894 women from the WHI-HT. A majority (87%) of the sub-sample was European American, 5% were African American, and 3% were Hispanic, and the rest were from other minority groups. The GARNET samples were genotyped using the Illumina Human Omni1_Quad_v1-0_B (Illumina, San Diego, CA) whole genome genotyping platform, which includes a total of 1,140,419 oligos/SNPs. Genetic data on this platform was available for 4,870 individuals who participated in the WHI-HT, prior to QC.

The second initiative that provides genetic data for the WHI-HT European American participants is the Women's Health Initative Memory Study + (WHI-MS). This study was designed to evaluate the long term effects of hormone therapy on cognitive abilities in the WHI-HT participants. This study has genotype data on 4,660 European American women participating in the WHI-HT who are not included in the GARNET study. This study additionally includes 1,178 European American women who are in the HT study but neither in the WHI-MS or the GARNET study. Samples for this study were genotyped using the Illumina Human Omni Express (Illumina, San Diego, CA) whole genome genotyping platform which includes 706,786

SNPs. Genetic data on this platform was available for 5,740 individuals who had also participated in the WHI-HT study, prior to QC.

All three of the data-sources described above are relevant to Aim 2, where we evaluate interactions between SNPs and parity and SNPs and BMI in European American, African American and Hispanic women. African American women for whom genetic data were available through SHARe and POP data were available through WHI-HT are additionally relevant to Aim 3, where we evaluate the association between global and local ancestry in relation to POP.

Methods for Specific Aim 2: To evaluate whether genetic variants modify the relationships between measures of obesity and pelvic organ prolapse, and parity and pelvic organ prolapse among European American, African American and Hispanic women

Hypothesis: We hypothesize that the association between measures of obesity and POP and association between parity and POP will be modified by SNPs.

Using data from the WHI-HT we conducted a nested case-control study to evaluate whether parity (an inciting risk factor) and/or BMI (a promoting risk factor) modify the association between select SNPs (selection criteria described below) and POP. POP cases and controls will be drawn from European American, African American, and Hispanic women who are participants in the WHI-HT trial, for whom GWAS data are available through the WHI SHARe initiative (for African Americans and Hispanics), WHI GARNET, or WHIMS+ (for European Americans). Briefly, GWAS data is not available for all individuals participating in the WHI-HT trial. These data are available for approximately more than half of the participants in the WHI-HT trial. Comparing baseline characteristics between all participants and the subset of participants with GWAS data available showed that the sub-set of individuals with GWAS data is a representative sample of all women participating in the WHI-HT study (Table 5-1). The only exception is that the African American and Hispanic populations are oversampled in the GWAS subset. The WHI-SHARe initiative had genotyped all African American and Hispanic participants from the WHI-HT and the Observational study. It should be noted that the GWAS subset compared in Table 5-1 represents all individuals from the WHI-HT for whom GWAS data is available, prior to sample- and genotype-QC and that this sub-study only utilizes data from European American, African American and Hispanic women.

Table 5-1. Comparing baseline-characteristics of WHI-HT participants by case-control status (at baseline) between all WHI-HT participants and participants with GWAS data

	WHI-HT All Individuals				WHI-HT participants with GWAS data			
	Cases		Controls		Cases		Controls	
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Age group								
50-59	3,085	28.4	5,676	34.8	1,620	23.1	2,977	29.0
60-69	5,148	47.4	7,137	43.8	3,313	47.3	4,569	44.5
70-79	2635	24.2	3482	21.4	2076	29.6	2712	26.4
Ethnicity								
European American*	8,852	81.5	13,039	80.0	5,351	76.3	7,496	73.1
African American*	805	7.4	1,910	11.7	697	9.9	1,701	16.6
Hispanic*	720	6.6	812	5.0	626	8.9	705	6.9
American Indian	50	0.5	80	0.5	42	0.6	73	0.7
Asian/Pacific Islander	266	2.4	256	1.6	246	3.5	239	2.3
Unknown	175	1.6	198	1.2	44	0.6	38	0.4
Body mass index (kg/m^2)								
Normal	2,289	21.2	4,915	30.3	1,489	21.2	3,050	29.7
Overweight	3,814	35.3	5,651	34.9	2,451	35.0	3,553	34.4
Obese	4,707	43.5	5,634	34.8	3,069	43.8	3,676	35.8
Parity								
0	512	4.2	2,094	14.8	334	4.8	1,307	12.8
1	650	5.3	1,509	10.7	400	5.8	995	9.8
2	2,194	17.9	3,576	25.3	1,359	19.5	2,184	21.4
3	4,616	37.6	3,749	26.6	1,633	23.5	2,287	22.4
≥4	4,827	39.3	5,280	37.4	3,231	46.4	3,427	33.6
Hysterectomy								
No	6,831	63.0	9,639	59.3	4,322	61.7	5,902	57.5
Yes	4,020	37.0	6,624	40.7	2,687	38.3	4,357	42.5
Hormone therapy use								
Never	7,309	67.3	10,410	63.9	3,681	54.1	5,201	52.3
Past	2,501	23.0	4,117	25.3	2,578	37.9	3,931	39.5
Current	1,056	9.7	1,759	10.8	540	7.9	809	8.1
Smoking								
Never	5,701	53.1	7,806	48.5	3,741	54.1	5,027	49.6
Past	4,111	38.3	6,426	39.9	2,608	37.7	3,929	38.8
Current	925	8.6	1,879	11.7	569	8.2	1,178	11.6

*Note that this study only focuses on European American, African American and Hispanic

Quality control (QC) for genotype data

A detailed flow-chart of quality control measures taken to prepare datasets for imputation is shown in Figure 5-3. Primary QC for genotype data was conducted using the PLINK software [114] in the following order for data generated from each of the genotyping platforms separately. We first removed a total of 8,957 individuals for whom data on POP was not available, from the WHI-MS, WHI-GARNET, WHI-SHARe (African American), and WHI-SHARe (Hispanic) datasets (9, 140, 6417, 2,391 individuals, respectively). Then, from the WHI-GARNET dataset, we removed 155 individuals who were already represented in the SHARe dataset and an additional 120 African American and 55 Hispanic individuals, since majority of the individuals sampled in this dataset were of European descent. We removed 1143 quality control SNPs from the WHI-MS dataset (the other datasets did not have quality control SNPs to remove). We then removed SNPs with minor allele frequencies less than 1%. Individual samples with genotyping call rates (GCR) of <95% were then removed from analysis. The GCR for a given sample (also called sample call rate) is the percentage of SNPs for which information is available for analysis out of the total SNPs genotyped in the assay. Specimens with low sample call rates may be indicative of errors associated with genotyping during the assay phase, and genotyping information available for that sample cannot be trusted with a high degree of confidence. We removed 104 individuals from the African American WHI-SHARe dataset and 73 individuals from the Hispanic WHI-SHARe dataset for low sample call rate. All individuals in the WHI-MS and WHI-GARNET dataset had sample call rates of 95% or more. After excluding these specimens, SNPs with genotyping rates less than 95% were then removed (the genotyping rate for a given SNP is the percentage of specimens for whom information on this SNP is available). When information on a particular SNP is not available for a relatively large percentage of individuals it is possible that the assay may not have been well equipped to detect

the SNP, and the information for this SNP may not have a high degree of certainty. We removed 9,287 SNPs, 1,077 SNPs, 19,094 SNPs and 26,325 SNPs from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively for low genotyping rates.

Using genetic information for alleles from non-autosomal chromosomes (sex chromosomes) with a minor allele frequency > 20%, it is possible to determine the sex of the given individual with a high degree of certainly. This was done by empirically estimating the sex of a given sample by comparing the estimates of homozygosity rate for the X chromosome in the sample using the PLINK software. Since males have only one X chromosome, one would expect males to have a homozygosity rate close to 1 for X chromosome markers that are not in the pseudo-autosomal regions of the Y chromosome. Similarly, females have a homozygosity rate of < 0.2 due to the presence of two X chromosomes. The program denotes individuals with homozygosity rate of greater than 0.8 to be genetically male, and denotes individuals with a homozygosity rate of less than 0.2 to be female. Generally, if the homozygosity rate is greater than 0.2 and less than 0.8, then the genotype determination of sex is considered to be inconclusive. Women were excluded from analysis if their homozygosity rate was ≥0.8. A total of 12 women, 0 women, 90 women and 50 women were removed for sex-check fail from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively.

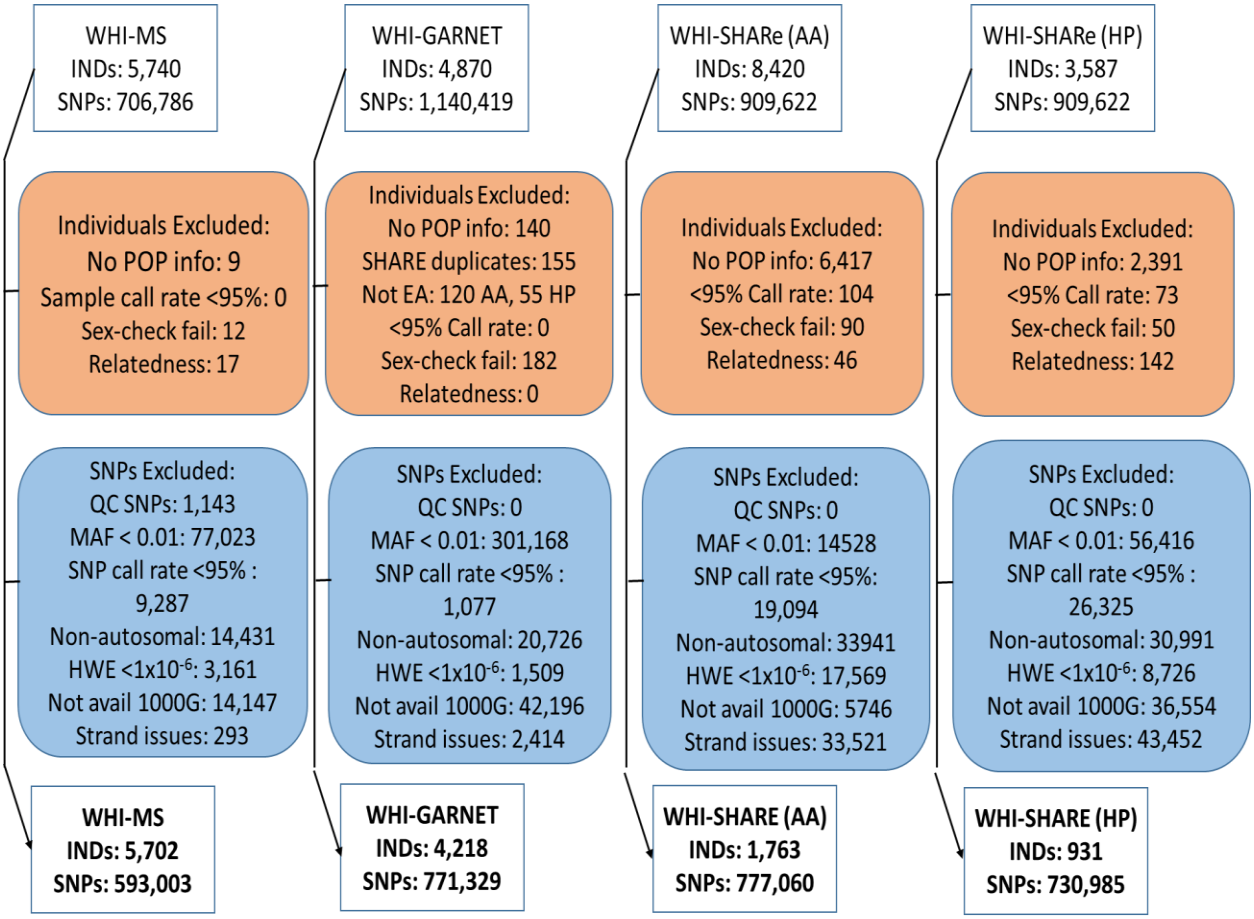
When analyzing a case-control study with the assumption of independence of observations, there is also a need to remove individuals who have familial relationships. The degree of relatedness, also called cryptic relatedness can be estimated to a high degree of certainty to the first cousin level with genetic data. Pairwise identity by descent (IBD) analyses

can be conducted to identify samples that are either monozygotic twins or duplicates, parent-child pairs, full siblings, half siblings or even first cousins. In general samples that show the probability of IBD greater than 20% were considered to have cryptic relatedness that needed to be removed from the samples to maintain the assumption of independent sampling. In the event monozygotic twins/duplicates are present in the sample, then both samples were removed as it may not be possible to identify if these are indeed monozygotic twins or mismatch/labeling errors during laboratory analysis. For samples that had some degree of cryptic relatedness, we dropped one sample from the pair, preferentially keeping cases over controls. If both samples in a given pair were cases or controls, then samples with the lowest sample call rate was excluded from the analysis. IBD was estimated using 60-80 thousand uncorrelated/independent autosomal SNPs after pruning SNPs for linkage disequilibrium. A total of 17 individuals, 0 individuals, 46 individuals and 142 individuals were removed for cryptic-relatedness from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively. Additionally, 14,431 non-autosomal SNPs, 20,726 non-autosomal SNPs, 33,941 non-autosomal SNPs and 36,554 non-autosomal SNPs were removed from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively. Finally, SNPs that violated the Hardy Weinberg equilibrium at a p-value threshold of $<1x10^{-6}$ were excluded from each of the datasets: 293 for WHI-MS, 2,414 for WHI-GARNET, 33,521 for WHI-SHARe (African American) and 43,452 for WHI-SHARe (Hispanic).

Pre-imputation QC

The WHI SHARe samples (Affymetrix 6.0), WHI GARNET samples (Illumina Omni Quad 1.0M) and the WHIMS+ samples (Illumina Omni Express) were genotyped on different platforms. Markers available in one panel may not necessarily be available in others. Therefore, imputation provides an efficient way of maximizing comparisons across datasets. For samples and genotypes that passed QC, we further removed SNPs which were available in the datasets but not available in the 1000 genomes phase 1 integrated reference panels [115],14,147 SNPs, 42,196 SNPs, 5,746 SNPs and 36,554 SNPs were removed from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively. We plotted the allele frequency estimates from our datasets against frequency estimates from 1000 genomes reference populations (of European origin, of African origin, or of Native American origin) to verify that the data are on the positive strand. SNPs that deviated by an absolute difference of 0.2 or more when comparing the allele frequency of SNPs in the datasets versus those in the 1,000 genome reference populations were removed prior to imputation. A total of 293 SNPs, 2,414 SNPs, 33,521 SNPs and 43,452 SNPs with unresolved strand issues either due to A/T, G/C base-pairs or delta > 0.2 were removed from the WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) datasets, respectively. After confirming that all remaining SNPs belonged to the positive strand, datasets were phased using 1,000 genomes mapping positions as the reference, using the software SHAPEIT2 [116].

Figure 5-3. Flow-chart depicting exclusion steps for SNPs and samples for WHI-MS, WHI-GARNET, WHI-SHARe datasets.



AA = African American; HP=Hispanic; POP=pelvic organ prolapse; SNP=single nucleotide polymorphism; QC = quality control; MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium; 1000G = 1000 genomes project

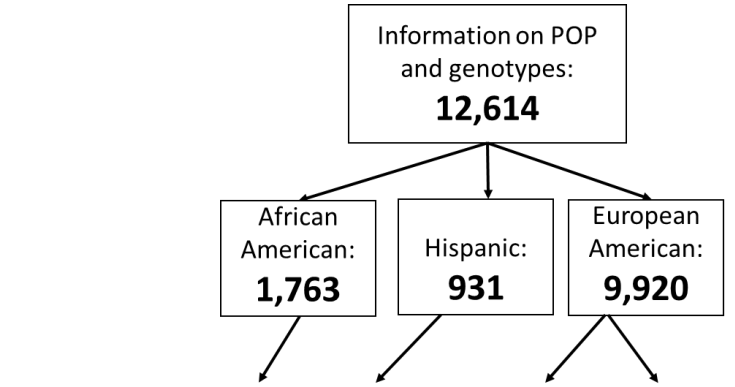
Imputation

Un-genotyped SNPs in the phased datasets were then imputed using the program IMPUTE2 [117]. The program uses genotypes from reference samples to predict (impute) genotypes that are not observed in the dataset to be assessed. The 1,000 Genomes integrated reference panel (phase 1, version 3) (cosmopolitan panel) was used as reference data for imputation, as studies have shown that using the entire reference panel increases imputation accuracy [117].

Primary analytic sample

Our primary analysis sample size was 12,614 women for whom information on POP and genetic data was available. Breakdown of the number of cases and controls in each dataset is provided in Figure 5-4. There were 1,763 African American women of whom 805 had prolapse of any grade and 156 had moderate to severe prolapse. A total of 958 women were classified as controls using the original control definition, of which only 344 had documented absence of any form of prolapse in two or more WHI-HT visits and were thus were eligible to be considered in the stringent-control category. Similarly, out of 941 Hispanic women, 621 women had any grade of POP, 168 women had moderate-to-severe POP, 310 were classified as controls according to the original control definition and 115 qualified as stringent-controls. There were a total of 9,920 European American women of whom 4,218 women belonged to the GARNET subset and 5,702 women belonged to the WHIMS subset. The GARNET subset included 2,491 women with POP of any grade, of whom 657 women had moderate to severe POP, and 1,727 controls of whom 534 qualified as stringent-controls. Similarly, the WHIMS subset included 3,679 women with POP of any grade of whom 1,116 women had moderate to severe POP, and 2,023 controls of whom 1,002 women qualified as stringent-controls. Our primary meta-analytic sample from four datasets for European American, Hispanic and African American women included 7,596 cases of grade 1-3 POP, 2,097 cases of grade 2-3 (moderate/severe POP), 5,018 controls (original definition) and 1,995 controls (stringent-control definition).

Figure 5-4. Ethnicity and genotyping platform-specific breakdown of individuals qualifying for primary analyses



WHI-HT Subsets	SHARe	SHARe	GARNET	WHI-MS	Total
Controls	958	310	1,727	2,023	5,018
Stringent Controls	344	115	534	1,002	1,995
Grades 1-3	805	621	2,491	3,679	7,596
Grades 2-3	156	168	657	1,116	2,097

SHARe: genotyped with Affymetrix 6.0; GARNET: genotyped with Illumina Human Omni1_v1-0_B; WHI-MS: genotyped with Illumina Human OMNI Express

Choice of SNPs for analysis

To test for gene-environment interactions between SNPs and BMI, and between SNPs and parity in relation to POP, we sought to evaluate interactions for SNPs that have been previously investigated in relation to POP, or may have etiological connections with POP. These include SNPs in or around collagen genes, matrix metalloproteinase genes, LOX genes, FIBULIN genes, BMP genes, estrogen and progesterone receptor genes and other genes that have been investigated in the past in either association studies, or tissue expression studies in relation to POP in humans or animal models. Genes which have been previously investigated for association with POP, and those which were investigated in this study are listed in Table 5-2. We also evaluated SNPs in or around genes that have been implicated of causing several connective tissue disorders, including genes for Elhers Danlos Syndrome, Marfan's Syndrome, among others [118], listed in Table 5-3. We additionally considered gene regions from SNPs which have

previously been associated with BMI or waist-to-hip ratio (Table 5-4). We did not limit our search to SNPs within these genes because SNPs in the promoter regions/transcription factor binding regions could be of equal importance in determining expression of genes. Therefore, for each gene we included SNPs that are 10 kilo-bases (kb) before or after the gene.

Table 5-2. Genes/loci specifically evaluated in the context of POP in GWAS, linkage studies, candidate gene studies, human tissue expression studies and knockout mice models in the literature

Gene/Locus name	Study Type	Ref
LAMC1	Linkage study; Candidate gene studies	[89;100;103]
COL3A1	Candidate gene studies	[42;48;49;56]
COL1A1	Candidate gene studies; Human tissue expression studies	[93-95;97;98;119]
MMP-1	Candidate gene studies; Human tissue expression studies	[95;99;119]
MMP-3	Candidate gene studies	[95;99]
MMP-9	Candidate gene studies	[50;95;101]
TIMP-1	Human tissue expression study	[120]
TIMP-3	Human tissue expression study	[120]
ER-beta	Candidate gene studies	[92]
ER-alpha	Candidate gene studies	[91]
PGR	Candidate gene studies	[121]
LOXL1	Candidate gene study; Human tissue expression study; mouse models	[58;59;62;63;96]
BMP1	Human tissue expression study	[61]
FBLN-5	Mice knockout study; tissue expression study	[59;60;64]
MYH11	Human tissue expression study	[122]
MYOM2	Human tissue expression study	[123]
MYO1E	Human tissue expression study	[123]
MYH3	Human tissue expression study	[123]
MYBPH	Human tissue expression study	[123]
ACTN3	Human tissue expression study	[123]

Table compiled from various studies

Table 5-3. Genes associated with various connective tissue disorders in the literature

Gene/Locus Name	Connective Tissue Disorders	OMIM ID	Mode of Transmission
ACTA2	Familial Thoracic Aortic Aneurysm and/or Dissection		Autosomal Dominant
ADAMTS2	Ehlers-Danlos, Dermatosporaxis	#225410	Autosomal Dominant
ADAMTS10	Weill-Marchesani Syndrome	#277600	Autosomal Recessive
ADAMTSL4	Ectopia Lentis, Familial	#129600	Autosomal Recessive
ATP6V0A2	Cutis Laxa Type II,	#219200	Autosomal Recessive
ATP7A	Cutis Laxa, X-linked	#304150	X-linked
B4GALT7	Ehlers-Danlos, Progeroid	#130070	Autosomal Recessive
CBS	Homocystinuria	+236200	Autosomal Recessive
COL1A1	Ehlers-Danlos, Arthrochalasia	#130060	Autosomal Dominant
COL1A2	Ehlers-Danlos, Arthrochalasia	#130060	Autosomal Dominant
	Ehlers-Danlos, Cardiac Valvular	#225320	Autosomal Recessive
COL2A1	Stickler Syndrome	#108300	Autosomal Dominant
	Aneurysms, Aortic Abdominal	#100070	Autosomal Dominant
COL3A1	Ehlers-Danlos, Vascular	#130050	Autosomal Dominant; Autosomal Recessive
	Aneurysms, Aortic Abdominal	#100070	Autosomal Dominant
COL9A1	Aneurysms, Aortic Abdominal	#100070	Autosomal Dominant
	Stickler Syndrome	#108300	Autosomal Recessive
COL5A1, COL5A2	Ehlers-Danlos, Classical	#130010	Autosomal Dominant
COL11A1, COL11A2	Stickler Syndrome	#108300	Autosomal Dominant
ELN	Cutis Laxa, Autosomal Dominant	#123700	Autosomal Dominant
FAA1 locus 11q23-q24	Familial Thoracic Aortic Aneurysm and/or Dissection	(multiple)	Autosomal Dominant
FBLN4	Cutis Laxa Type I, Autosomal Recessive	#219100	Autosomal Recessive
FBLN5	Cutis Laxa Type I, Autosomal Recessive	#219100	Autosomal Recessive
	Cutis Laxa, Autosomal Dominant	#123700	Autosomal Dominant

(Continued)

Gene/Locus Name	Connective Tissue Disorders	OMIM ID	Mode of Transmission
FBN1	Ehlers-Danlos, Arthrochalasia	#130060	Autosomal Dominant
	Familial Thoracic Aortic Aneurysm and/or Dissection	(multiple)	Autosomal Dominant
	Marfan Syndrome	#154700	Autosomal Dominant
	MASS Phenotype	#604308	Autosomal Dominant
	Mitral Valve Prolapse Syndrome	#157700*	Autosomal Dominant?
	Shprintzen-Goldberg Syndrome	#182212	Autosomal Recessive
	Weill-Marchesani Syndrome	#608328	Autosomal Dominant
FBN2	Congenital Contractural Arachnodactyly	+121050	Autosomal Dominant
FGFR3	CATSHL Syndrome	#610474	Autosomal Dominant
LTBP-2	Ectopia Lentis, Familial	#129600	Autosomal Recessive
MYH11	Familial Thoracic Aortic Aneurysm and/or Dissection	(multiple)	Autosomal Dominant
	Persistent PDA with Familial Thoracic Aneurysm	#132900	Autosomal Dominant
NOTCH1	Bicuspid Aortic Valve with Thoracic Aortic Aneurysm	#109730	Autosomal Dominant
P5CS	Cutis Laxa Type II, Autosomal Recessive	#219200	Autosomal Recessive
PLOD1	Ehlers-Danlos, Kyphoscoliotic	#225400	Autosomal Recessive
PLOD3	LH3 Deficiency Syndrome	#612394	Autosomal Recessive
PYCR1	Cutis Laxa Type II, Autosomal Recessive	#219200	Autosomal Recessive
RIN2	MACS Syndrome	*610222	Autosomal Recessive
SLC2A10	Arterial Tortuosity Syndrome	#208050	Autosomal Recessive
SLC39A13	Ehlers-Danlos, Spondylocheiro Dysplastic	#612350	Autosomal Recessive
TAAD1 locus 5q13-q14	Familial Thoracic Aortic Aneurysm and/or Dissection	(multiple)	Autosomal Dominant
TGFB1	Camurati-Engelmann Disease	#131300	Autosomal Dominant

(Continued)

Gene/Locus Name	Connective Tissue Disorders	OMIM ID	Mode of Transmission
TGFBR1	Ehlers-Danlos, Spondylocheiro Dysplastic	#612350	Autosomal Dominant
	Loeys-Dietz Syndrome Type I	#609192 #610168	Autosomal Dominant
	Loeys-Dietz Syndrome Type II	#608967 #610380	Autosomal Dominant
TGFBR2	Ectopia Lentis, Familial	#129600	Autosomal Dominant
	Familial Thoracic Aortic Aneurysm and/or Dissection	(multiple)	Autosomal Dominant
	Loeys-Dietz Syndrome Type I	#609192 #610168	Autosomal Dominant
	Loeys-Dietz Syndrome Type II	#608967 #610380	Autosomal Dominant
TNXB	Ehlers-Danlos-like Syndrome, Tenascin-X	#606408	Autosomal Recessive

Table adapted with permission from Murphy-Ryan, Maureen, Apostolos Psychogios, and Noralane M. Lindor. "Hereditary disorders of connective tissue: a guide to the emerging differential diagnosis." *Genetics in Medicine* 12.6 (2010): 344-354. [118]

Table 5-4. Genes/nearby loci previously reported to be associated with measures of obesity

Gene/Locus Name	Obesity trait
FTO	BMI
TMEM18	BMI
MCAR	BMI
GNPDA2	BMI
BDNF	BMI
NEGR1	BMI
SH2B1	BMI
ETV5	BMI
MTCH2	BMI
KCTD15	BMI
SEC16B	BMI
TFAP2B	BMI
FAIM2	BMI
NRXN3	BMI; WHR; waist-circumference
RBJ	BMI
GPRC5B	BMI
MAP2K5	BMI
QPCTL	BMI
TNNI3K	BMI
SLC39A8	BMI
FLJ35779	BMI
LRRN6C	BMI
TMEM160	BMI
FANCL	BMI
CADM2	BMI
PRKD1	BMI
LRP1B	BMI
PTBP2	BMI
MTIF3	BMI
ZNF608	BMI
RPL27A	BMI
NUDT3	BMI

Gene/Locus Name	Obesity trait
RSP03	WHR
VEGFA	WHR
TBX15-WARS2	WHR
NFE2L3	WHR
GRB14	WHR
LYPAL1	WHR
DNM3-PIGC	WHR
ITPR2-SSPN	WHR
LY86	WHR
HOXC13	WHR
ADAMTS9	WHR
ZNRF3-KREMEN1	WHR
NISCH-STAB1	WHR
CPEB4	WHR
BTNL2	WHR
ZEB1	WHR

BMI related loci extracted from Speliotes et al. [124]; WHR related loci extracted from from Heid et al. [125]

There were a total of 96 genes for which we extracted SNPs which were ± 10 kb from a given gene. The final list of genes with range of base-pair positions for each gene is presented in Table 5-5. These 96 genes provided 168,731 SNPs which were included in the 1000 genomes phase 1 reference dataset. 59,432 of these 168,731 SNPs, were available in our imputed datasets. We then limited our analysis to only those SNPs that had minor allele frequencies of > 2.5% in the larger datasets (WHI-MS and WHI-GARNET), and > 5% in the smaller datasets (WHI-SHARe African American and Hispanic). This left us with a total of 33,515 SNPs which were assessed for interaction with BMI in all four datasets.

Table 5-5. Details of gene loci evaluated for interactions

Chromosome	BP Begin - 10,000	BP End + 10,000	Gene Name	Tied to:
chr1	11984262	12045595	*PLOD1*	CTD
chr1	71851623	72758417	*NEGR1*	BMI
chr1	74691085	75020112	*TNNI3K*	BMI
chr1	97177221	97299294	*PTBP2*	BMI
chr1	103332023	103584052	*COL11A1*	CTD
chr1	119415669	119542179	*TBX15*	WHR
chr1	119563839	119693294	*WARS2*	WHR
chr1	150511845	150543413	*ADAMTSL4*	CTD
chr1	171800638	172397606	*DNM3*	WHR
chr1	172329329	172423226	*PIGC*	WHR
chr1	177883091	177963438	*SEC16B*	BMI
chr1	203126939	203154969	*MYBPH*	POP
chr2	657335	687439	*TMEM18*	BMI
chr2	58376378	58478507	*FANCL*	BMI
chr2	74749541	74792817	*LOXL3*	POP
chr2	140978992	142899270	*LRP1B*	BMI
chr2	165339322	165488358	*GRB14*	WHR
chr2	189829046	189887472	*COL3A1*	POP; CTD
chr2	189886622	190054605	*COL5A2*	CTD, POP
chr3	30637994	30745634	*TGFBR2*	CTD
chr3	52479134	52537087	*NISCH*	WHR
chr3	52519354	52568511	*STAB1*	WHR
chr3	64491330	64683676	*ADAMTS9*	WHR
chr3	84998132	86133579	*CADM2*	BMI
chr3	185754097	185838107	*ETV5*	BMI
chr4	1785034	1820599	*FGFR3*	CTD
chr4	41248898	41280446	*UCHL1*	POP

chr4	44674217	44738612	*GNPDA2*	BMI
chr4	103162198	103362415	*SLC39A8*	BMI
chr5	121388890	121423980	*LOX*	POP
chr5	123962606	124094500	*ZNF608*	BMI
chr5	127583601	128004878	*FBN2*	CTD
chr5	173305283	173398979	*CPEB4*	WHR
chr5	177017101	177047348	*B4GALT7*	CTD
chr5	178527852	178782431	*ADAMTS2*	CTD
chr6	6578341	6665216	*LY86*	WHR
chr6	31947373	32171814	*TNXB*	CTD
chr6	32299951	32461069	*BTNL2*	WHR
chr6	33042085	33340639	*COL11A2*	CTD
chr6	34237456	34370451	*NUDT3*	BMI
chr6	43727921	43764224	*VEGFA*	WHR
chr6	50776436	50825326	*TFAP2B*	BMI
chr6	70914764	71022786	*COL9A1*	CTD
chr7	26181860	26236745	*NFE2L3*	WHR
chr7	73432119	73494237	*ELN*	POP; CTD
chr7	94013873	94070544	*COL1A2*	CTD
chr7	100839258	100871701	*PLOD3*	CTD
chr8	1983082	2103380	*MYOM2*	POP
chr8	22012249	22079839	*BMP1*	POP
chr9	101856320	101926474	*TGFBR1*	CTD
chr9	137523620	137746689	*COL5A1*	CTD
chr9	139378896	139450314	*NOTCH1*	CTD
chr10	31597424	31828742	*ZEB1*	WHR
chr10	90684831	90761147	*ACTA2*	CTD
chr11	8693958	8746306	*RPL27A*	BMI
chr11	27666440	27753605	*BDNF*	BMI
chr11	47418683	47448052	*SLC39A13*	CTD
chr11	47628867	47674175	*MTCH2*	BMI
chr11	66304312	66340799	*ACTN3*	POP
chr12	26264924	26462223	*SSPN*	WHR
chr12	26479448	26996131	*ITPR2*	WHR
chr12	48356748	48408269	*COL2A1*	CTD
chr12	50250679	50308000	*FAIM2*	BMI
chr12	54322549	54350328	*HOXC13*	WHR
chr12	124186865	124256302	*ATP6V0A2*	CTD
chr13	27999780	28034728	*MTIF3*	BMI
chr14	30035685	30671104	*PRKD1*	BMI
chr14	74954873	75089081	*LTBP2*	CTD
chr14	78698734	80344633	*NRXN3*	BMI
chr14	92325756	92424331	*FBLN5*	POP
chr15	35070297	35097927	*ACTC1*	CTD
chr15	39863280	39899668	*THBS1*	CTD
chr15	48690503	48948046	*FBN1*	CTD
chr15	59418168	59675071	*MYO1E*	POP
chr15	67825047	68109461	*MAP2K5*	BMI

chr15	93568503	93642433	*RGMA*	POP
chr16	15786992	16046023	*MYH11*	CTD, POP
chr16	19858013	19907489	*GPRC5B*	BMI
chr16	28847921	28895533	*SH2B1*	BMI
chr16	53727875	54165853	*FTO*	BMI
chr17	10521843	10570626	*MYH3*	POP
chr17	48250650	48288993	*COL1A1*	POP, CTD
chr17	79880260	79910288	*PYCR1*	CTD
chr19	8635126	8685620	*ADAMTS10*	CTD
chr19	34276838	34316668	*KCTD15*	BMI
chr19	41797492	41869816	*TGFB1*	CTD
chr19	46185741	46217247	*QPCTL*	BMI
chr19	47539165	47561888	*TMEM160*	BMI
chr20	19857165	19993101	*RIN2*	CTD
chr20	45328126	45374986	*SLC2A10*	CTD
chr21	44463301	44507053	*CBS*	CTD
chr22	29269580	29463475	*ZNRF3*	WHR
chr22	29459066	29574321	*KREMEN1*	WHR

CTD=connective tissue disorders; BMI=body mass index; WHR=waist-to-hip ratio

Statistical analysis

Association Analysis

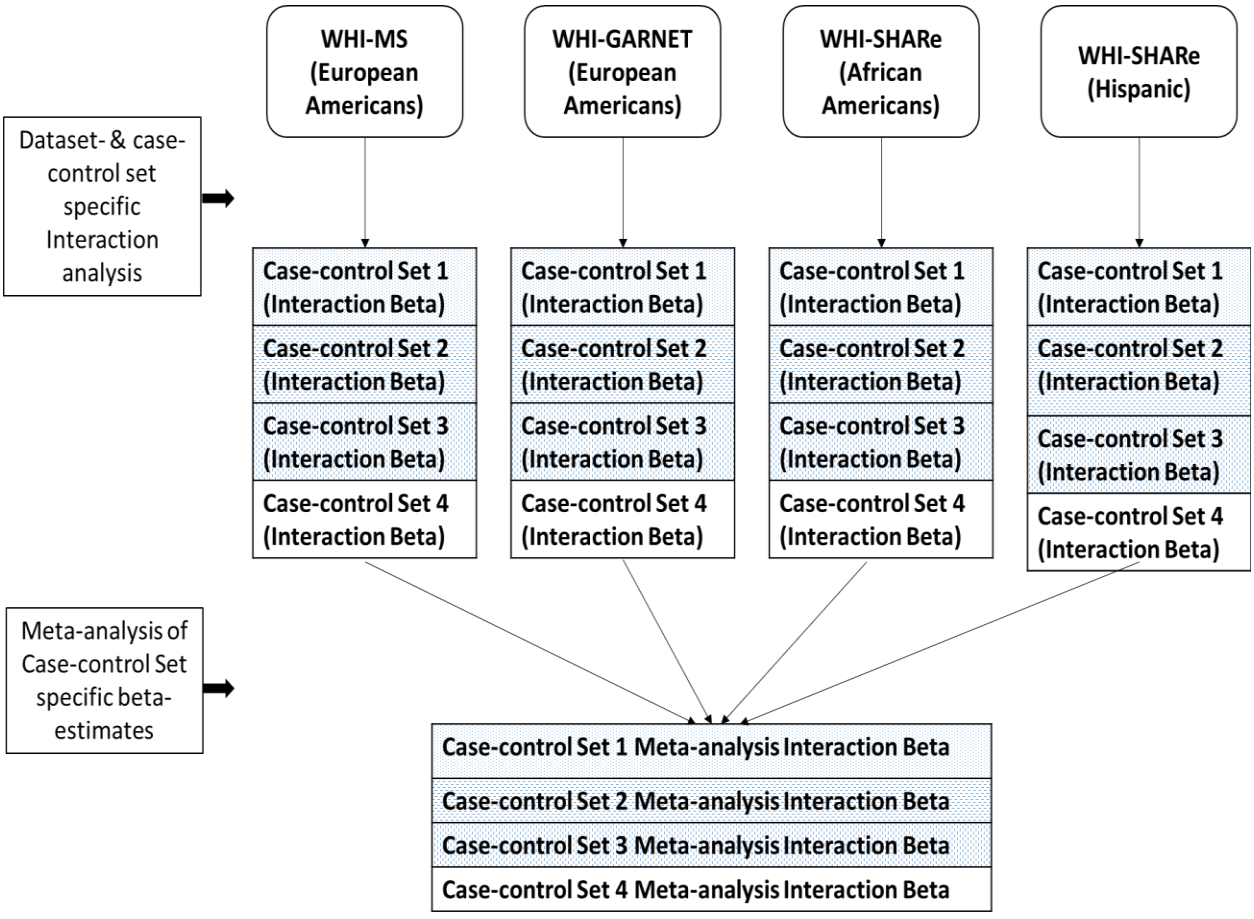
In this study we evaluated interaction between SNPs and BMI, and SNPs and parity in relation to any POP (grades 1-3) and moderate-severe POP (grades 2-3) in European American, African American and Hispanic women. As previously described, we chose two methods of defining controls. The original definition included women who went through at least 1 pelvic exam for assessment of POP and was found to have grade 0 POP. The modified control definition was more stringent: where women had to have at least two pelvic assessments for POP and were confirmed to have grade 0 POP. The stringent-control group was constructed to evaluate the potential impact of reduced misclassification of outcome on interaction effect estimates. We therefore assessed for interactions using four analysis models each for BMI and SNP interaction and parity and SNP interaction (Table 5-6). A schematic representation of primary analysis steps is provided in Figure 5-5.

Table 5-6. Case-control combination sets used for assessing interaction between BMI and SNPs and parity and SNPs in relation to POP

Case-control Sets	Analysis Models
Set 1	Grade 0 (All Controls) vs. Any POP
Set 2	Grade 0 (Stringent Controls) vs. Any POP
Set 3	Grade 0 (All Controls) vs. Mod/Sev POP
Set 4	Grade 0 (Stringent Controls) vs. Mod/Sev POP

Any POP = Grade ≥ 1; Mod/Sev POP = Grade ≥ 2; All Controls = At least 1 visit with no POP; Stringent Controls = At least 2 visits with no POP

Figure 5-5. Schematic representation of analysis steps utilized in primary analyses



Using the 33,515 SNPs from our individually imputed datasets, we first evaluated interaction between SNPs and BMI, and SNPs and parity in each of the individual datasets (WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic)) using the four case-control sets described above. Dataset-specific interaction analyses were conducted using the ProbABEL software, which is capable of handling both imputed and genotyped SNPs [126]. For each of the four sets described above, POP status was regressed onto a two way continuous-interaction term (SNP x BMI term, or SNP x parity term) while adjusting for confounders and key covariates. Interaction models for BMI and SNP were adjusted for age, parity and genetic-ancestry components. Interaction models for parity and SNP were adjusted for age, BMI and genetic-ancestry components. A description of how confounders and key covariates were chosen is detailed later in a subsection dedicated to assessment of confounding. In each of the four datasets and for all four combinations of case-control definitions, interactions between BMI and SNPs and interactions between parity and SNPs were assessed using the following modeling strategies:

$$\text{Logit(POP)} \approx \beta 1 * SNP + \beta 2 * BMI + \beta 3 * (BMI * SNP) + \beta 4 * Age + \beta 5 * Parity + \beta 6\left(MDS_{component1}\right) + \beta 7\left(MDS_{component2}\right)$$

$$\text{Logit(POP)} \approx \beta 1 * SNP + \beta 2 * Parity + \beta 3 * (Parity * SNP) + \beta 4 * Age + \beta 5 * BMI + \beta 6\left(MDS_{component1}\right) + \beta 7\left(MDS_{component2}\right)$$

We then performed random-effects meta-analysis of the continuous interaction term beta-coefficients obtained from the individual WHI datasets (WHI-MS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic) to present an aggregated meta-analysis-interaction effect estimate for each of the four case-control analysis sets. The same

procedure was followed for the BMI and parity interaction models. Random-effects meta-analyses were conducted using the Metasoft software [127]. The linear interaction terms were primarily used for detection purposes, since using continuous terms provides the greatest statistical power assuming that the variables have been modeled correctly and that estimates are not confounded.

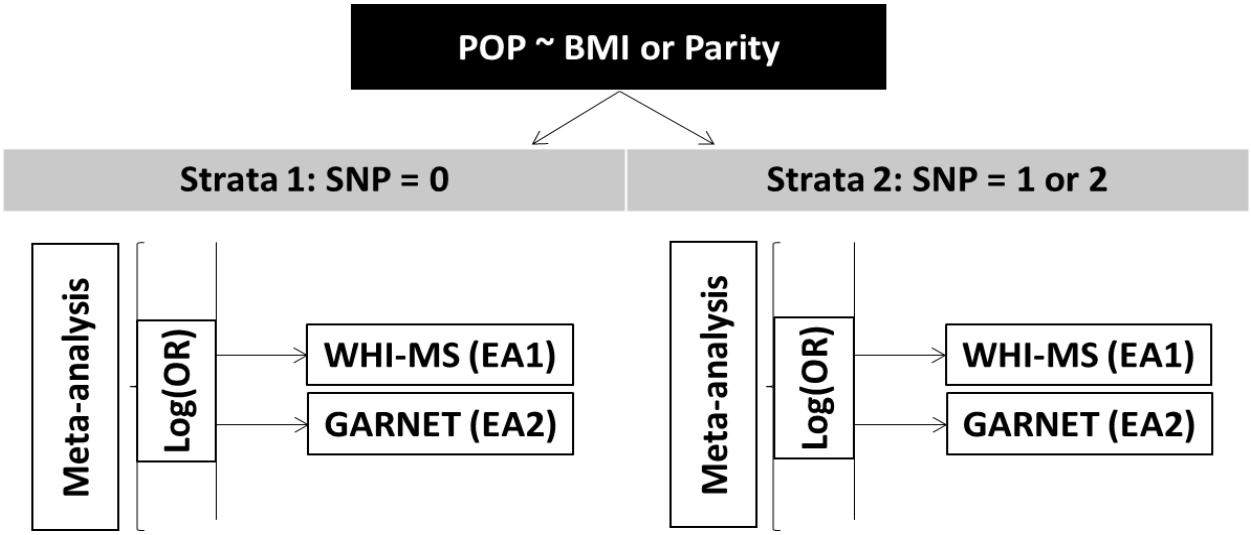
Then, for SNPs which were the most statistically significant in our primary continuous interaction analyses ($p < 2.18 \times 10^{-4}$), we performed interaction analyses for BMI and parity by strata of SNPs for ease of interpretation. Schematic representation of stratum-specific analysis plan is shown in Figure 5-6. In these analyses, we used the case-control sets with the stringent controls for any POP and moderate/severe POP. Since we imputed genotype data, information on SNPs were available in dosage format as probabilistic predictions of the genotype in each individual. As a result, SNP data were not only in integer format (0, 1 or 2), but also included probabilistic values between these integers. Therefore, in order to create strata for SNPs, we opted to take the following approach. SNPs with dosage estimates ≤ 0.5 were classified as "0" and dosage estimates greater than 0.5 were classified as "1", where 0 indicates two reference alleles and 1 indicates the presence of one or more effect alleles (dominant model). Stratum specific analyses were limited to WHI-MS and WHI-GARNET datasets, as only these datasets had sample sizes large enough to allow for reliable estimation of stratum-specific odds ratios. Histograms of SNPs for the WHIMS and WHI-GARNET datasets are provided in Figure 5-7 for SNP evaluated for interaction for BMI and in Figure 5-8 for SNPs evaluated for interaction with parity. The majority of the SNP dosages were distributed around 0, 1 or 2.

BMI was modeled as a dichotomous variable (BMI <25 kg/m^2, and BMI ≥ 25 kg/m^2) to compare odds of POP between normal-weight and overweight/obese individuals by strata of

SNP. Parity was modeled as a continuous variable to provide odds of POP for each unit increase in parity by strata of SNP. The interpretation of each additional birth is not only straightforward but also clinically meaningful. Instead of providing estimates for BMI and parity in relation to POP by stratum of SNP, we could have alternatively chosen to provide estimates for the SNP by strata of BMI or strata of parity. However, we chose the former method of presenting results since the interpretation is easier. For example, the odds/risk of POP for BMI or parity is higher/lower for individuals with a given genotype than individuals with a second genotype. It should be noted that we did not perform formal statistical tests for interaction for stratum specific analyses since the power to detect associations would be lower using dichotomous variables, and we had already formally tested these interactions as continuous variables.

Stratum-specific analyses were then aggregated using inverse-variance weighted fixed-effects meta-analysis. Since the distribution of imputed SNPs were similar for both datasets (WHI-MS and WHI-GARNET), stratum specific analyses were also conducted by pooling the two datasets while additionally adjusting for the source of the data (WHI-MS or WHI-GARNET as a dichotomous variable) to account for between-dataset heterogeneity. Evaluation for interaction in the pooled datasets were conducted by comparing the negative log-likelihoods from models which included SNP (dichotomous) x BMI (dichotomous) interaction term or SNP (dichotomous) x parity (continuous) interaction term with models without the interaction term. All stratum specific analyses, subsequent meta-analyses and tests for interaction for the stratum specific estimates were conducted using STATA [128].

Figure 5-6. Schematic representation of strata-specific meta-analyses by strata of SNP category



EA = European Americans

Figure 5-7. Dataset specific histograms of top seven independent SNPs identified in BMI-SNP interaction analyses

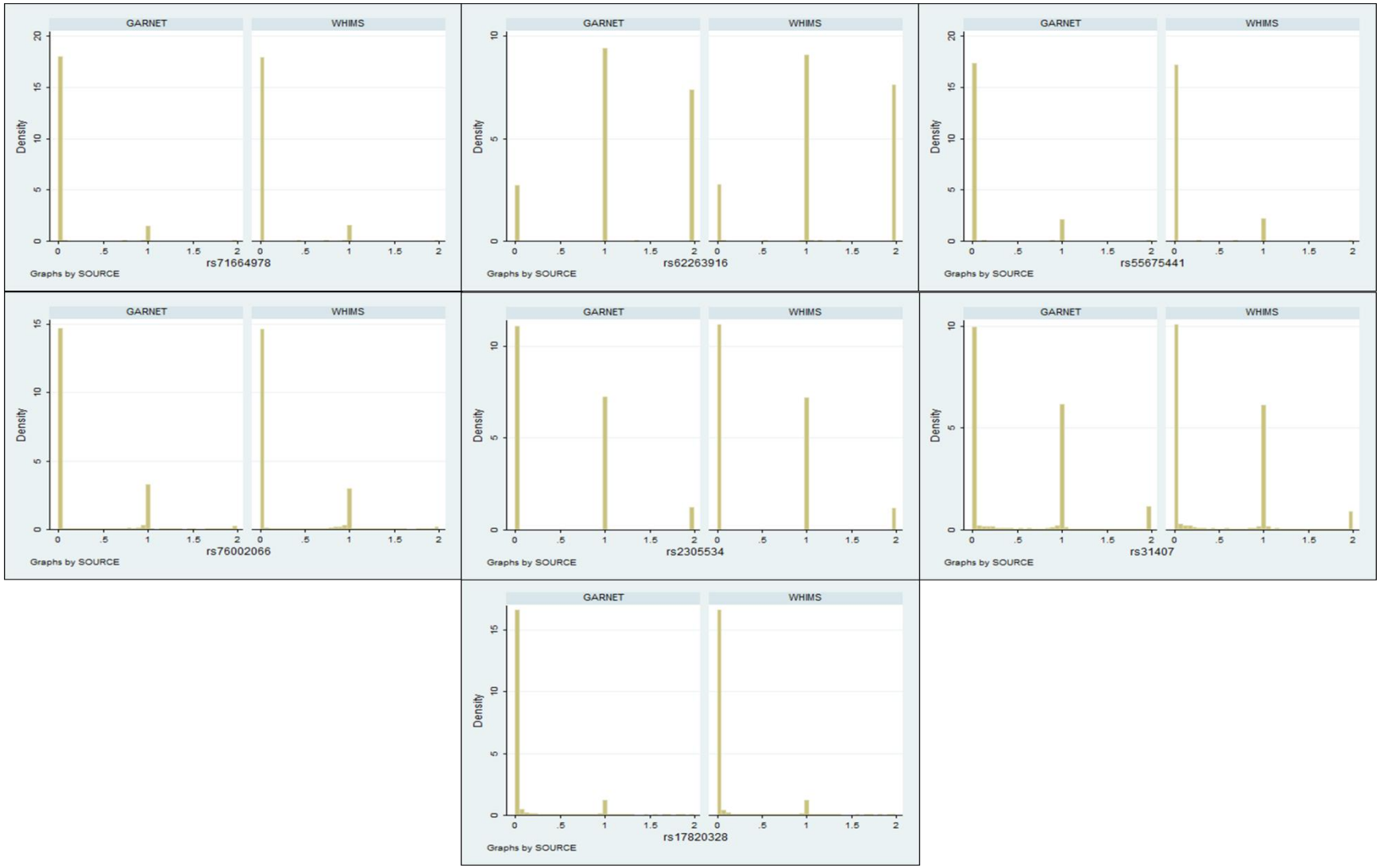
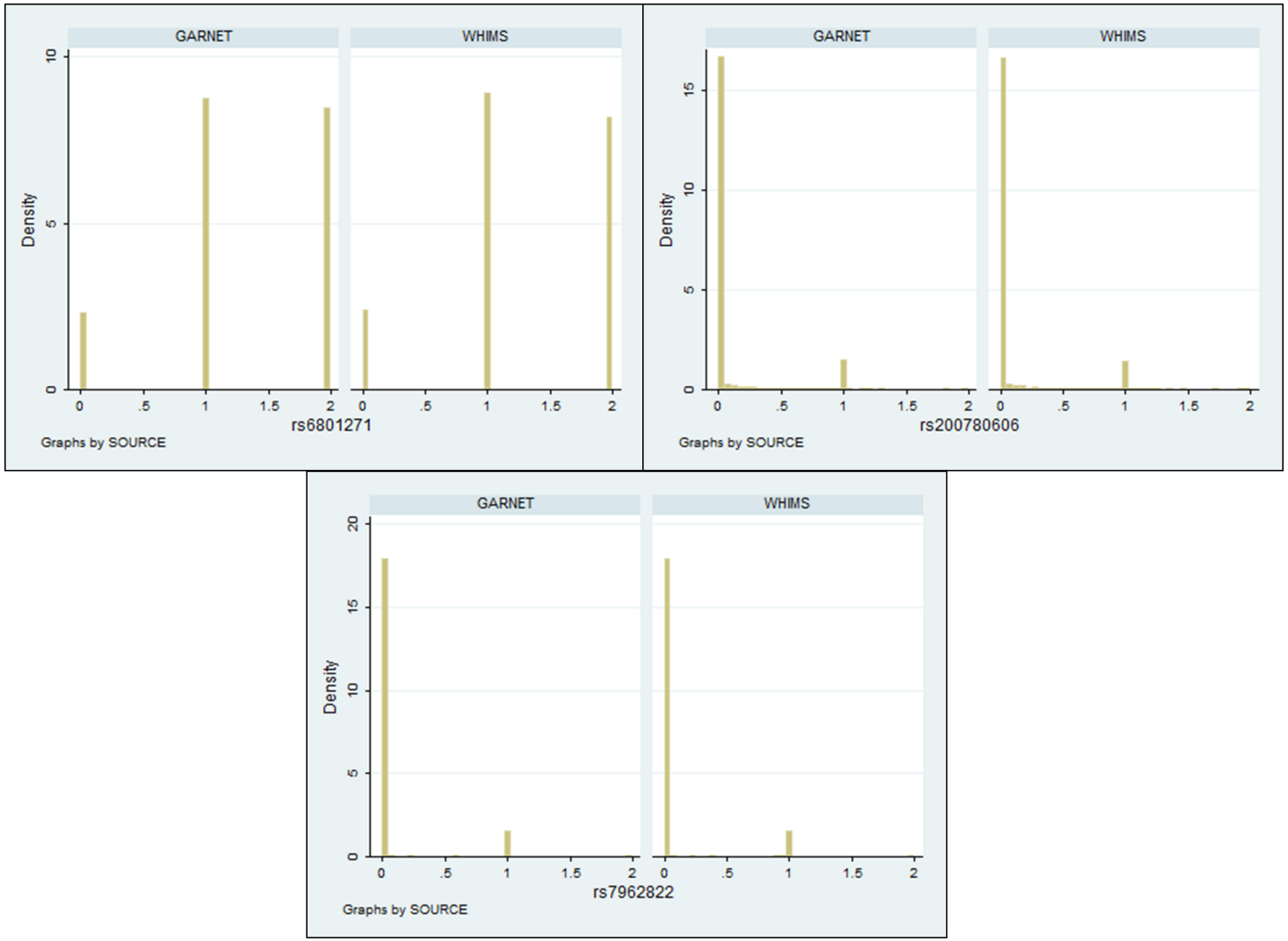


Figure 5-8: Dataset specific histograms of top seven independent SNPs identified in parity-SNP interaction analyses



Modeling assumptions

All models were tested using a log-additive assumption for the genotypes as this has shown to be a robust assumption when conducting a large number of *a priori* tests. In these primary models we used BMI and parity as continuous terms after checking for the assumption of linearity in the logit. It is important to assess this assumption prior to modeling a continuous variable in a regression framework, since assuming the presence of a linear relationship when it is not true can lead to biased effect-estimates and potentially spurious associations when the assumption is grossly violated. One way to assess this assumption is to break the continuous variable into quartiles and assess if the association between the variable of interest and disease is monotonic, that is if it progressively increases or decreases with every unit increase in quartile. The absence of a monotonic relationship would indicate that the variable should not be modeled as a continuous variable. To assess linearity in the logit for BMI, the variable was first broken down into quartiles, which were then assessed for association with POP as dummy variables (lowest quartile served as the reference group). Linearity in the logit for BMI was also assessed according to the world health organization (WHO) categorization of obesity (BMI <25 kg/m^2 serving as the referent category, BMI ≥ 25 – <30 kg/m^2, and BMI ≥30 kg/m^2). Linearity in the logit for parity, which only had a range from 0 to 5, was assessed as dummy variables (4 dummy variables for 5 categories) to produce 5 logits. No gross violations of this assumption were noted.

Treatment of confounding

In epidemiology, confounding is defined as the phenomenon that occurs when an extraneous factor that is not an intermediate in the association between an exposure and outcome, is independently a cause of both the exposure and also independently a cause of the outcome. Failure to account for this extraneous factor may lead to the observance of a spurious

association between exposure and outcome in one extreme, or the observance of a lack of association between exposure and outcome in the other extreme when a true association in fact exists.

In the investigation of genetic factors in a case-control design, only a few scenarios may qualify under this traditional definition of confounding. In most investigations the presence of genetic markers precedes any other extraneous factor, and if the markers are indeed associated with the extraneous factor, then the factor would be considered to be an intermediate in the pathway between the genetic marker and outcome of interest.

Population stratification occurs due to the presence of systematic differences in allele frequencies between populations with disease prevalence disparity [129;130]. Population stratification is of concern to studies evaluating the association between allele frequency and disease when the systematic difference in genetic markers is also correlated with another environmental variable and the different sub-populations that are being examined as one population has different disease rates [131-133]. An association analysis that has not taken population stratification into consideration may spuriously come to the conclusion that a given gene marker is associated with disease, when in fact this is not the case [130]. For example, let us consider a study sample that consists of two sub-populations: sub-populations A and B. Sub-population A may have higher rates of disease Y, higher rates of exposure to an unknown causal environmental variable and may have higher frequency of allele Z that is not causal for disease Y. Allele Z and the environmental exposure are highly correlated. Analysis of this sample of individuals may spuriously reveal an association between allele Z and disease Y simply by the virtue that that subpopulation A has higher rates of disease Y and allele Z and it is correlated with the causal environmental exposure.

Similarly, confounding can also occur in analysis of admixed populations in case-control framework. Admixture is the result of gene flow between historically isolated populations that have been separated for long periods of time [134]. Gene flow between these historically isolated populations leads to offspring with chromosomal admixture with extended blocks of chromosomes being contributed from one ancestral population or the other. Over a period of generations, recombination events and further gene flow will lead to shorter blocks of chromosomal blocks originating from one ancestry or another. The length of ancestry segments is a function of time since the initial admixture, and the extent of gene flow. For example, modern African Americans represent a heterogeneous pool of individuals that may have varying degrees of African ancestry and varying degrees of European ancestry. On average, approximately 80% of the chromosomal segments in modern African Americans are of African origin, and 20% of European origin. The degree of admixture in any given African American could theoretically range between 1% and 99%. Similarly, the population termed as Hispanic also represents a heterogeneous group of individuals who have varying degrees of Native American, Caucasian, and African Ancestry. Since gene regions are often inherited in long fragments, in such admixed populations a spurious association between a marker that is not causal and the phenotype of interest may arise simply by the presence of excess ancestry from a given ancestral population in cases versus controls [129;135].

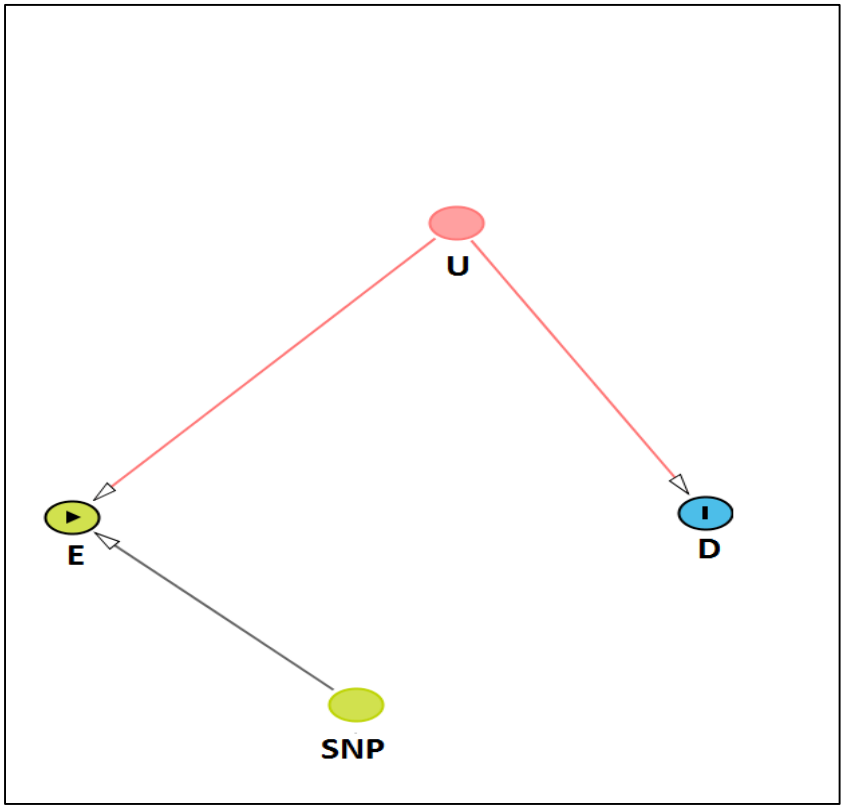
Population stratification can either be assessed by performing stratum specific analyses by strata of ancestral population, or by strata of percentage ancestry (in the case of admixed populations). If the SNP to disease association is persistent across the different strata, then this is more convincing evidence for a causal association, assuming all other confounders have been adjusted for. An additional method of dealing with confounding by population stratification is to

compute variables using genetic data that effectively capture the systematic genetic differences. These variables can then be used to adjust for confounding due to ancestry in association analyses; a technique that has been shown to be successful [136].

The relative degree of admixture in each individual was assessed using multi-dimensional scaling in the PLINK software package [114]. We first LD-pruned the datasets using 80 SNPs per-window, 15 SNPs per shift, and a r-squared threshold of 0.15 between SNPs to obtain a random sample of independent autosomal SNPs (ranging from 40,000 to 60,000 SNPs) with MAF > 2%. These SNPs were then used to generate multidimensional scaling (MDS) components for each sample. Plots of MDS components from the sample datasets were produced along with the European American, African American, African and Hispanic International HapMap Project Phase 3 populations (CEU: Utah residents with Northern and Western European ancestry; ASW: African ancestry in Southwest USA; MXL: Mexican ancestry from Los Angeles, CA; LWK: Luhya in Webuye, Kenya; MKK: Maasai in Kinyawa, Kenya; YRI: Yoruban in Ibadan, Nigeria) to visualize racial genetic differences. Four MDS components were generated for the African American and Hispanic samples, and two MDS components were generated for the European American samples. These components were then used as covariates in single SNP association analyses to adjust for confounding due to ancestry in the African American and Hispanic samples. Adjustment for these four MDS components should be more than sufficient in controlling for confounding due to admixture in African American and Hispanic populations, since the first two MDS components generally capture majority of geographic variation in genetic ancestry.

Treatment of confounding in analyses that evaluate gene-environment/SNP-environment interactions is more complicated than treatment of confounding in an analysis that evaluates main effects [137;138]. Collider stratification is one way through which associations with gene-environment interactions may be confounded (Figure 5-9). Let us consider a scenario where the SNP effect (S) is associated with the main exposure of interest (E). However, S and E are not marginally associated with disease D, and there is an unmeasured confounder U that is associated with E and D. In this scenario, where all variables are considered to be binary, and where S causes E and U causes E, conditioning on E, which is the common effect for S and U will lead to a situation described as collider stratification. In a logistic model where we input a term for E, a term for S, and an interaction term for SxE, S and D will be conditionally associated within at least one stratum of E [138]. When U is not adjusted for, this model would imply that (at least) the coefficient for the SNP term or the coefficient for the interaction term should be non-zero, even though there is no actual relationship. The implication of not adjusting for U would be irrelevant in this scenario only if G and E are not associated with each other as well, in which case, the association between G and D would be null across all strata of E. These scenarios may not apply to our investigation of interest as we know that our exposures, BMI and parity are all marginally and conditionally associated with POP.

Figure 5-9. Directed acyclic graph of hypothetical collider stratification



E = Exposure; D = Disease; SNP = Polymorphism/gene of interest; U = Unknown Confounder

In a scenario where SNPs are associated with our exposures, the exposures are associated with disease, and the SNPs are also independently associated with the disease of interest, the detection of a statistically significant interaction could be due to the presence of uncontrolled confounding (U) between exposures and POP. When U is not controlled for, the effect of E on D is biased and this bias will also be reflected on the SxE parameter. This scenario is depicted using direct acyclic graphs for BMI and parity in Figures 5-10 A-B. VanderWeele and colleagues conducted simulation studies with various scenarios where they show that in the presence of uncontrolled confounding U, and simultaneous absence of UxE interaction or UxS interaction, the type I error for the SxE interaction term is inflated only in the presence of SxE

interaction and extreme uncontrolled confounding between E and D (confounding OR of 5.0) [138]. With the exception of parity, which is highly associated with POP (OR as high as 10.0), the literature does not identify any other risk factor with magnitudes that are this high. Additionally, if there was indeed an unmeasured factor that has such a large association with POP, it would likely have been detected.

In a scenario where the SNP is not associated with the exposure of interest, (BMI or parity), but is independently associated with POP, if confounders between the association between exposure and disease are not adjusted for, although the SxE term would not necessarily be biased, the main-effect of exposure and disease would certainly be biased. This would most likely be seen as biased stratum specific estimates (even though the ratio of the two stratum specific odds ratios may not be biased). This scenario of independence between SNP and exposure using depicted as directed acyclic graphs for BMI and parity in Figures 5-11 A-B, respectively. Since we are testing a large number of SNPs it is not always possible to know if the scenario of independence or non-independence between SNP and exposure holds. Therefore, in our models assessing BMI x SNP interaction, we adjust for age, parity, and continuous axes of ancestry. In our models assessing parity x SNP interaction, we adjust for age BMI and continuous axes of ancestry.

Figure 5-10. Directed acyclic graphs showing hypothetical scenarios for BMI (A) and parity (B) where SNP-exposure relationship is non-independent

a b

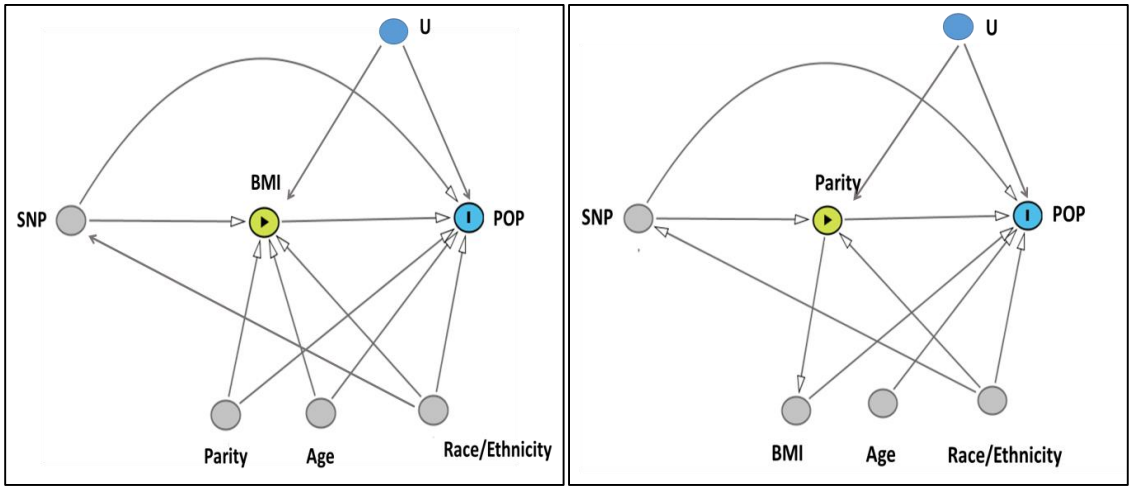
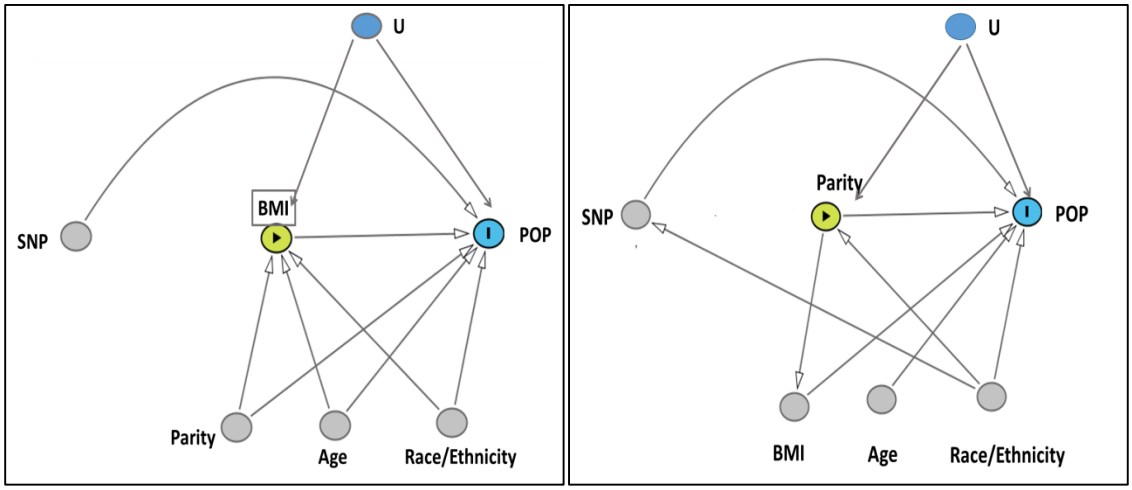


Figure 5-11. Directed acyclic graphs showing hypothetical scenarios for BMI (A) and parity (B) where SNP-exposure relationship is independent

a b



Multiple testing

We tested 33,515 SNPs from 96 genes for two exposures of interest (BMI and parity), leading to a total of 67,030 tests. Since multiple formal comparisons were made, it is no longer valid to assume that the family-wise error rate for these tests is 0.05, as this is the threshold for significance for conducting one independent test. It is necessary to now control for the family wise error rate, that is, the probability of making one or more type I errors among all of the hypotheses tested. One simple method of correcting for the multiple tests would be to perform a Bonferonni correction, where the new threshold for significance would be 0.05/67,030. However, the Bonferonni approach would be an extremely conservative approach in this situation, since there is most likely a considerable amount of correlation between the SNPs we tested, especially given that we tested imputed SNPs in gene regions. In a scenario such as ours, one approach of correcting for the familywise error rate would be to perform a permutation procedure. A permutation procedure is conducted by randomly shuffling phenotype status to generate a large number of phenotype sets. The maximum test statistic or the minimum p-value across all tests conducted for each permuted set is then extracted. For example, if we performed 10,000 permutations, we would end up with 10,000 maximum T statistics. These test statistics are then ranked in descending order. The test-statistic corresponding to the 5^{th} percentile would then be the new-threshold for declaring statistical significance. The purpose of the permutation procedure is to estimate the true type I error under the null-distribution, that is if there was no association between SNPs tested and phenotype of interest. However, the permutation procedure is computationally intensive. Additionally, in a meta-analysis setting it is not clear how the aggregated test-statistics would be permuted.

An alternate approach is to estimate the effective number of independent tests (Meff) that were conducted in a given dataset in order to estimate the true FWER rate. SimpleM is an approach devised by Gao and colleagues which estimates Meff for genotype data using composite LD structure between SNPs of interest to generate principal components which estimate 99.5% of the variability in the dataset [139]. This procedure has been shown to closely approximate the permutation method (although slightly more conservative) and is highly efficient computationally [139].

Using SimpleM we estimated the effective number of tests for each of the four datasets for the 33,515 tests we conducted (Table 5-7). The WHI-SHARe (African American) dataset had the largest Meff of 11,478. Taking a conservative approach we then assumed the WHI-SHARe (African American) Meff to be the effective number of tests to be used for each of the interaction analysis. Since we investigated interactions with BMI and parity, we estimated the total number of effective tests for this study to be 22,956. We divided 0.05 by this number to obtain the new threshold of significance for this study to be 2.18×10^{-6}. P-values of 2.18×10^{-4} or less were considered suggestive signals.

Table 5-7. Dataset-specific estimates for M_{eff}

Dataset	M_{eff}
WHI-MS (European American)	6,392
WHI-GARNET (European American	6,386
WHI-SHARe (African American)	11,478
WHI-SHARe (Hispanic)	8,470

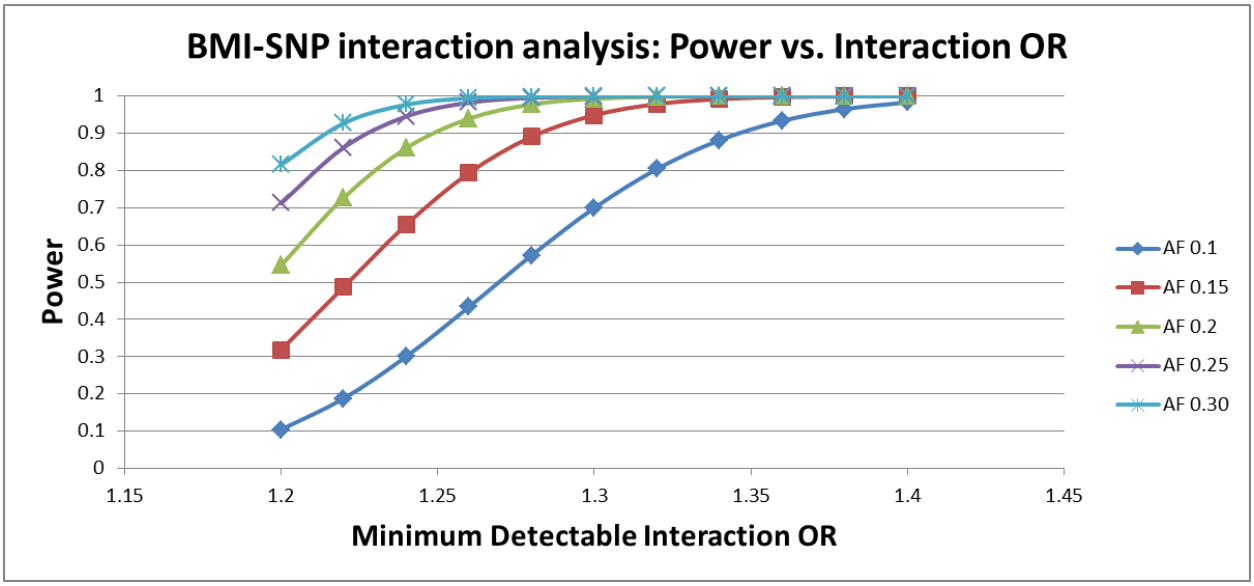
M_{eff} = effective number of independent tests

Power

All *a priori* power calculations were conducted using QUANTO [140]. We first calculated the power to detect gene-environment interactions for European American, African American and Hispanic populations separately, followed by a pooled power analysis to reflect power with meta-analysis. Assuming 80% power, a p-value threshold of 1×10^{-4}, MAF of 30%, odds ratio per unit increase in BMI of 1.03 and SNP main effect of 1.20, we will have the ability to detect an interaction odds ratio of 1.44 or higher for the African American population (806 cases; 963 controls), and an interaction odds ratio of $\geq$1.76 for the Hispanic population (621 cases; 311 controls). For European American, conservatively estimating 50% of European American participants have prolapse (including prevalent and incident cases), and assuming 80% power, p-value threshold of 5×10^{-8}, MAF of 30%, odds ratio per unit increase in BMI of 1.03 and SNP main effect of 1.20, we will have the ability to detect an interaction odds ratio of 1.22 or higher. It is clear that the African American and Hispanic samples alone do not have sufficient power to detect associations for 1,000 or more independent markers. However, a meta-analysis of all three populations would provide us with sufficient power to detect small to moderate associations at the genome-wide significance level. For our meta-analysis, assuming a p-value threshold of 5×10^{-8}, MAF of 30%, odds ratio per unit increase in BMI of 1.03 and SNP main effect of 1.20 we expect to have 80% to detect an interaction odds ratio of 1.19 or higher. Similarly for our meta-analysis for parity-SNP interaction terms, assuming a p-value threshold of 5×10^{-8}, MAF of 30%, and a per child birth odds ratio of 1.5 (used as a discrete variable), and SNP main effect of 1.20, we expect to have 80% power to detect an interaction odds ratio of 1.22 or higher. Graphical representation of *a priori* power calculations showing power vs. minimum

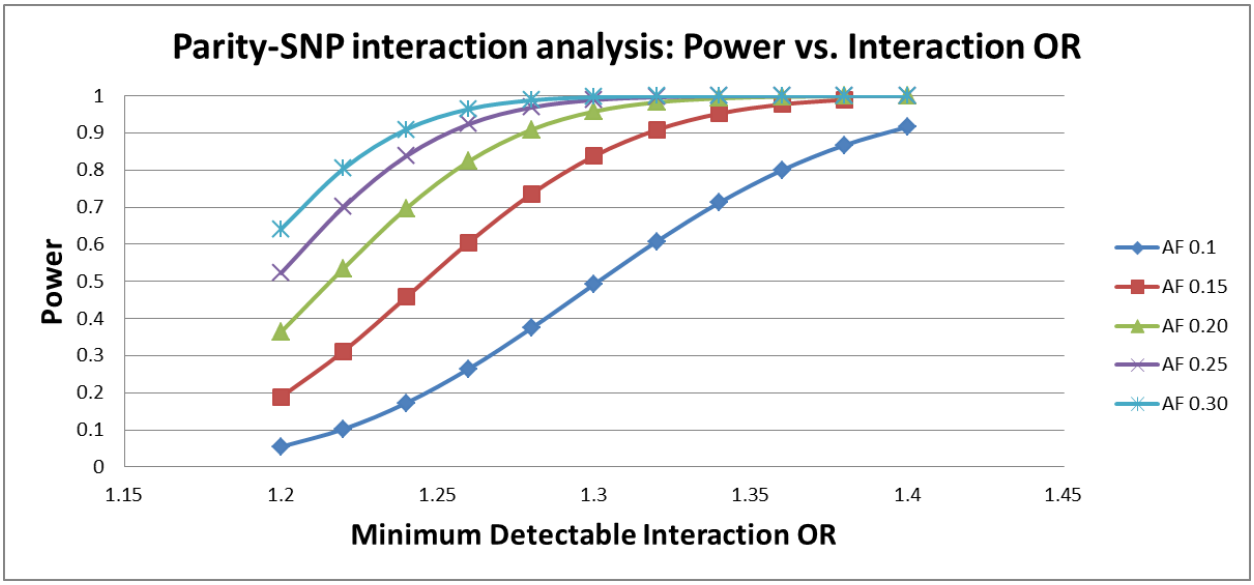
detectable odds ratio for various allele frequencies are shown in Figure 5-12 for BMI and Figure 5-13 for parity.

Figure 5-12. Power curves for BMI-SNP interactions



AF = allele frequency

Figure 5-13. Power curves for parity-SNP interactions



AF = allele frequency

Methods for Specific Aim 3: To evaluate the relationship between individual ancestry proportion (global ancestry) and local ancestry (admixture mapping) in relation to pelvic organ prolapse in African American women.

Hypothesis: The WHI-HT study and other studies have reported lower prevalence of POP in African American women compared with European American women. We hypothesize that biological differences represented by genetic polymorphisms may in part explain this discrepancy. Specifically, African American women with higher levels of European ancestry on average will have higher odds of having POP. Additionally, African or European specific local ancestry estimates will be associated with POP.

Overview of admixture and admixture mapping

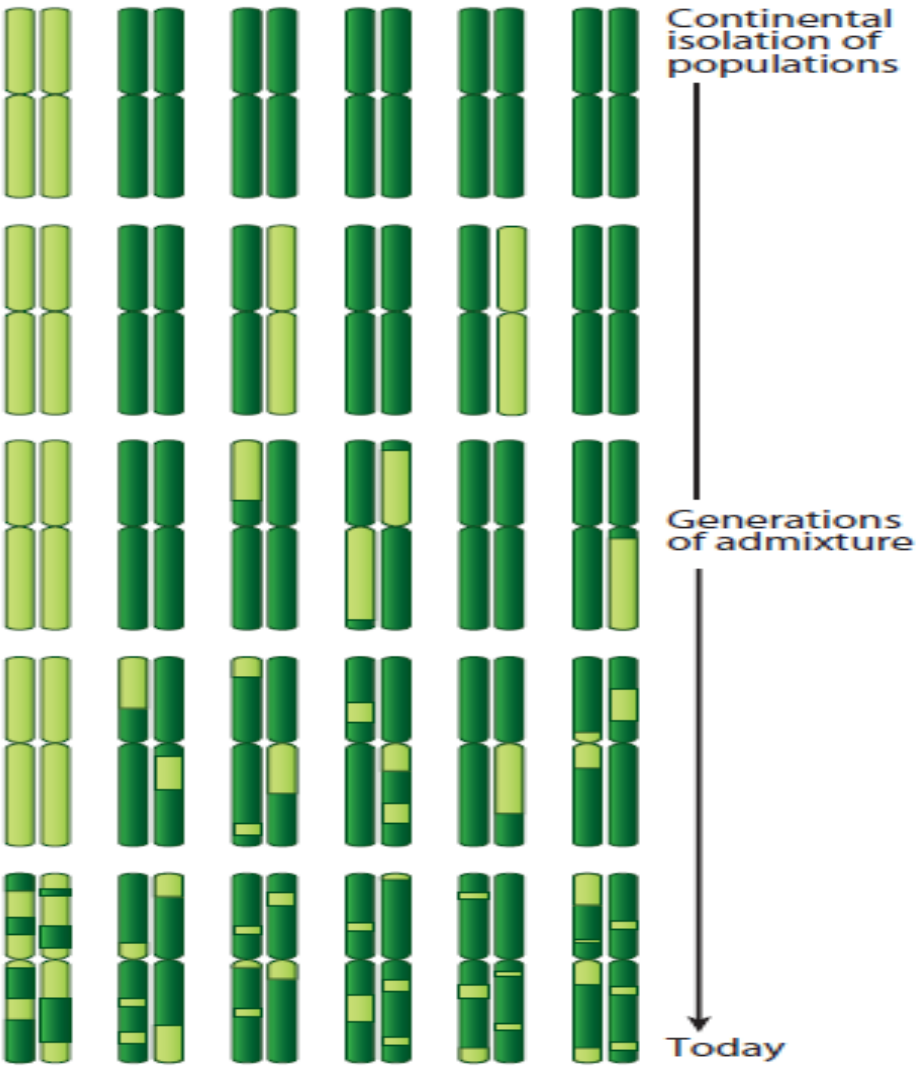
Migrations from Africa 60-80 thousand years ago led to the existence of several geographically isolated populations that have distinct phenotypic and genotypic features. Admixture is simply a phenomenon in which reproductively active individuals from historically and geographically isolated populations interbreed to form offspring that have a mixture of genetic material that are distinct and unique to the historically isolated populations [134;141]. The modern African American population is a result of such a process for which recent gene flow from the African continent to the Americas started approximately 400 to 500 years ago.

From a biological/genetic perspective admixture can be described in the following manner. Two populations (A and B), both of the same species have genetic segments that are mostly shared across both populations. Geographic isolation, bottleneck effects, varying effective population sizes for founders, genetic drift, and selective pressures due to environmental changes have elicited several distinct genetic features that are unique to each of the sub-populations over time. Thus individuals from each sub-population have unique but predictable haplotype signatures (sequences of DNA that allow us to clearly delineate between

the two populations) [134;141]. The longer the isolation time for a population, the more likely it is that the haplotype frequency distribution for one population is unique and distinguishable from another population. When an individual from population A mates with an individual from population B, each individual contributes its own unique chromosome to its offspring. Thus, in the first generation of interbreeding, the offspring has 50% of its whole chromosomes that came from population A and the 50% that came from population B. During meiosis, segments of chromosomes have the tendency to cross over, which in an admixed individual would now create gametes with chromosomes that are a mosaic of genetic segments from populations A and B. After each additional generation of interbreeding, the offspring of admixed populations will have chromosomes with ancestral segments that are increasingly fragmented [134]. A schema of chromosomal admixture and how ancestry specific chromosomal blocks get shortened over time is depicted in Figure 5-14.

Evidence suggests that historically isolated populations not only have these subtle yet important genetic differences, but that the prevalence of some diseases in these populations is dramatically different. For example, European Americans have higher rates of multiple sclerosis and several other autoimmune disorders than those with African ancestry. Africans have been reported to have higher rates of fibro-proliferative diseases, cardiovascular disease, and obesity than those with European ancestry. It is possible that subtle genetic differences attributed to ancestry may in part be causally associated with these disease traits.

Figure 5-14. Schematic representation of chromosomal admixture of historically isolated populations



Reprinted from Winkler et al. 2010 [134]

Admixture mapping

Admixture mapping of disease is a procedure that utilizes the premise that disease prevalence rates vary in ancestral populations and leverages our ability to detect/estimate haplotype switches in chromosomal regions that belong to ancestral populations in admixed populations [134;141;142]. The basic hypothesis behind an admixture mapping study is that

ancestry-specific genetic segments that are causally associated with the disease of interest (higher frequency in a given ancestral population) will be found in higher proportion in admixed individuals who have the disease of interest than in admixed individuals who do not have the disease. However, this considers a simplistic scenario where only one loci is causally associated with disease. For complex diseases, such as POP, where individuals from both African and European ancestry have the condition, but the prevalence is higher in European women than African American women, it is also possible that multiple loci are specific to African or European women may increase POP risk. Therefore, as long as allele-frequency differences exist for causal loci between ancestral populations, it is possible to detect these causal alleles by utilizing admixed populations regardless of the direction of excess ancestry. For complex polygenic-traits it should also be appreciated that any risk differential by ancestry could be due to combination of environmental and genetic differences. Therefore, for complex traits, the presence of risk differential by ancestry does not guarantee the success of an admixture mapping study or even the direction of association between ancestry and disease in the event there is an association [141]. Conversely, the absence of risk differential by ancestry does not guarantee that admixture mapping will fail, since genetic factors causally associated with ancestry could exist but be mitigated/masked by environmental factors [141].

An admixture mapping procedure can be applied in a case-only framework and a case-control study framework, as depicted in Figure 5-15. A case-only framework compares the difference between the proportions of ancestry A, at a given marker (m_1) to the average proportion of ancestry A across all other unlinked markers throughout the genome in cases. This can then be converted into a z-score by dividing the difference with the variance of proportion of ancestry A across the genome:

$$Z_A(m_1) = \frac{p_A(m_1) - \frac{\sum p_A(m_i)}{m_i}}{sqrt(\sigma_A(p_A(m_i))}$$

A case-control admixture mapping framework, compares the proportion of cases with a specific ancestry A at a marker (m_1) with the proportion of controls with a specific ancestry A at the same marker (m_1). Mathematically, this too can be converted into a score statistic:

$$Z_A(m_1) = \frac{p_A(m_{1cases}) - p_A(m_{1controls})}{sqrt(q_1 q_2 (m_{1cases} + m_{1controls}/2m_{1cases}m_{1controls})}$$

Where $p_A\left(m_{1cases(controls)}\right)$ = the proportion of cases (controls) with ancestry A at marker 1, where q_1 = the proportion of ancestry A across the genome and proportion of ancestry B across the genome q_2 is (1- q_1).

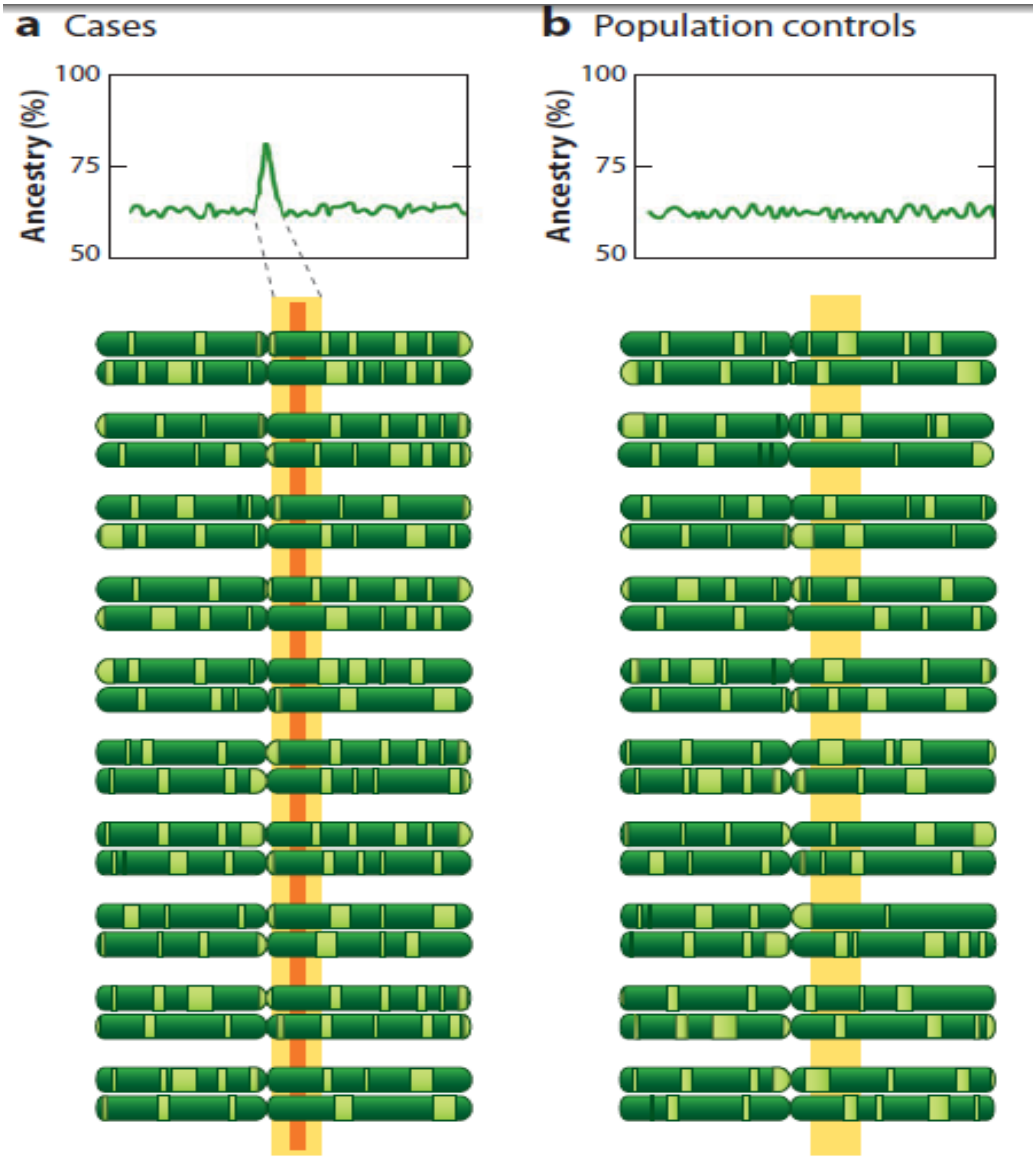
Genetic variation between ancestral populations is usually measured through SNPs which represent the most abundant type of genetic variation in human DNA. In extreme scenarios, 100% of a variant may be present in one ancestry where as 0% of a variant may be present in another ancestry. Such is the case with the null duffy antigen, which is present in 100% of the west-African population but not present at all in the European population [143]. The search for causal SNPs that are fixed for alternate alleles in two ancestral populations would be impossible to detect in studies that evaluate the associations in the two populations separately. An admixed population on the other hand will be likely to include both versions of the variant and would therefore allow for detection of the variant.

Ancestry at a given locus cannot be observed in humans and therefore must be inferred through probabilistic methods. Identification of chromosomal haplotypes which are specific to ancestry allows for assigning ancestry at individual SNPs, which must originate from one or the other ancestral population, assuming a two-way admixture model. An African American individual can then have either 0, 1 or 2 copies of a given allele originating from either European or African ancestry. In general the following pieces of information are necessary or aid in inference of local ancestry: estimate of time since original admixture event (number of generations), an estimate of the recombination rate, average estimate of global ancestry (population average of ancestry proportion) and estimates of ancestry-specific allele frequencies or phased data on ancestral-proxy populations. Several complex locus-ancestry estimation methods have been formulated in the recent past, details of which are beyond the scope of this study. Briefly, some methods such as ANCESTRYMAP, ADMIXMAP and MALDSOFT utilize hidden markov models (HMM) to combine data across highly differentiated independent markers in ancestral populations to infer ancestry at each locus. However, these programs are not equipped to handle densely mapped panels of markers throughout the genome and are not computationally efficient. Other software programs such as HAPMIX use the HMM framework to model ancestral linkage disequilibrium from haplotype structures using phased ancestral population data. These methods of inferring local ancestry have been reviewed in depth by Seldin and colleagues [144] and Smith and colleagues [145]. For this aim we will use Local Ancestry in admixed Populations (LAMP) to infer local ancestry [146]. LAMP uses a computationally efficient approach to infer local ancestry. Briefly, it uses a sliding window of contiguous SNPs with low correlation to infer ancestry at each locus, and then uses a majority voting system between overlapping windows to finally assign ancestry at each locus [146]. An

additional advantage of LAMP is that it does not require data (haplotypes or allele-frequencies) for ancestral populations in order to assign local ancestry in the admixed population. However, in the event these data are available, LAMP is able to use allele-frequency estimates from these ancestral-proxy populations to provide an even more accurate inference of local ancestry in the admixed population. Another advantage of LAMP is that it is able to handle dense panels of SNPs such as the Affymetrix 6.0 in a computationally efficient manner. Intuitively, the larger the numbers of SNPs that are utilized, the more number of overlapping windows are used, which in turn provides a more accurate estimate when using the majority vote process.

Once local ancestry calls are made, this output can then be used to compute a global ancestry variable per admixed individual, which denotes the percentage of SNPs that belong to a given ancestry for that individual. Then the global ancestry or local ancestry variables can be used in a generalized-linear regression model framework of choice to model the association between global or local ancestry with disease/trait of interest.

Figure 5-15. Heuristic representation of admixture mapping

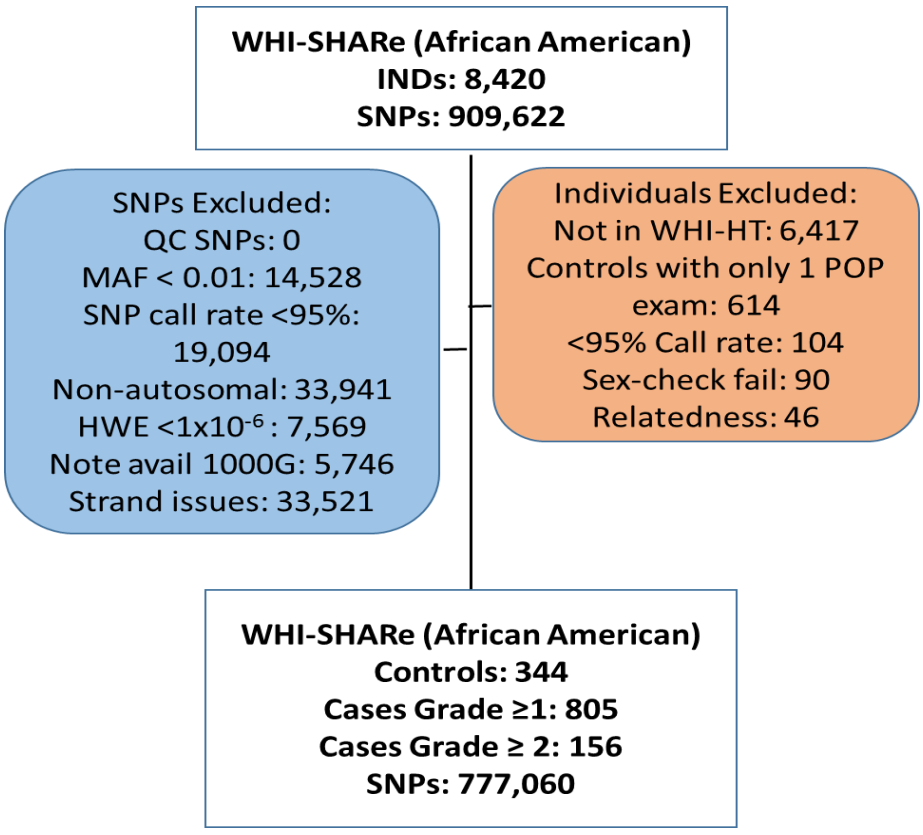


Reprinted from Winkler et al. 2010 [134]

Case-control selection and QC for genetic data

Case control selection strategy and QC for genetic data have been described previously in Chapter V sub-section Parent Study for Aims 2 and 3. Only "stringent controls" were utilized for this aim as this method was shown to provide the least amount of misclassification. Flow-chart specific to this study is shown below in Figure 5-16

Figure 5-16. QC flow chart for African American cases and controls



Statistical analysis

Global and local ancestry inference

We used the LAMP software to estimate local and global ancestry [146]. The modern African American population is composed of varying degrees of genetic contributions from two broadly historically isolated ancestral populations: Africans and Europeans [147;148]. We used the 1000 genomes African (AFR) and European (EUR) allele frequencies as proxies for estimates of ancestral allele frequencies [115]. The allele-frequencies for the European reference population were calculated from individuals originating from the following European populations: British in England and Scotland (GBR: N= 94), Finnish in England (FIN: N=100), Toscani in Italy (TSI: N=110), Iberian Population in Spain (IBS: N=107) and Utah residents with Northern and Western European Ancestry (CEU: N=103). Similarly, allele-frequencies for the African reference population were calculated from individuals originating from the following African populations: African ancestry in Southwest US (ASW: N=66), Luhya in Webuye, Kenya (LWK: N=116), and Yoruba in Idadan, Nigeria (YRI: N=116). Using a total of 777,060 markers for all cases (805 any POP) and 344 controls, we ran LAMP-ANC assuming the following parameter configurations: fraction of overlap between windows (offset=0.2), number of generations since admixture (g=7), recombination rate ($1x10^{-8}$), average African to European admixture ratio (0.8:0.2), and LD-pruning cut-off (R^2=0.1). After local-ancestry estimates were inferred, ancestry estimates across all SNPs were averaged for each individual to obtain proportion of average African ancestry (% global ancestry) per-sample. In this case we will use % European ancestry as the exposure of interest. Since the premise of association testing with local-ancestry estimates is based on the assumption that there are marked differences in ancestry-specific allele frequencies in cases versus controls, we extracted local-ancestry estimates for SNPs which were highly differentiated in the ancestral populations. Specifically, we extracted

local-ancestry estimates for SNPs for which the absolute value for the allele frequency difference (delta) between the EUR and the AFR 1000 genomes reference populations was 0.4 or more. There were a total of 39,546 SNPs in our dataset for which the absolute value of delta was 0.4 or higher.

Associations with global and local ancestry

We used multivariable logistic regression to evaluate the association between estimated global ancestry (% European) and any POP and the association between global ancestry and moderate/severe POP, while adjusting for age at POP ascertainment (continuous), parity (continuous), and BMI (continuous).

The association between local ancestry and POP (any POP and moderate/severe POP) was tested in a case-only and a case-control design for comparison purposes. Briefly, a case-only design only utilizes the case-population in a study and compares the deviation in the frequency of ancestry at each marker compared with the genome-wide average in cases. We computed a Z-statistic for each loci and calculated two-sided p-values to represent case-only admixture mapping peaks. The case-only design makes the assumption that under the null hypothesis there are no systematic deviations in ancestry frequency across cases and controls. Under this assumption adding controls to the mix would only contribute to extra noise to the detected signal. This is a rather stringent assumption to make and so deviation in ancestry frequency in any loci compared with the genome average will appear as a signal. However, in the scenario where both cases and controls may have deviations in ancestry which are also in the same direction compared with genome-wide averages, there is the possibility of false positive results. We then also conducted case-control admixture mapping analyses in a logistic regression framework using PLINK, where we modeled POP (any POP and moderate/severe POP) with local ancestry

(0, 1 or 2 copies of European Ancestry in an additive model) while adjusting for age at ascertainment (continuous), parity (continuous), BMI (continuous) and four multi-dimensional scaling (MDS) components representing continuous axes of genetic ancestry. To determine the threshold for statistical significance after accounting for multiple testing, we conducted 10,000 permutations to approximate the null distribution. The p-value for statistical significance was established at 1.82×10^{-5}.

The resolution of admixture mapping studies is highly dependent on the number of markers utilized during ancestry estimation and also during association testing. Association testing of local ancestry estimates in recently admixed populations is only helpful in locating broad genetic regions in the genome which could potentially harbor causal SNPs associated with disease. In general, since a given marker is representative of an ancestry call for a specific genetic segment due to the presence of long-range (ancestral) LD, admixture mapping alone is not sufficient to hone in on causal variants which may be associated with disease [141]. We juxtaposed signals from case-only and case-control analyses to evaluate overlapping signals between two methods of assessment. Regions from admixture mapping analyses which showed overlaps between case-only and case-control methods with peak p-values $<5 \times 10^{-4}$ (suggestive peaks) were probed for further investigation. Genotype data for broad regions (10 to 20 mega-base pair regions) below these peaks were imputed using the 1000 genomes cosmopolitan reference panels using IMPUTE2 [117]. We then evaluated the association between imputed SNPs and POP (any POP and moderate/severe POP) using multivariable logistic regression adjusting for age at ascertainment, BMI, parity and the four MDS components to representing continuous axes of genetic ancestry. Then, to evaluate if any of the SNPs investigated in the regions of interest explained the broad admixture mapping peaks, we performed logistic

regression between local ancestry and POP with and without adjustment for the most-statistically significant SNPs in the region while also adjusting for the aforementioned covariates.

Multiple testing

In our analyses we tested a total of 39,546 local ancestry estimates in relation to POP. Although these SNPs have minimal short-range LD, they have extensive long-range (ancestral) LD due to haplotype structures originating from European or African ancestry. This results in long stretches of ancestry calls which are of either African or European origin before a recombination switch occurs. Consequently, a Bonferroni correction to correct for the family-wise error rate is too conservative since it assumes that the multiple tests being conducted are independent, which is not the case with admixture mapping. Therefore, to estimate the null distribution, we conducted 10,000 permutations in 20 parallel sets of 500 permutations per set using the max(T) permutation procedure in PLINK for analyses evaluating moderate/severe POP and controls, which had the strongest signals. The maximum chi-squared statistic across local ancestry calls for each of the 10,000 permuted replicates were extracted and sorted in descending order. The test statistic at the 500^{th} position (5% mark across 10,000 replicates) was considered the threshold for statistical significance according to the null distribution. The p-value for statistical significance was established at 1.82×10^{-5}.

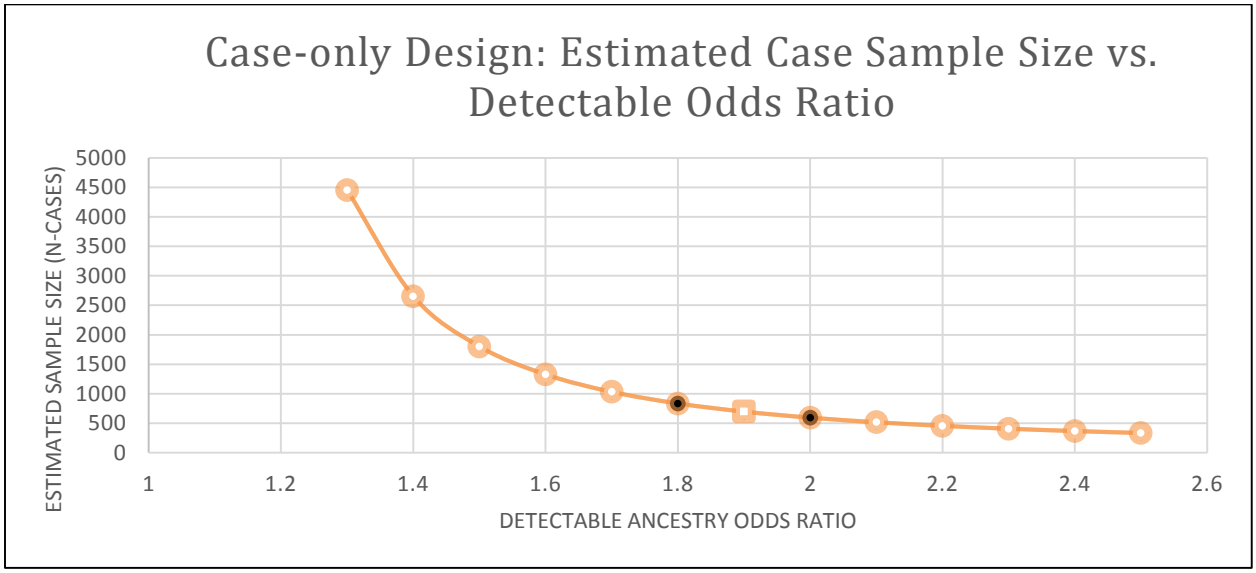
Power

A priori, we estimated a conservative sample size required for a case-only design for the African American population. Assuming an average admixture proportion of 20% European ancestry [147-149], a two sided p-value of $5x10^{-5}$ (approximately 1,000 effective tests), detecting an ancestry odds ratio of 1.8 with 80% power would require approximately 800 cases. By only testing independent markers that are informative of ancestry (delta $\geq$0.4), the number of markers that are tested in admixture mapping is reduced by a magnitude of >19 fold (from 777,060 to 39,546 markers). This significantly decreases the number of independent tests conducted and hence also decreases the significance threshold. The effective number of tests which are based on local ancestry calls is even lower due to admixture linkage disequilibrium (large chunks of chromosomes belonging to a specific ancestry that are jointly inherited) and correcting for the number of markers used in the admixture mapping procedures (39,546 markers in this case) would be highly conservative [150]. From our laboratory's experience doing admixture mapping studies of uterine fibroids in BioVU, we had previously used permutation testing to estimate the effective number of independent tests in African American admixture mapping studies to be approximately 1,000 tests. This was what was used to conduct *a priori* power calculations.

Sample size requirements, as estimated using techniques detailed by Hoggart and colleagues [150;151], for a wider range of effect estimates are provided (Figure 5-17). In a study using simulated data, Patterson and colleagues demonstrated that for any given effect size, power is similar for populations with admixture proportions ranging from 10 to 90% [150;152]. Previous studies of admixture mapping for other traits have been successful in detecting signals using smaller numbers of individuals than those being used in our study [150;153]. As the first

admixture mapping study for POP, our goal is to provide exploratory results which may then be used to design future studies to replicate our findings.

Figure 5-17. Sample size estimation as a function of minimum-detectable ancestry odds ratio for case-only admixture mapping



Black dots: Expected range of detectable associations considering sample size

CHAPTER VI

Findings for Specific Aim 1: Systematic review and meta-analysis on the relationship between obesity and pelvic organ prolapse

Abstract

Background and Motivation: Obesity is most likely one of the few modifiable risk factors for pelvic organ prolapse (POP). Several studies have evaluated the association between body mass index (BMI) and POP, and have reported a wide range of effect estimates ranging from negative and null to positive associations. The objectives for this study were to perform a systematic review and meta-analysis of articles published in the medical literature to: 1) provide a quantitative summary of the direction and magnitude of measures between of obesity and POP, and 2) identify potential factors which may explain heterogeneous findings across studies.

Methods: We searched PubMed for original research articles which evaluated the association between measures of obesity and POP up to June 18, 2015. Eligible studies for meta-analysis were analytical observational studies published in English that reported the risk ratios (relative risk, odds ratio or hazard ratio) for categories of body mass index (overweight and obese) in relation to POP. Inverse variance weighted random-effects models were used to evaluate and report the associations with POP from case-control, cross-sectional and cohort studies for overweight and obese BMI categories compared with women in normal-weight category (BMI < 25 kg/m^2). Visual examination of funnel plots and Egger's test were used to evaluate publication bias. Sub-group analyses were performed to evaluate potential sources of heterogeneity for the following study attributes: method of POP assessment (objectively measured POP of any grade, symptomatic self-reported POP, objectively measured clinically significant POP), whether

studies adjusted for key covariates (no, yes), percent of post-menopausal women in study (<33%, 33%-<67%, and ≥ 67%), whether studies used standard World Health Organization BMI categories (no, yes) and study design (cross-sectional, cohort, and case-control).

Results: A total of 22 eligible studies provided 21 effect estimates for meta-analysis of the overweight BMI category and 14 effect-estimates for meta-analysis of the obese BMI category, both compared with the normal weight category (referent: BMI < 25 kg/m^2). Compared with the referent BMI category, women in the overweight and obese category had meta-analysis risk ratios of at least 1.33 (95% Confidence interval [CI]: 1.16, 1.52; I^2: 63%) and 1.41 (95% CI: 1.24, 1.60; I^2: 39%), respectively. We report some evidence of publication bias in one of our overweight category meta-analysis set. Subgroup analyses showed that eligible case-control studies were more likely to report larger effect estimates than cross-sectional or cohort study designs. Risk ratios for objectively measured clinically significant POP were higher than risk ratios for other measurements of POP, both in the overweight and obese analysis categories. Studies with smaller percent of post-menopausal women tended to report larger effect estimates for overweight and obese categories than studies with larger percentage of post-menopausal women. Only one eligible study included in the meta-analysis performed a prospective assessment of BMI and POP. Only two studies in the literature reported associations between waist-circumference and POP.

Conclusions: In conclusion, our meta-analysis suggests being overweight or obese increased risk for having POP compared with women who had BMI in the normal range. Mechanistic studies and larger prospective investigation of obesity measures in addition to BMI are needed to understand underlying mechanisms and establish causality.

Introduction

In POP, one or more of the intra-pelvic organs including the uterus, bladder, rectum and the urethra descend into the vaginal space, presumably due to deficiencies in the pelvic support system which normally provides sustained support [1;2]. POP is a highly prevalent condition in women with prevalence rates ranging from 10% in younger women up to 50% in post-menopausal women [3-5;22]. While not all women with POP are symptomatic, affected women may experience a range of debilitating symptoms which impair quality of life. These include but are not limited to the feeling of pressure or bulge in the pelvic area, pain, impaired sexual function, and urinary and fecal incontinence [1;6]. It is estimated that one in ten women will undergo surgical correction for POP in their lifetime [34]. The reason for POP is likely multifactorial with a host of complex factors combining to manifest POP including genetic predisposition, weakening of the connective tissue support matrix attributed to aging and childbirth, and factors that exert excessive strain or pressure on the pelvic floor support system such as obesity. Aging and parity have been most consistently associated with POP [12-20;25], however, these factors can hardly be considered modifiable, if at all.

Obesity appears to be a targetable and modifiable risk factor which may be influenced on a population level to reduce the burden of POP, both from a public health and economic viewpoint. Literature identifies obesity as a potential risk factor POP, however studies evaluating the relationship between obesity and POP do not always report consistent conclusions. Effect estimates for POP in obese women (body mass index [BMI] $\geq$ 30 kg/m^2) range from null to a 2.5 fold increase in risk, when compared with women of normal weight [3;5;12-16;18-24;38-46]. In a recent literature review, Vergeldt and colleagues recently evaluated 30 risk factors for POP published in the literature from ten studies and concluded that among other risk factors, BMI

should be considered a risk factor for POP, on the basis that two or more articles reported statistically significant results [25]. While the assessment provided by Vergeldt and colleagues is useful in several ways, the authors did not perform a meta-analysis and we are not aware of any study that quantitatively assesses the strength and consistency of associations between obesity and POP in the literature. A meta-analysis of measures of obesity and its relationship to POP may not only serve to bring the scientific community closer to a consensus on this issue, but also may also help identify reasons for the heterogeneity in effect estimates presented in the literature.

Therefore, the goals of this review are twofold. First, we aim to provide overall effect estimates for POP in relation to various degrees of obesity, as measured by categories of body mass index. We additionally aim to evaluate study level characteristics which may in part help to explain the heterogeneous effect estimates reported by studies examining obesity and POP.

Methods

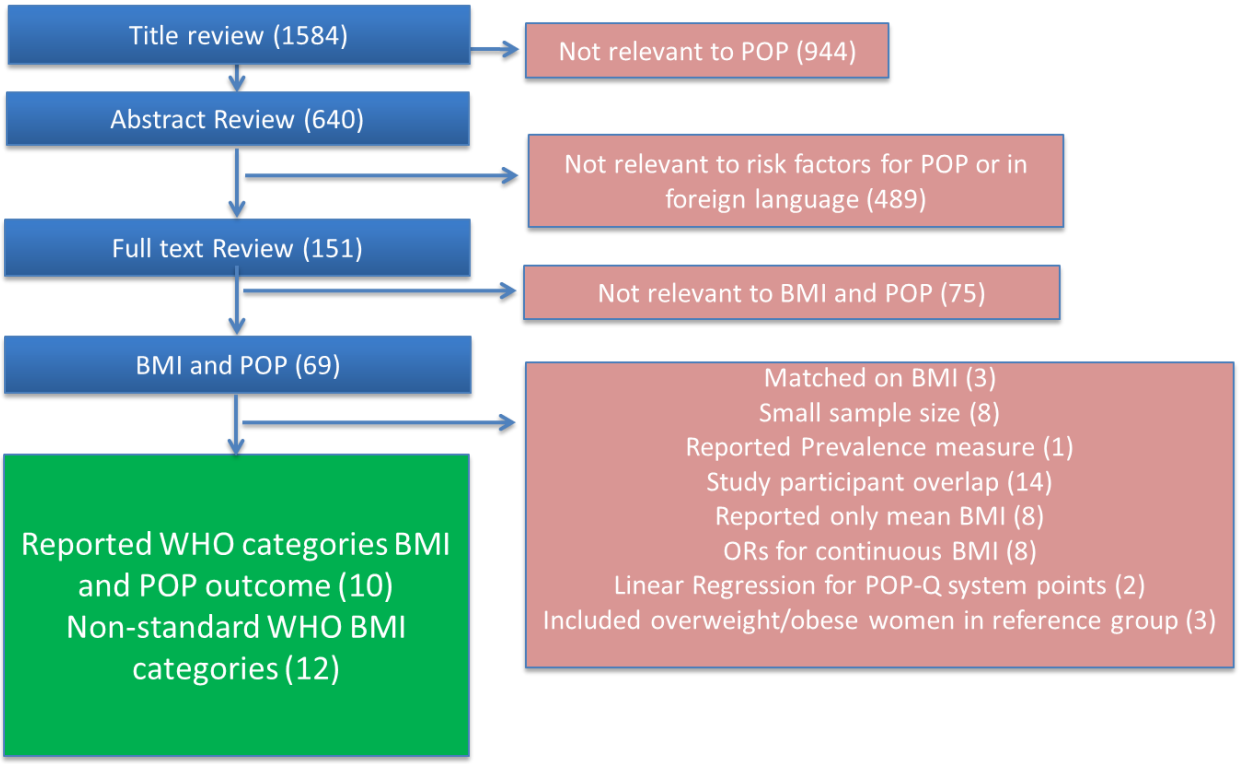
For detailed description of methods utilized for this Specific Aim please revisit Chapter V. Sub-section:

Methods for Specific Aim 1 (page numbers: 65-72)

Results

We identified 69 original-research articles which evaluated BMI and POP. Of these 69 studies, 22 studies with non-overlapping populations that reported risk ratios between categories of BMI and POP or provided numbers which allowed for risk ratio calculation were identified. A flow diagram of the selection process and reasons for ineligibility for primary meta-analysis are presented in Figure 6-1.

Figure 6-1: Flow chart of inclusion/exclusion of studies for meta-analysis



Basic characteristics and a description of abstracted information from the selected studies are listed in Table 6-1. Of the 22 studies which were considered eligible for meta-analysis, 20 studies provided 21 effect estimates from non-overlapping populations which could be utilized in the overweight vs. normal weight meta-analysis set, and 13 studies provided 14 effect estimates from non-overlapping populations which could be utilized in the obese vs. normal weight meta-

analysis set. Of the 22 studies, nine studies were based within a cohort [12;14-16;19-21;23;40], nine studies were cross-sectional [3;24;39;43-45;154-156] and 4 studies were case-control [38;46;85;90], based on the authors' classification of their own study or the reviewer's assessment when authors did not clearly state a study design. However, it is of note that only one study provided a hazard ratio [22], and only one study provided relative risk [16] based on Poisson regression; all other studies either provided odds ratios from logistic regression or ORs were calculated based on raw numbers. A total of 10 out of the 22 studies presented risk ratios based on the WHO BMI categorization criteria. Of the 22 studies, 11 studies reported a statistically significant association (with a risk ratio greater than unity) either for the overweight and/or the obese meta-analysis set. Seven out of the 22 studies did not provide adjusted risk ratios. All other studies appropriately adjusted for at least age and parity as confounding variables, with the exception of studies which evaluated only primi-parous women, where authors adjusted for mode of delivery or presented effect estimates for BMI and POP by strata of mode of delivery. Four studies presented one or more risk ratios for varying methods of POP assessment. The 22 studies included in the meta-analysis contributed more than 82,383 participants and 12,415 POP cases (regardless of measurement method), of which 4,322 cases were considered clinically significant POP (defined as Baden-Walker Halfway grade 2 or more, POP-Q Stage II or more, POP at or below hymen, or POP which required surgical treatment), and 2,359 cases were considered symptomatic POP (based on self-reported questionnaire). Sample size utilized for meta-analysis for each study is provided in Table 6-2. The total number of total participants and POP-cases included in the overweight and obese category could not be determined as not all studies provided stratum specific numbers, which is also the reason the reviewers relied on inverse-variance weighted meta-analysis.

Table 6-1. Description of study characteristics of the 22 studies eligible for meta-analysis

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results RR/OR/HR (95% CI)	Comments
Mant et al. (1997) [16]	Population-based longitudinal study; 17,032	Age: >25 yrs; % post-menopausal not provided and could not be inferred	English and Scottish; 100% European	BMI: <20 (Ref); 20-21.9; 22-23.9; 24-25.9; 26-27.9; ≥28	ICD9 codes for POP; no degree staging information provided	Age, parity, and calendar period	RR Ref BMI: <20: 1.0; RR BMI > 28: 1.31 (0.9-1.81)	Used RR calculated from poisson regression model as presented by authors to be used in the normal weight vs. obese analysis
Chiaffarino et al. (1999) [38]	Hospital based case-control; 208	Cases: 58.5; 81% post-menopausal. Controls: 59.8; 88% post-menopausal	Italians; Possibly 100% European	BMI: ≤ 23 (Ref); 24-26; >26	Baden-Walker classification for both cases and controls (Cases Grade II or III uterine or cystocele)	Age	OR Ref BMI <23: 1.0; OR BMI 24-26: 1.1 (0.5-2.1); OR BMI >26: 0.9 (0.5-1.7)	Meta-analyst recalculated OR to make between-study comparisons similar: BMI Ref: ≤ 26 vs. BMI >26
Parazzini et al. (2000) [44]	Multi-center cross-sectional study of non-hysterectomized women; 21,449	Age category (%): ≤51: 39.8; 52-55: 28.5; ≥56: 30.9; % post-menopausal not specified; Inferred: 59% ≥52 years of age	Italian women; Ethincity not specified; most likely mostly European	BMI: <23.8 (Ref); 23.8-27.2; >27.2	POP measured using Baden Walker classification system, uterine prolapse; Grade 0 - ≥ II; Analysis classification Grade 0 vs. Grades ≥I; Grade 0 vs. Grade I; Grade 0 vs. Grade ≥ II	Age, education, and parity	Grade 0 vs. ≥I; OR BMI <23.8 (Ref); OR BMI 23.8-27.2: 1.4 (1.2-1.7); OR BMI >27.2: 1.6 (1.3-1.8); Grade 0 vs. I: OR BMI <23.8 (Ref); OR BMI 23.8-27.2: 1.3 (1.1-1.6); OR BMI >27.2: 1.5 (1.2-1.8); Grade 0 vs. ≥II: OR BMI <23.8 (Ref); OR BMI 23.8-27.2: 1.6 (1.2-1.8); OR BMI >27.2: 1.8 (1.3-2.4)	Meta-analyst used Grade 0 vs. Grade I->II and Grade 0 vs. Grade >II analyses odds ratios as presented by authors for normal vs. overweight and normal vs. obese analysis categories;

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Scherf et al. (2002) [45]	Population-based cross-sectional study of 40 villages; 1067	Mean age: 32.6; range 25-44 yrs; 0% post-menopausal	Gambian; 100% West African	BMI: < 18; 18-25 (Ref); >25	Objectively measured prolapse using mild, moderate and severe	Unadjusted	OR Ref BMI: 18-25: 1.0; OR BMI > 25: 1.33 (0.86-2.04)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis
Fornell et al. (2004) [24]	Population-based cross-sectional study if 40 year olds and 60 year olds; 1336	Age: 40-year olds (48.6%); 60 year-olds; 51.4% post-menopausal (inferred)	Swedish; possibly 100% European	BMI: <25 (Ref); 25-30; >30	Symptomatic POP measured through standardized questionnaire	Univariate OR	OR BMI < 25 (Ref): 1.0; OR BMI 25-30: 1.6 (0.9-3.0); OR BMI >30: 1.2 (0.5-3.2)	Authors measured three tyupes of genital prolapse symptoms: pelvic heaviness, genital bulge and digitation by defecation; Meta-analyst used OR for genital bulge
Swift et al. (2005) [3]	Population-based multicenter cross-sectional study; 1004	Mean age (SD): 42.7 (13.9); 40% post-menopausal	US; 42% white, 24% black, 29% Hispanic, 2% Asian, and 2% other	BMI: <25 (Ref); 25-30; >30	POP-Q system; Used leading edge of prolapse at -0.5 or greater for defining POP	Age, race, parity, gravidity, number of vaginal delivery, weight of vaginally delivered infant, hormone therapy, labor related employment, income categories, and smoking	OR BMI < 25 (Ref): 1.0; OR BMI 25-30: 2.51 (1.18-5.35); OR BMI >30: 2.56 (1.23-5.35)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis and normal weight vs. obese analysis categories;

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Jun Tae Seo et al. (2006) [156]	Hospital-based cross-sectional, annual gynecologic examinations; 713	41.6 (10.2); 29% post-menopausal	Koreans; Possibly 100% Asian	BMI: ≤18.5; 18.6-22.9; 23-24.9; ≥25	POP-Q system; Stages 0-3; POP-Q stage 0-1 vs. stage ≥ 2	Authors provided raw numbers only	BMI and waist circumference had statistically significant trend toward increased POP-Q state; P < 0.001; Calculated univariate OR BMI < 25 (Ref): 1.0; OR BMI ≥25: 2.85 (1.72-4.71)	Meta-analyst calculated OR from raw numbers for BMI < 25 vs. BMI ≥25
Tegerstedt et al. (2006) [46]	Population-based case-control study; 554	Age range: 30-79 yrs; exact number not provided; 67.5% ≥50 years of age (inferred)	Swedish; Possibly 100% European	BMI: <20; 20-24.9 (Ref); ≥25;	Self-reported symptomatic POP was used for OR calculation; measured POP-Q on a smaller subset of women	Age and parity	OR Ref BMI: 20-24.9: 1.0: OR BMI ≥25: 1.3 (0.5-5.7)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis
Rortveit et al. (2007) [19]	RRISK; population-based cohort study; Independent group of women than RRISK2; 2,001	Mean age for RRISK (SD): 55.6 (8.6); 66% post-menopausal	US; 47% white; 19% African American; 17% Asian; 17% Latina; 1% Native American/other	BMI: < 25(Ref); 25-<30; 30-<35; 35-<40; ≥ 40	Symptomatic POP measured through standardized questionnaire	Authors present raw numbers only	OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 0.53 (0.33-0.84); OR BMI ≥30: 0.92 (0.59-1.43)	Meta-analyst calculated OR from raw numbers for BMI < 25 vs. BMI 25-<30 and BMI <25 vs. BMI ≥30
Forsman et al. (2008) [21]	Twin-Cohort Study; 16,886	Mean age (SD): 64.1 (9.2); 66% post-menopausal at least; not specified; although majority most likely post-menopausal; (inferred)	Swedish twins, both monozygotic and di-zygotic; most likely mostly European	BMI: <25 (Ref); 25-30; >30	POP assessed through Swedish inpatient register data, using Swedish Classification of Operations and Major Procedures	Age, and childbirth (ever/never)	OR BMI < 25 (Ref): 1.0; OR BMI 25-30: 1.1 (0.8-1.5); OR BMI >30: 1.4 (0.7-2.8)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis and normal weight vs. obese analysis categories;

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Whitcomb et al. (2009) [20]	Reproductive Risks for Incontinence Study at Kaiser 2 (RRISK2); population-based cohort study; 2,270	Mean age for RRISK2 (SD): 55.0 (9); 72% post-menopausal	US; 44% white; 20% African American; 18% Asian; 18% Latina/Hispanic/Native American/other	BMI: <25 (Ref); 25-<30; ≥30	POP measured in two ways; Symptomatic POP in 2270 women and through POP-Q examination in 1137 women	Age, race/ethnicity, education, parity and diabetes	Analysis in women with symptomatic prolapse: OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 1.03 (0.53-2.00); OR BMI ≥30: 1.43 (0.76-2.68); Analysis in women with prolapse at or below hymen (POP-Q): OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 1.46 (1.05-2.02); OR BMI ≥30: 1.67 (1.22-2.39); Analysis in women with prolapse ≥ Stage II (POP-Q): OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 1.06(1.01-1.11); OR BMI ≥30: 1.09 (1.04-1.14)	The authors presented three types of adjusted odds ratios for both overweight and obese analyses; Meta-analyst used Symptomatic Prolapse and Prolapse at or below the hymen as it is more advanced prolapse than just POP-Q Stage II
Chen Huey-Yi et al. (2009) [90]	Hospital-based case-control; 237	Cases: ≥54 yrs 72%; 71% post-menopausal. Controls: ≥54 yrs 31.3%; 44% post-menopausal	Taiwanese; possibly 100% Asian	BMI: < 23.6 (Ref); ≥ 23.6	POP-Q system; Stages 0-3; POP-Q stage 0-1 vs. stage ≥ 2	Age, parity, BMI, menopause	OR Ref BMI <23.6: 1.0; OR BMI ≥23.6: 1.99 (1.08-3.70)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis
Braekken et al. (2009) [85]	Hospital-based case-control; 98	Mean age: 47.1 (10.57); 36.7% post-menopausal	Norwegian; possibly 100% European	BMI: ≤25 (Ref); > 25	POP-Q system; Stages 0-4; POP-Q stage 0-1 vs. stage ≥ 2	socioeconomic status and heavy work	OR Ref BMI ≤25: 1.0; OR BMI >24: 5.0 (1.1-23.0)	Matched for age and parity; Used OR as presented by authors

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Fritel et al. (2009) [40]	Cross-sectional analysis of Cohort study; 2,640	Median Age: 54 (range: 50-61); 100% post-menopausal	French; ethnicity mostly European	BMI: <25 (Ref); ≥25	Symptomatic POP measured through standardized questionnaire	Mode of delivery	OR: Ref BMI <25: 1.0; OR BMI ≥25: 1.41 (1.01-1.97)	Authors only included variables in model which were statistically significant; Only mode of delivery was statistically significant
de Araujo et al. (2009) [39]	Population-based cross-sectional, sexually active women; 377	Mean age (SD): 31 (15); %post-menopausal not provided; 14.32% ≥50 (inferred)	Brazilian; Ethnicity stated as indigenous Xingu women; most likely native	BMI: ≤25 (Ref): >25	POP-Q exam; Stage 0-3; Stage 0-1 vs. ≥2; Also Ba point ≥ 0 as cases	Does not explicitly report adjusted variables; most likely age, vaginal delivery, resting pressure and maximum pressure	Analysis Stage 0-1 vs. Stage ≥ II; OR: Ref BMI ≤25 1.0; OR BMI >25: 1.05 (0.60-1.82); Analysis leading edge Ba < 0 vs. ≥0; OR Ref BMI ≤25: 1.0; OR BMI >25: 1.33 (0.79-2.24)	Authors presented adjusted odds ratios for two methods of POP classification as noted in the POP-measurement column; Both were considered
Chen CC et al. (2009) [155]	Hospital-based study comparing obese vs. non-obese women; 427	Obese Mean age (Range): 45 (15-71); % post-menopausal 47%; Non-obese Men age (Range): 43 (19-90); 32% post-menopausal	US; 61.3% white; 22.7%; remaining other	Non-obese (Mean BMI: 23) (Ref); Obese (Mean BMI: 50	POP defined by self-report of POP and previous history of POP surgery or treatment	Unadjusted; authors provided raw numbers;	Statistically non-significant p-value for comparing self-reported POP and self-reported surgery/treatment	Meta-analysis calculated crude odds ratio combining self-reported POP and self-reported surgery/treatment for POP between obese and non-obese women

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Miedel et al. (2009) [43]	Population-based cross-sectional study; 442	Mean age cases (SD): 53.3 (12.3) Mean age controls (SD): 49.1 (13.5); % post-menopausal status not explicitly stated; inferred based on hormone therapy use: 28% in cases and 29% in controls	Swedish; ethnicity not provided; most likely 100% European	BMI: <19; 19-25 (Ref); 26-30); >30	Self-reported symptomatic POP was used for OR calculation; measured POP-Q on a smaller subset of women	Age, parity, hernia, family history of POP, heavy lifting, and constipation	OR BMI 19-25 (Ref): 1.0; OR BMI 26-30: 1.88 (1.15-3.08); OR BMI >30: 2.07 (0.95-4.5)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis and normal weight vs. obese analysis categories;
Dolan et al. (2010) [12]	Cross-sectional analysis of registry-based records; 1,787	Mean age: 45.7 (4.8); % post-menopausal not provided; Inferred: at most <33%	English; ethnicity not provided, but most likely mostly European	BMI: <25 (Ref); 25-30; >30	Symptomatic POP measured through standardized questionnaire	Age, social class, parity, birth weight, mode of 1st delivery length of 1st labor, length of 2nd stage labor, epidural/caudal, and perineum status upon delivery	OR BMI < 25 (Ref): 1.0; OR BMI 25-30: 0.98 (0.72-1.34); OR BMI >30: 1.30 (0.89-1.88)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis and normal weight vs. obese analysis categories;

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Kudish et al. (2011) [23]	Prospective-cohort study; 12,649	Age range: 50-79; 100% post-menopausal	US; 88.4% white; 6.3% black, and 5.3% Hispanic	BMI: <25 (Ref); 25-<30; ≥30	POP measured using WHI POP-Grading System (Grades 1-3 as POP); Analysis provided for Grade 0 vs. Grade 1-3; and Grade 0 vs. Grade 2-3; Analyses provided in all samples and by race/ethinicty status	Age, ethicity, parity, smoking, constipation, asthma, emphysema, hormone therapy use history, estrogen+progesterone hormone treatment vs placebo, Incontinence, waise circumference and physical activity	Combined Analyses for all ethnicities Grade 0 vs. Grade 1-3 POP: OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 1.07 (0.99-1.17); OR BMI ≥30: 1.16 (1.02-1.30); Combined Analyses for all ethnicities Grade 0 vs. Grade 2-3 POP: OR BMI < 25 (Ref): 1.0; OR BMI 25-<30: 1.25 (1.08-1.44); OR BMI ≥30: 1.27 (1.05-1.54); Ethnicity specific analyses also presented	
Glazener et al. (2012) [14]	Cross-sectional analysis of 13 year longitudinal study; 3,763	Mean age at birth index (SD): 29.2 (4.9); Mean age at follow-up (range): 42 (26-58); % post-menopausal not provided, mostly pre-menopausal	English; 95.7% non-Asian	BMI: <18.5; 18.5-24.9 (Ref); 25-29.9); ≥ 30	POP-Q system; used leading edge of prolapse at or beyond hymen for defining POP	Age at first birth, and total number of births	OR BMI 18.5-<24.9 (Ref): 1.0; OR BMI 25-29.9: 1.33 (0.90-1.96); OR BMI ≥30: 1.48 (0.91-2.40)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis and normal weight vs. obese analysis categories;

Author (Publication Year) [Ref]	Study Design, sample size	Mean Age (SD)/Age Category(%); %post-menopausal	%Race/Ethnicity or Country of Study	Obesity Measure	POP Measurement	Covariates adjusted for	Results	Comments
Gyhagen et al. (2012) [15]	Registry-based national cohort study of primiparous women; 5,236	Maternal age range at birth: <23 - ≥ 35; Evaluation of POP 20 years later; Maternal age range during self-reported POP evaluation <43 - ≥55; % post-menopausal at POP evaluation not provided; mostly, pre-menopausal	Swedish; possibly 100% European	BMI: <25 (Ref); 25-29.9; ≥30	Symptomatic POP measured through standardized questionnaire	Age at delivery and infant birth weight	Analysis in women with Caesarean section: OR BMI < 25 (Ref): 1.0; OR BMI 25-29.9: 1.70 (0.99-2.94); OR BMI ≥30: 1.60 (0.86-2.96); Analysis in women with vaginal delivery: OR BMI < 25 (Ref): 1.0; OR BMI 25-29.9: 1.33 (1.08-1.63); OR BMI ≥30: 1.74 (1.38-2.18)	Authors presented adjusted odds ratios for women with C-section and women with vaginal delivery separately. Therefore, two independent odds ratios are provided for normal vs. overweight and normal vs. obese, each
Awwad et al. (2012) [154]	Population-based cross-sectional study; 557	Cases Mean age: 40.42 (9.34); 21.1% post-menopausal; Controls Mean age: 31.78 (9.56); 7.5% post-menopausal	Rural Lebanese women; Ethnicity unknown	BMI: ≤24 (Ref); >24	POP-Q system: Stages 0-4; POP-Q stage 0-1 vs. stage ≥ 2	Age, miscarriage, vaginal parity , age by parity interaction	OR Ref BMI ≤24: 1.0; OR BMI > 24: 1.6242 (1.00-2.63)	Used OR as presented by authors to be used in the normal weight vs. overweight analysis

BMI measured in kg/m^2

Table 6-2. Sample size estimates from studies utilized in meta-analyses

Author (Publication Year)	N-Total POP	N-Significant POP	N-Symptomatic POP	Total N
Mant et al. (1997) [16]	106	106	-	NR
Chiaffarino et al. (1999) [38]	105	-	108	208
Parazzini et al. (2000) [44]	1,086	379	-	19,310
Scherf et al. (2002) [45]	407	-	-	911
Fornell et al. (2004) [24]	53	-	53	1,336
Swift et al. (2005) [3]	203	203	-	943
Jun Tae Seo et al. (2006) [156]	114	114	-	713
Tegerstedt et al. (2006) [46]	184	-	184	317
Rortveit et al. (2007) [19]	117	-	117	1989
Forsman et al. (2008) [21]	1,099	1,099	-	29881
Whitcomb et al. (2009) [20]	257	257	74	1,137/2,269[a]
Chen Huey-Yi et al. (2009) [90]	87	87	-	237
Braekken et al. (2009) [85]	49	49	-	98
Fritel et al. (2009) [40]	152	-	152	3,114
de Araujo et al. (2009) [39]	245	245	-	377
Chen CC et al. (2009) [155]	48	-	-	427
Miedel et al. (2009) [43]	265	-	265	532
Dolan et al. (2010) [12]	250	-	250	1,788
Kudish et al. (2011) [23]	6,002	1,353	-	12,650
Glazener et al. (2012) [14]	179	179		752
Gyhagen et al. (2012) [15]	1,156	-	1,156	5,159
Awwad et al. (2012) [154]	251	251	-	504
Total	12,415	4,322	2,359	>82,383[b]

NR = Not recorded because it could not be determined; [a] Used two different sample sizes; [b] Exact sample size could not be determined; provides a conservative estimate

Meta-analysis results

Results from primary meta-analyses for the minimum scenarios (includes lowest of the two or more obesity-category-specific estimates for studies that presented more than one risk ratio) and the maximum scenarios (includes the largest of the two or more obesity-category-specific estimates for studies that presented more than one risk ratio) are presented in Table 6-3. Compared with women in the normal weight (referent) category, meta-analysis risk ratio for women in the overweight category ranged from 1.33 (95% CI: 1.16, 1.52) for the minimum analysis set, to 1.41 (95% CI: 1.24, 1.60) in the maximum analysis set (Table 6-3 and Figure 6-2: A-B). The heterogeneity statistics suggested considerable heterogeneity for effect estimates in the minimum overweight analysis scenario (I^2 = 63%) and moderate heterogeneity for effect estimates in the maximum overweight analysis scenario (I^2=52%). Examination of the funnel plots (Figure 6-3: a-b) for both the minimum and maximum scenarios suggested some evidence of publication bias (Egger's test p: 0.03 for the minimum scenario) (Table 6-3); smaller published studies tended to report larger effect estimates in the positive direction than larger studies.

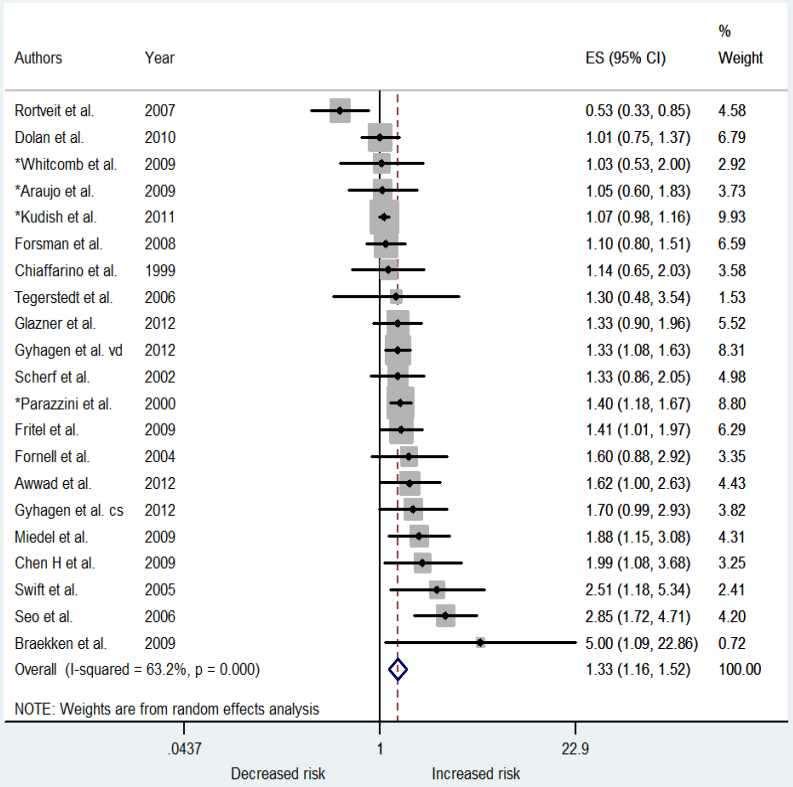
Table 6-3: Main meta-analysis results evaluating obesity categories in relation to POP

Scenario	BMI Category	N	Risk Ratio (95% CI)	p-value	I^2	Small-Study Bias-p-value
Minimum						
	Over-weight	21	1.33 (1.16, 1.52)	<0.001	63%	0.03
	Obese	14	1.41 (1.24, 1.60)	<0.001	39%	0.26
Maximum						
	Over-weight	21	1.39 (1.22, 1.58)	<0.001	52%	0.11
	Obese	14	1.47 (1.31, 1.64)	<0.001	14%	76

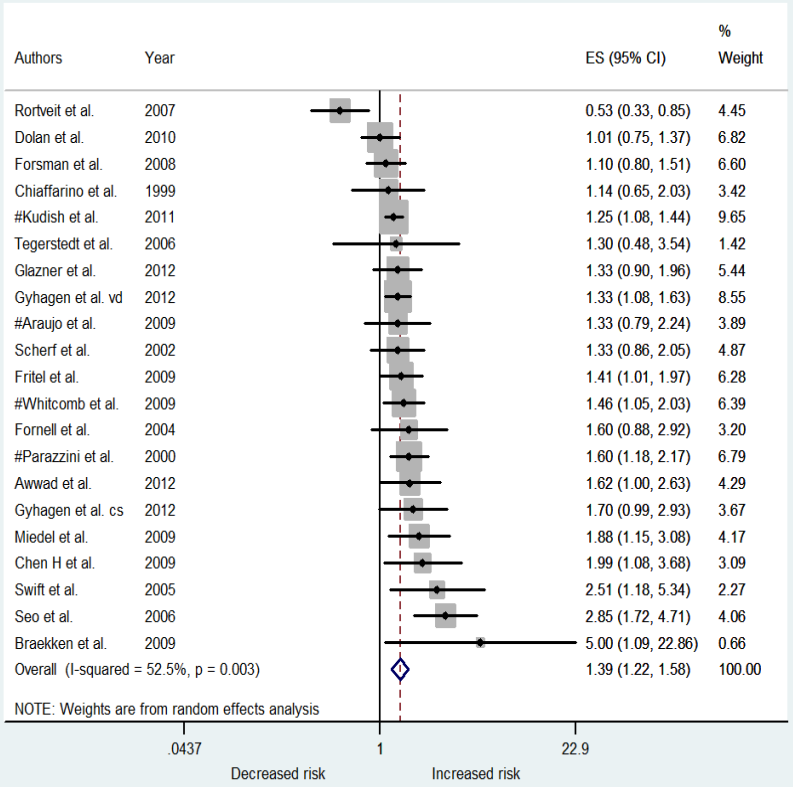
p-value = tests the null hypothesis that risk ratio = 1; I2 = % heterogeneity attributed to factors other than chance; Minimum = represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by each study in the scenario when studies reported two or more effect estimates; Maximum = represents the meta-analysis risk ratio when comparing the largest effect estimates provided by each study in the scenario when studies reported two or more effect estimates

Figure 6-2: A-B. Main-analyses: Forest plots for studies evaluating normal-weight vs. over-weight categories in relation to POP

A



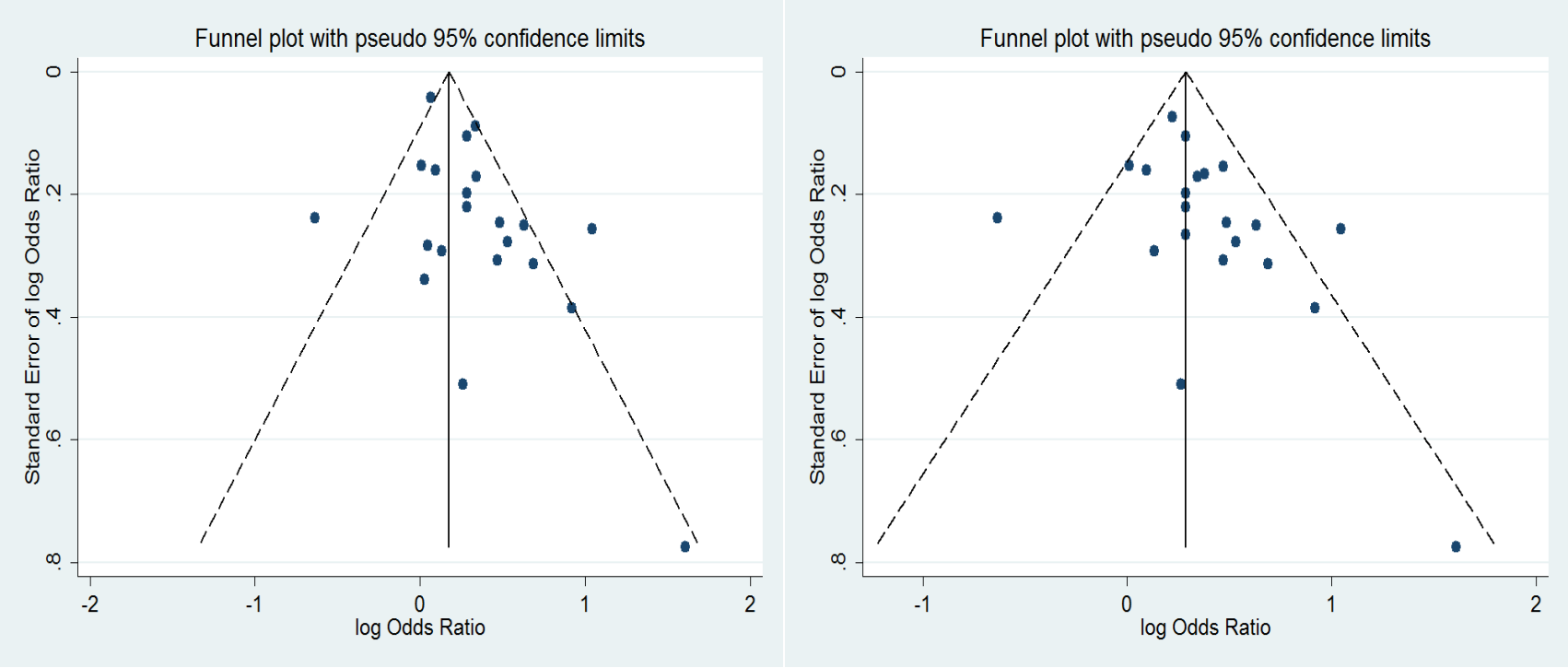
B



A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-3: A-B. Main-analyses: Funnel plots for studies evaluating normal-weight vs. over-weight categories meta-analyses in relation to POP

A **B**



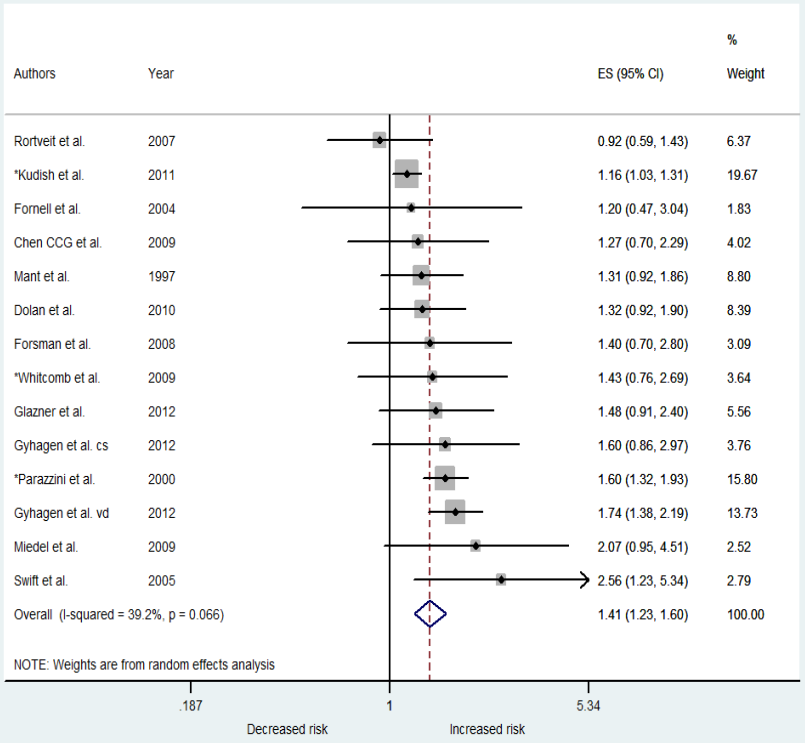
A: Minimum Scenario= represents the meta-analysis when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates

Similarly, compared with women in the normal weight (referent) category, meta-analysis risk ratio for women in the obese category ranged from 1.41 (95% CI: 1.24, 1.60) for the minimum analysis set, to 1.47 (95% CI: 1.31, 1.64) in the maximum analysis set (Table 6-3 and Figure 6-4: a-b). There was considerably less heterogeneity in the obese-category analyses; I^2 estimates for the

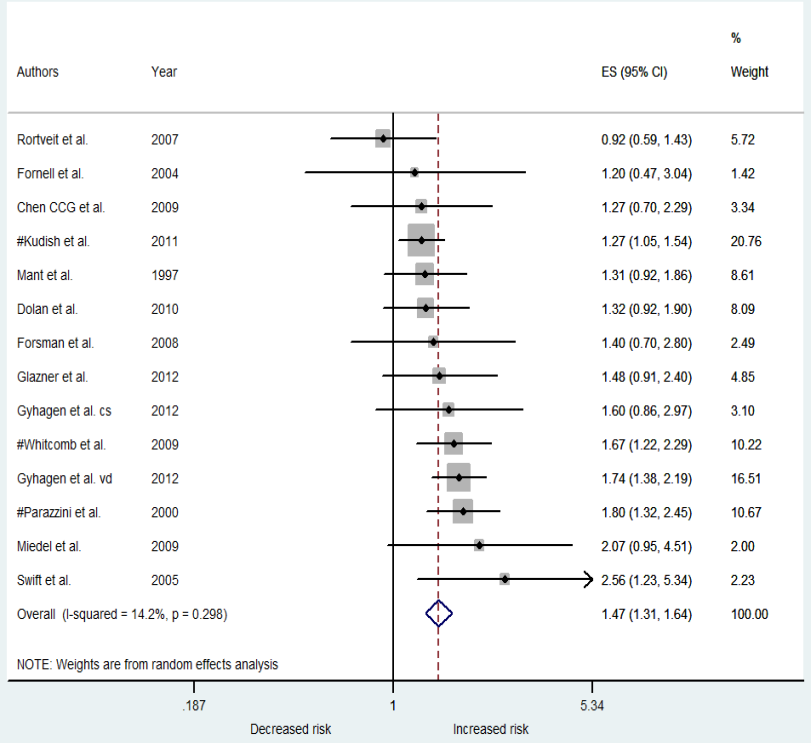
minimum and maximum scenarios were 39% and 14% respectively. Examination of funnel plots (Figure 6-5: a-b) and the Egger's test (Table 6-3) did not show much evidence for publication bias for the obese-category analyses.

Figure 6-4: A-B. Main-analyses: Forest plots for studies evaluating normal-weight vs. obese categories in relation to POP

A



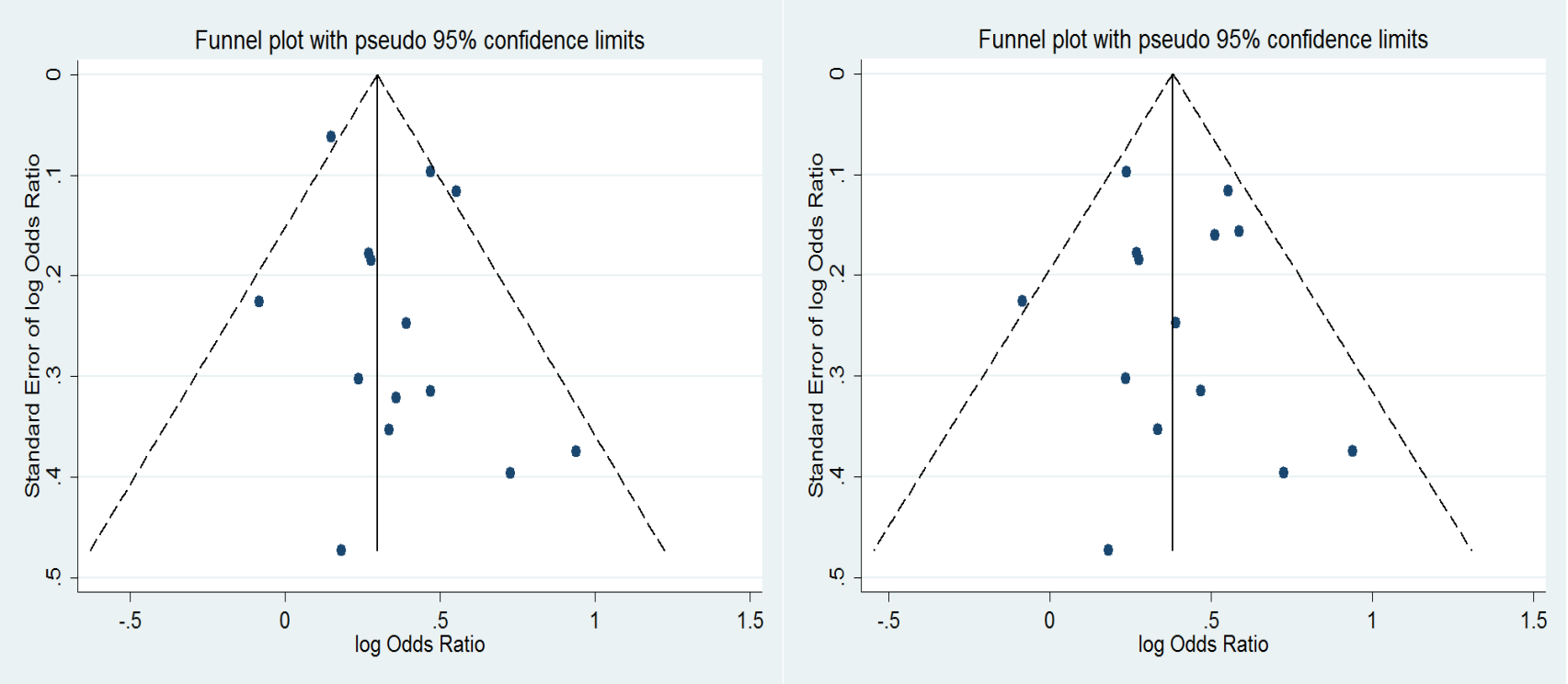
B



A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-5: A-B. Main-analyses: Funnel plots for studies evaluating normal-weight vs. obese categories meta-analyses in relation to POP

A **B**



A: Minimum Scenario= represents the meta-analysis when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates

We performed several sensitivity analyses by strata of study attribute in an effort to identify sources of heterogeneity. Results for the over-weight category and obese category by strata of study characteristic are presented in Table 6-4 and Table 6-5, respectively. Forest plots for sub-group analyses are shown in Figure 6-6 through Figure 6-15. As would be expected, effect estimates summarizing clinically significant objective POP were considerably larger than effect estimates summarizing any grade/stage of objectively measured POP or symptomatic self-reported POP for both the overweight and obese analysis categories. Compared with women in the normal weight category, women in the over-weight category had a meta-analysis risk ratio of 1.23 (95% CI: 0.99, 1.53) if any grade of objectively measured POP was assessed, 1.23 (95% CI: 0.97, 1.55) if self-reported symptomatic POP was assessed and 1.48 (95% CI: 1.25, 1.74) if objectively measured clinically significant POP was assessed (Table 6-4; Figure 5.6: A-B). Similarly, compared with women in the normal weight category, women in the obese category had a meta-analysis risk ratio of 1.34 (95% CI: 1.06, 1.69) if any grade of objectively measured POP was assessed, 1.44 (95% CI: 1.21, 1.72) if self-reported symptomatic POP was assessed and 1.53 (95% CI: 1.29, 1.82) if objectively measured clinically significant POP was assessed (Table 6-5; Figure 6-7). The heterogeneity statistics were low for the symptomatic POP and clinically significant objective POP, 12% and 26%, respectively (Table 6-5). Statistical significance for between-study heterogeneity by strata of POP measurement was not calculated as there was population overlap between groups (within groups, the study populations were independent).

Table 6-4: Sensitivity analyses for normal weight vs. over-weight categories in relation to POP by study characteristic

		Minimum Scenario				Maximum Scenario			
Study Characteristic	**N**	**Risk Ratio (95% CI)**	**p-value**	**I^2**	**Het-p***	**Risk Ratio (95% CI)**	**p-value**	**I^2**	**Het-p***
POP Measurement					NA				NA
Objective (Any grade POP)	3	1.23 (0.99, 1.53)	0.06	74%		-	-	-	
Symptomatic POP	9	1.23 (0.97, 1.55)	0.08	61%		-	-	-	
Objective (Clinically Significant)	12	1.48 (1.25, 1.74)	<0.001	48%		1.49 (1.27, 1.75)	<0.001	46%	
Analysis Adjusted					0.63				0.58
No	6	1.29 (0.78, 2.13)	0.32	79%		-	-	-	
Yes	15	1.32 (1.16, 1.51)	<0.001	54%		1.37 (1.24, 1.52)	<0.001	21%	
% Post-menopausal					0.001				0.078
<50%	12	1.54 (1.28, 1.86)	<0.001	50%		1.56 (1.30, 1.87)	<0.001	47%	
≥50%	9	1.14 (0.96, 1.36)	0.14	61%		1.23 (1.03, 1.48)	0.025	56%	
% Post-menopausal					0.003				0.645
<33%	9	1.44 (1.19, 1.74)	<0.001	49%		1.46 (1.22, 1.75)	<0.001	46%	
≥33% - <67%	7	1.38 (0.97, 1.96)	0.08	75%		1.44 (0.96, 2.15)	0.08	76%	
≥67%	5	1.09 (1.01, 1.18)	0.04	0%		1.29 (1.15, 1.45)	<0.001	0%	
Reported WHO BMI Categories					<0.001				0.109
No	10	1.51 (1.27, 1.80)	<0.001	31%		1.59 (1.34, 1.88)	<0.001	17%	
Yes	11	1.20 (1.02, 1.43)	0.03	32%		1.27 (1.08, 1.58)	0.004	59%	
Reported Study Design					<0.001				0.003
Case-control	4	1.61 (1.01, 2.57)	0.04	27%		-	-	-	
Cohort	9	1.13 (0.97, 1.32)	0.10	57%		1.20 (1.03, 1.40)	0.02	57%	
Cross-sectional	8	1.61 (1.32, 1.97)	<0.001	39%		1.70 (1.43, 2.02)	<0.001	7%	

p-value = tests the null hypothesis that risk ratio = 1; I^2 = % heterogeneity attributed to factors other than chance; Minimum = represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; Maximum = represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * = p-value for between group heterogeneity; NA= Between group heterogeneity not valid due since different effect estimates from some studies are represented in more than one group; - = if no studies report two or more estimates then, minimum = maximum

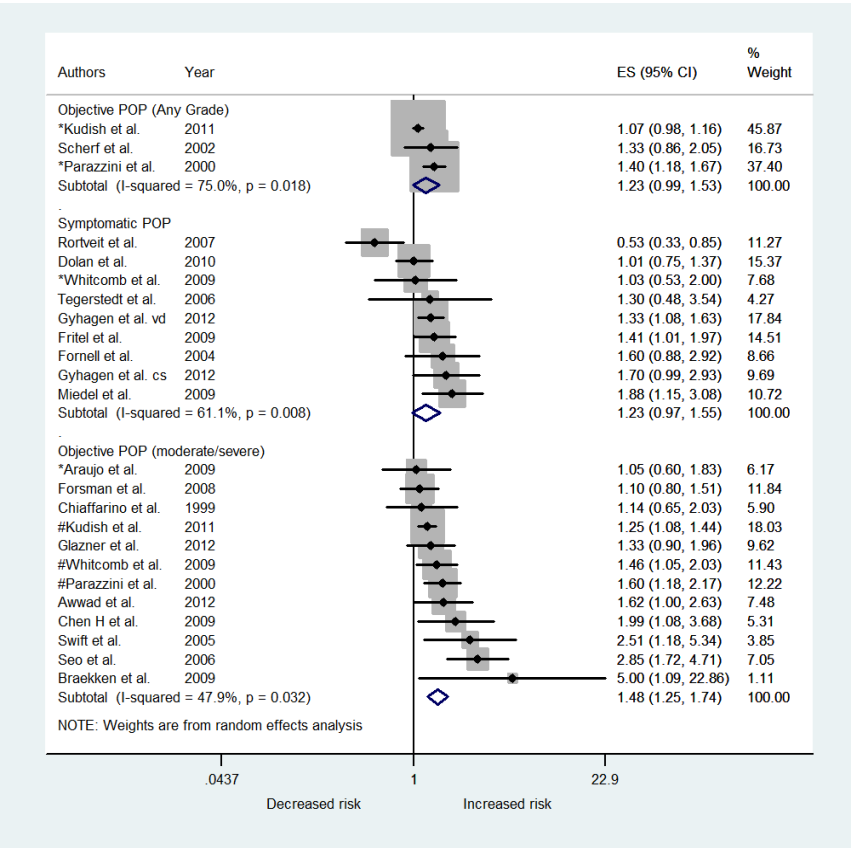
Table 6-5: Sensitivity analyses for normal weight vs. obese in relation to POP by study characteristic

		Minimum Scenario				Maximum Scenario			
Study Characteristic	**N**	**Risk Ratio (95% CI)**	**p-value**	**I^2**	**Het-p***	**Risk Ratio (95% CI)**	**p-value**	**I^2**	**Het-p***
POP Measurement					NA				NA
Objective (Any grade POP)	3	1.34 (1.06, 1.69)	0.014	75%		-	-	-	
Symptomatic POP	8	1.44 (1.21, 1.72)	<0.001	12%		-	-	-	
Objective (Clinically Significant)	6	1.53 (1.29, 1.82)	<0.001	26%		-	-	-	
Analysis Adjusted:					0.13				0.04
No	3	1.05 (0.76, 1.47)	0.77	0%		-	-	-	
Yes	11	1.47 (1.24, 1.60)	<0.001	45%		1.51 (1.36, 1.68)	<0.001	1%	
% Post-menopausal					0.008				0.145
<50%	7	1.62 (1.38, 1.90)	<0.001	0%		-	-	-	
≥50%	6	1.28 (1.05, 1.56)	0.014	50%		1.40 (1.14, 1.70)	0.001	39%	
% Post-menopausal					0.004				0.457
<33%	6	1.58 (1.35, 1.86)	<0.001	0%		-	-	-	
≥33% - <67%	5	1.42 (1.04, 1.94)	0.03	46%		1.47 (1.02, 2.11)	0.04	52%	
≥67%	2	1.17 (1.04, 1.32)	0.01	0%		1.41 (1.09, 1.84)	0.01	53%	
Reported WHO BMI Categories					0.010				0.681
No	3	1.51 (1.28, 1.77)	<0.001	0%		1.52 (1.20, 1.91)	<0.001	10%	
Yes	11	1.40 (1.18, 1.66)	<0.001	43%		1.46 (1.27, 1.67)	<0.001	22%	
Reported Study Design					0.02				0.11
Case-control	0	NA	NA	NA		NA	NA	10%	
Cohort	9	1.33 (1.15, 1.54)	<0.001	37%		1.41 (1.25, 1.60)	<0.001	17%	
Cross-sectional	5	1.61 (1.36, 1.91)	<0.001	0%		1.74 (1.38, 2.20)	<0.001	0%	

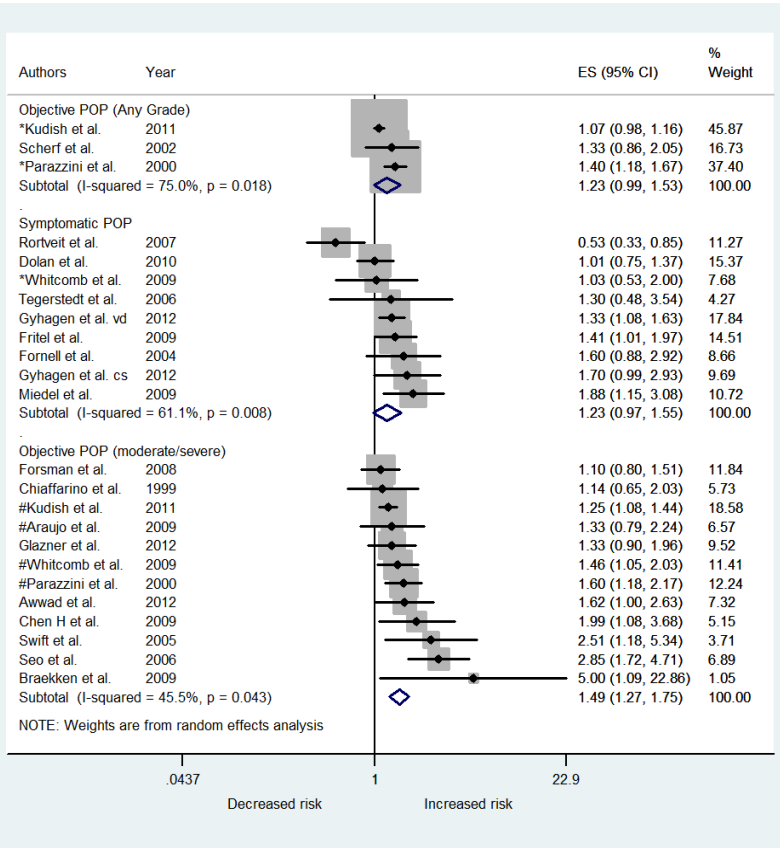
p-value = tests the null hypothesis that risk ratio = 1; I^2 = % heterogeneity attributed to factors other than chance; Minimum = represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; Maximum = represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * = p-value for between group heterogeneity; NA= Between group heterogeneity not valid due since different effect estimates from some studies are represented in more than one group; - = if no studies report two or more estimates then, minimum = maximum

Figure 6-6 A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. over-weight categories in relation to POP by POP measurement criteria

A

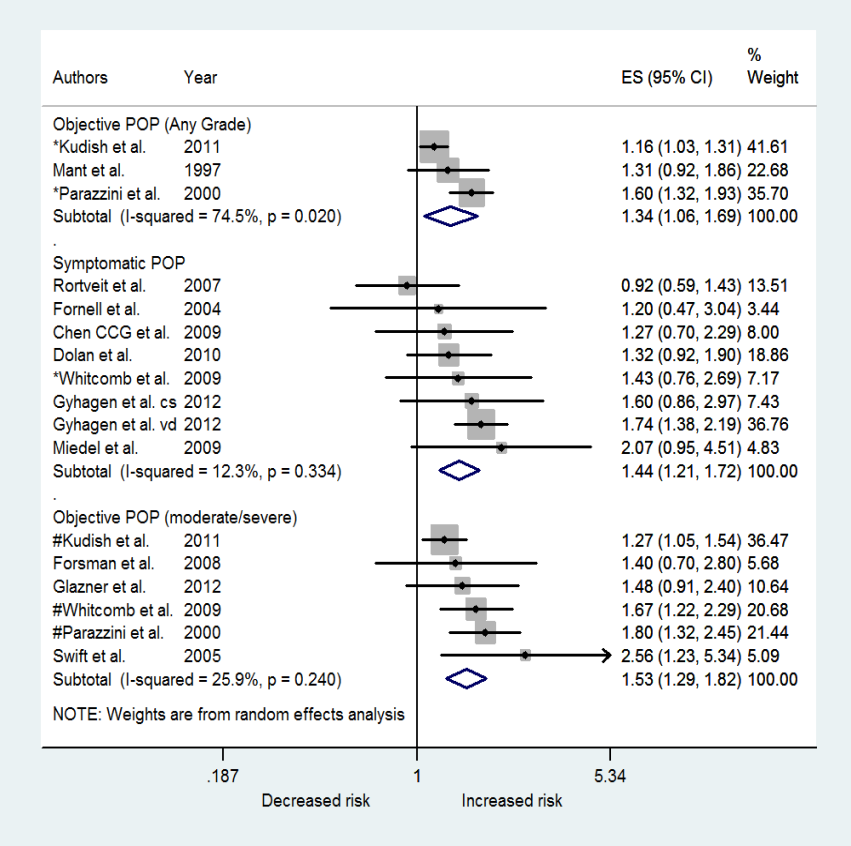


B



A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-7. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by POP measurement criteria



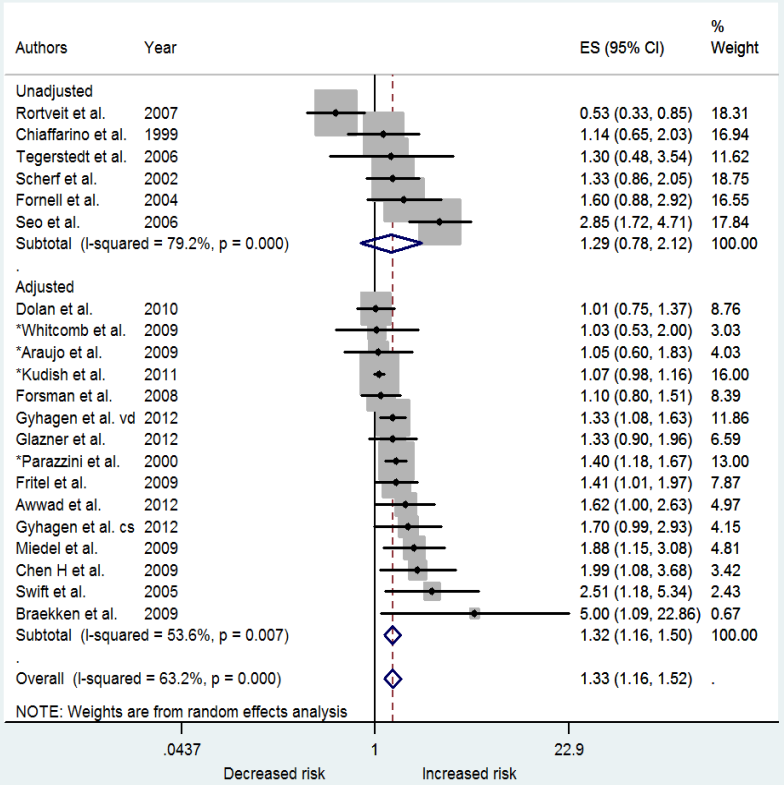
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

It is of interest that studies presenting adjusted analyses were more likely to report higher effect estimates than studies that presented unadjusted estimates; this was true for both the overweight (Table 6-4; Figure 6-8: A-B) and obese category (Table 6-5; Figure 6-9: A-B) analyses. The most-profound difference was found in the maximum scenario for the obese category analysis where the meta-analysis risk ratios for unadjusted group and adjusted group were 1.05 (95% CI: 0.76, 1.47) and 1.51 (95% CI: 1.36, 1.68), respectively. Next we evaluated whether the effect estimates of studies differed by the percent of post-menopausal women included in their study (Table 6-4 for overweight category and Table 6-5 for obese category), grouped in two ways: <50%, ≥50% (Figure 6-10 A-B for over-weight category and Figure 6-11 A-B for obese category), and <33%, ≥33%-<67% and ≥67% (Figure 6-12 A-B for over-weight category, and Figure 6-13 A-B for obese category). Studies with a lower percent of post-menopausal women had the highest meta-analysis risk ratio, with a consistent decrease in meta-analysis risk ratio as the categories for percentage of post-menopausal women increased. This effect was most evident for the obese category analysis in the minimum scenario where category specific risk ratios for <33% post-menopausal women, >33%-<67% post-menopausal women and ≥67% post-menopausal women were 1.58 (95% CI: 1.35, 1.86), 1.42 (95% CI: 1.04, 1.94) and 1.17 (95% CI: 1.04, 1.32). The first and the last percentage of post-menopausal category had the least within-group heterogeneity and the mid-category had moderate heterogeneity. Studies utilizing BMI categories similar to the WHO criterion reported lower effect estimates than studies reporting non-WHO categories of BMI (Figure 6-14 A-B for over-weight category and Figure 6-15 A-B for obese category); this difference was most notable in the over-weight analyses. In this meta-analysis, only one study presented hazard ratio and one study presented a relative risk. Excluding these effect estimates from the meta-analysis sets to only include odds

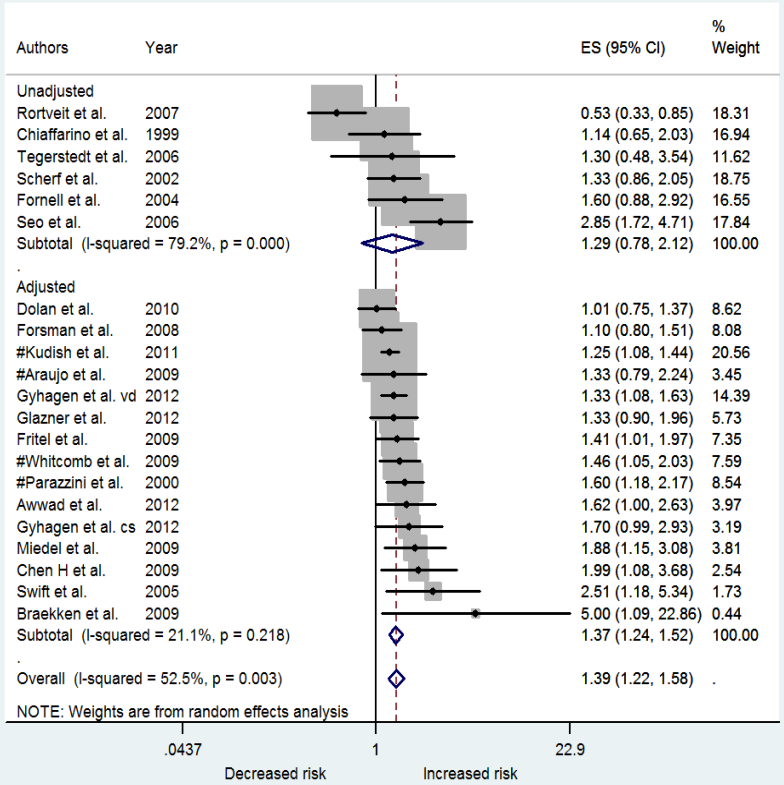
ratios did not change the meta-analysis effect estimates considerably. Case-control and cross-sectional studies were more likely to report higher effect estimates than studies that were identified as originating from a cohort design (Figure 6-16 A-B for over-weight category and Figure 6-17 A-B for obese category).

Figure 6-8. A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. over-weight categories in relation to POP by adjustment status (yes, no)

A



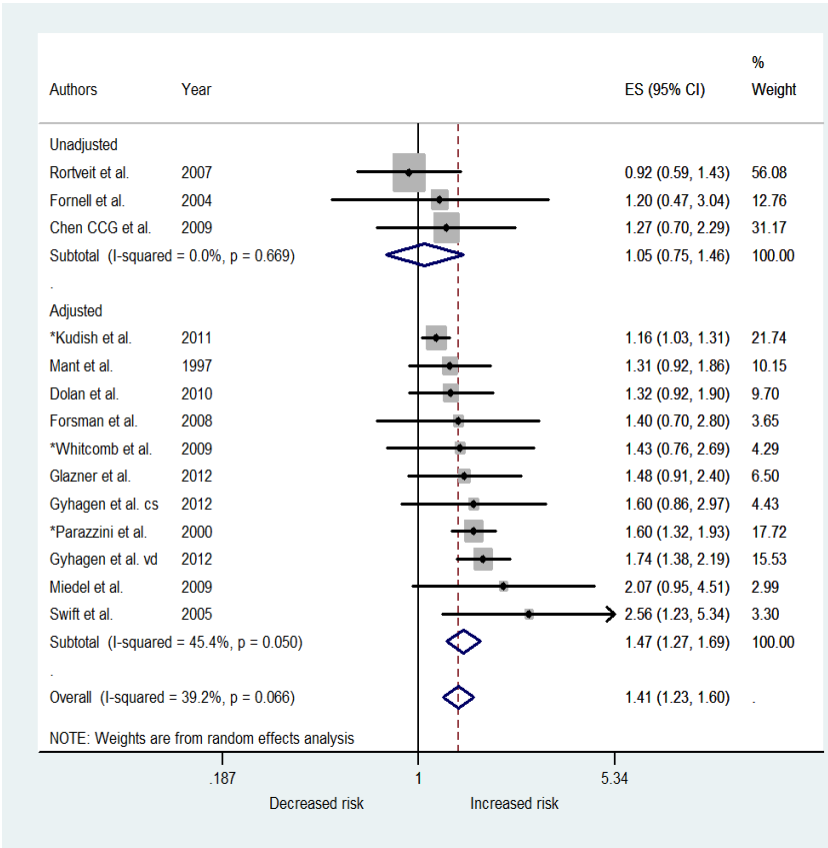
B



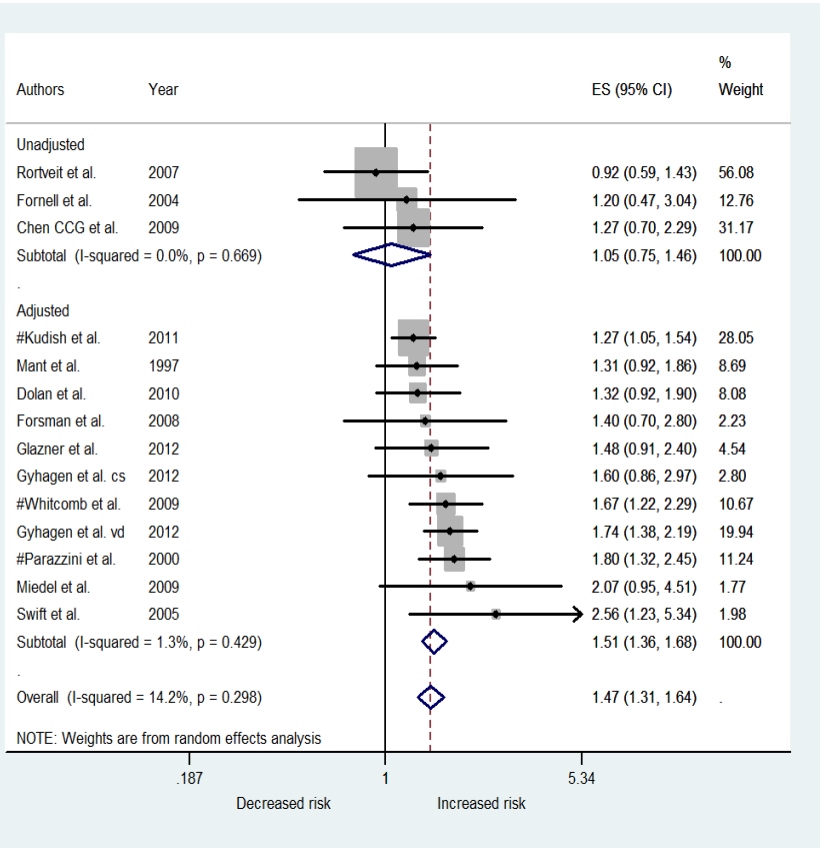
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-9: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by adjustment status (yes, no)

A



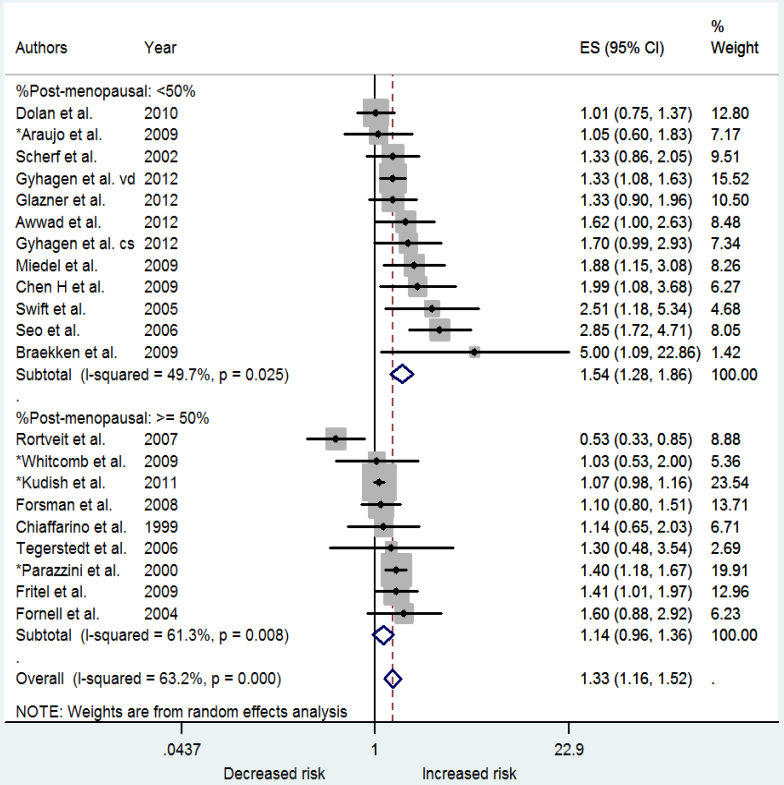
B



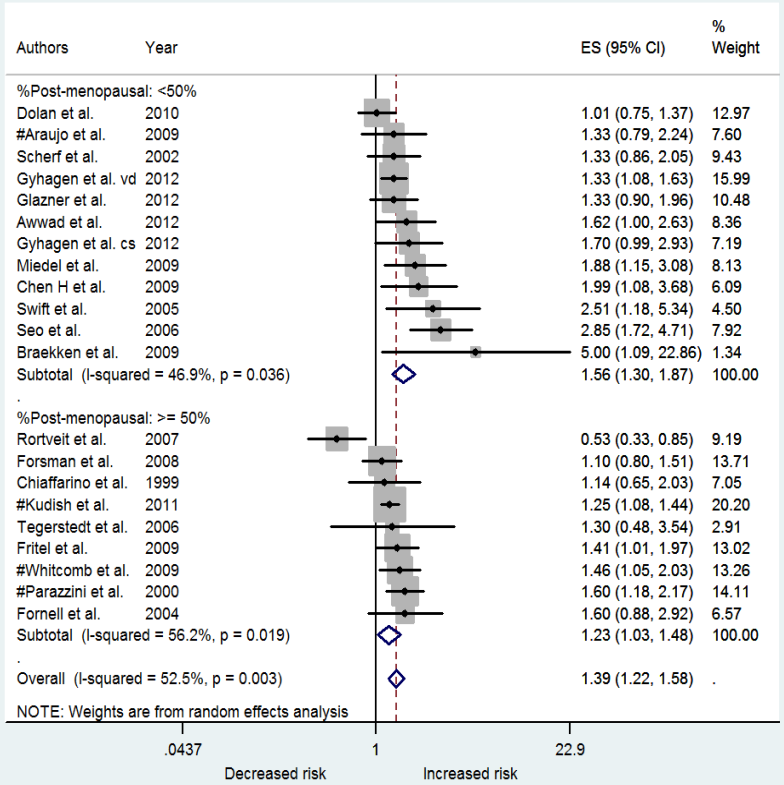
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-10: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. over-weight categories in relation to POP by category of % post-menopausal women in study (<50%, ≥50%)

A



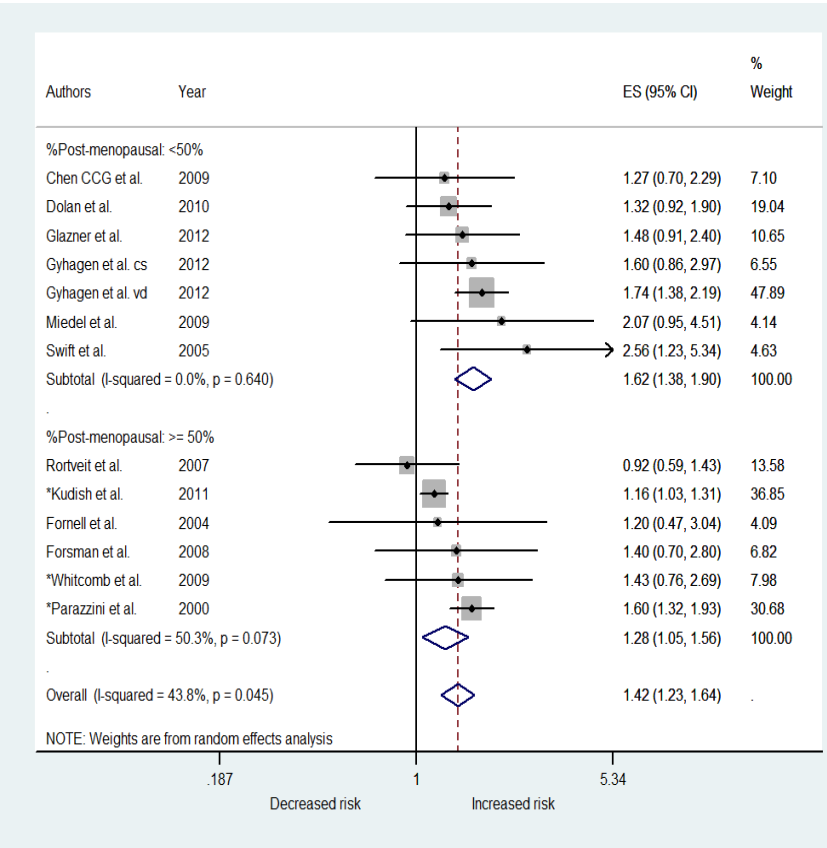
B



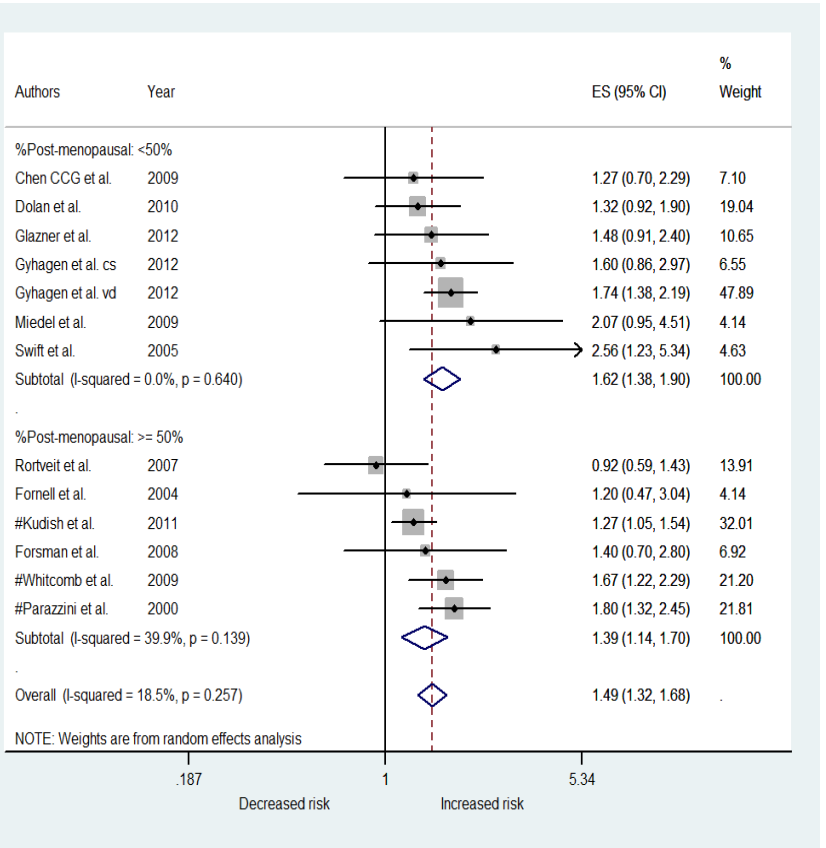
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-11: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by category of % post-menopausal women in study (<50%, ≥50%)

A



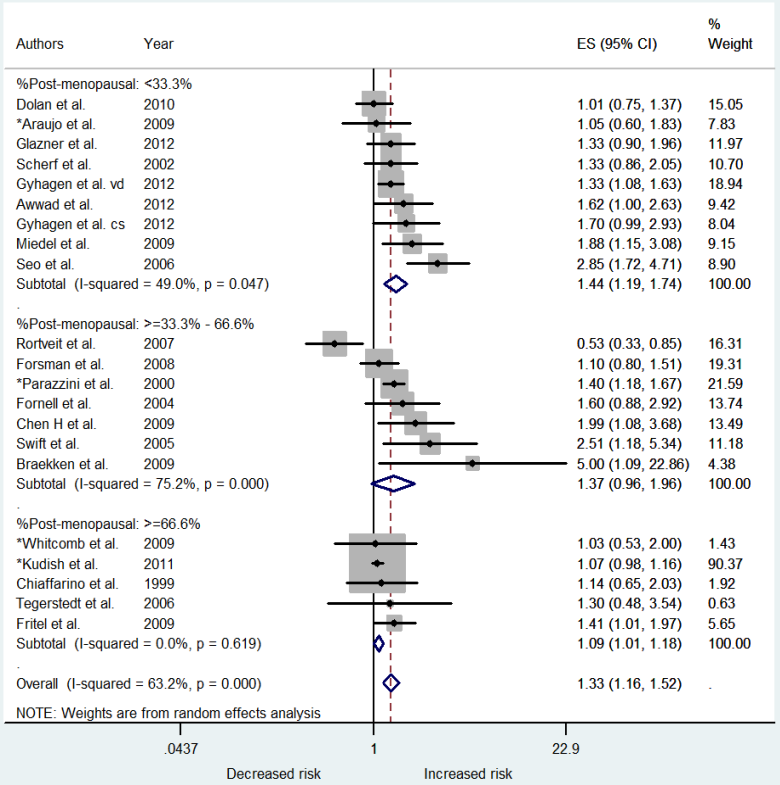
B



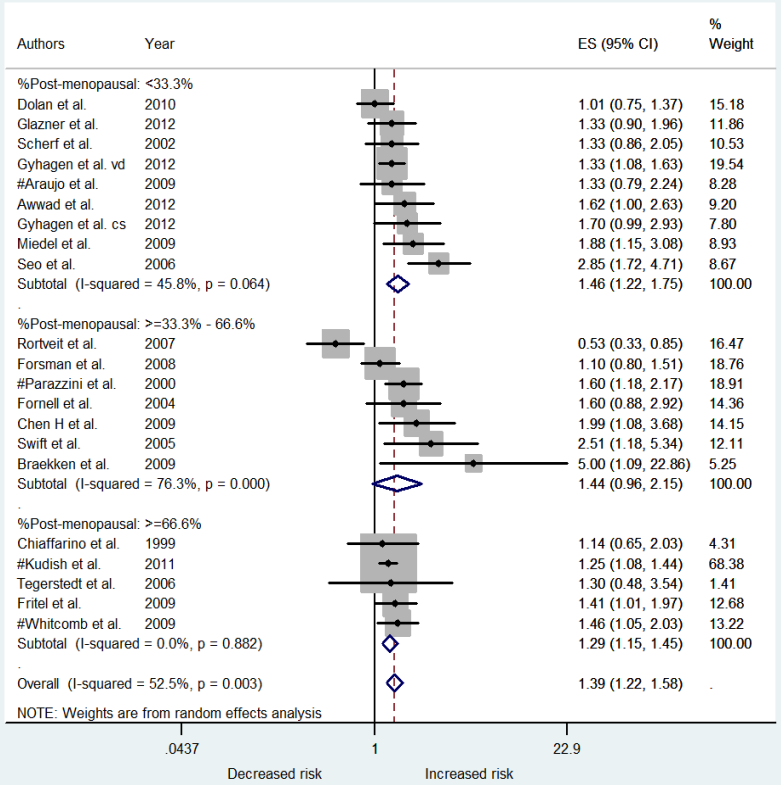
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-12: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. normal-weight categories in relation to POP by category of % post-menopausal women in study (<33%, ≥33%-<67% ≥67%)

A



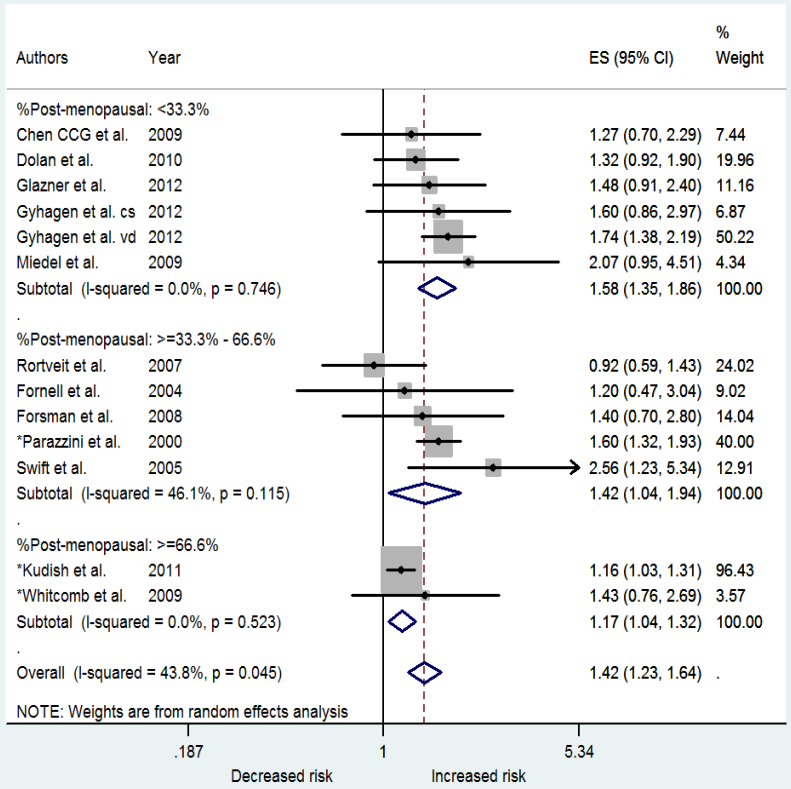
B



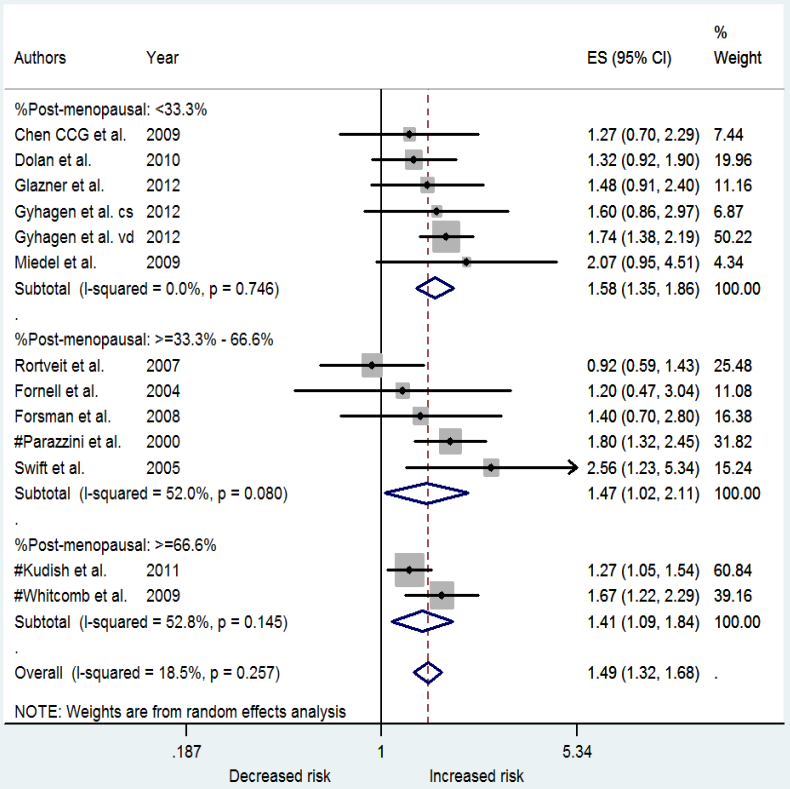
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-13: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by category of % post-menopausal women in study (<33%, ≥33%-<67% ≥67%)

A



B

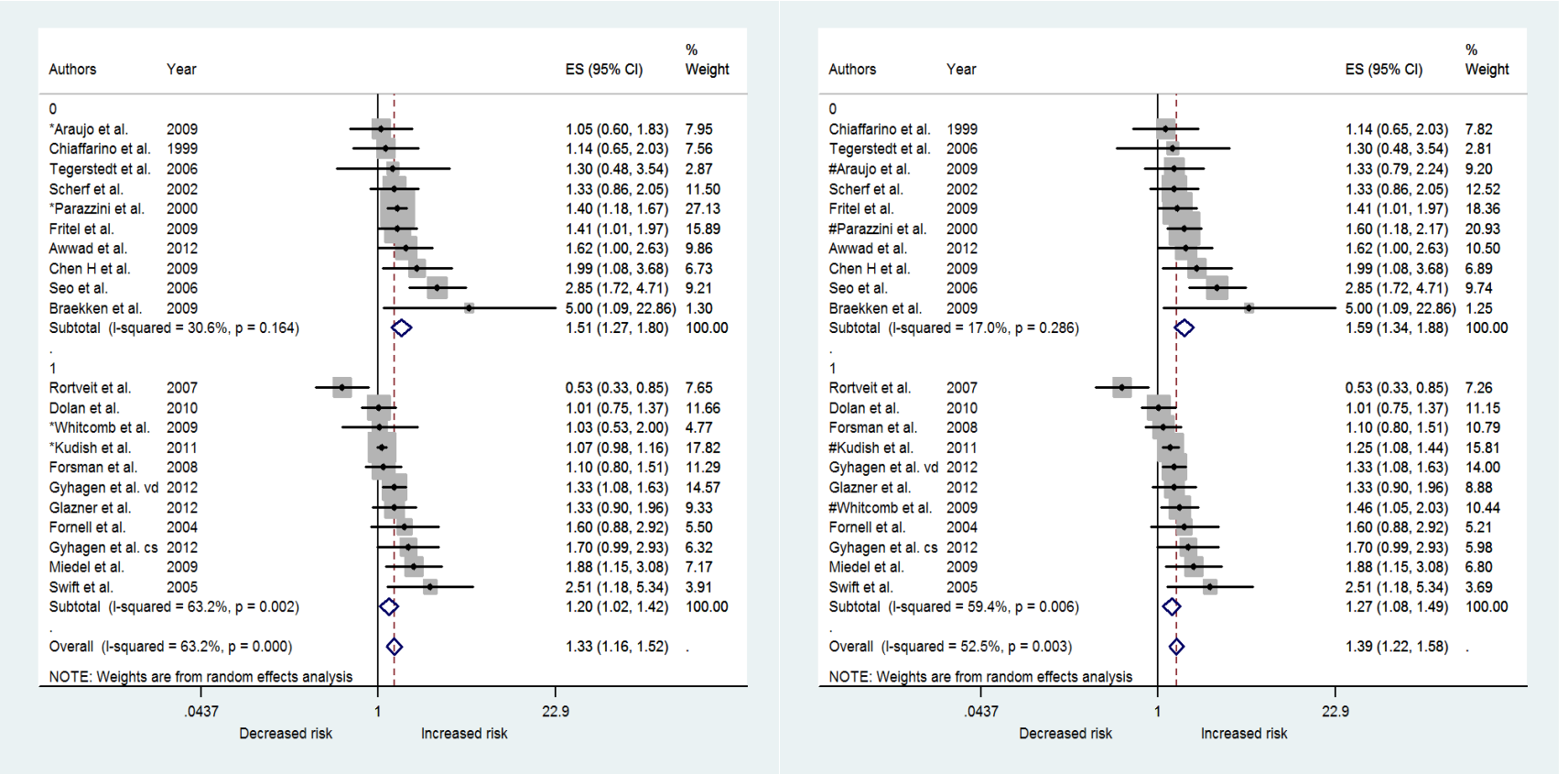


A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-14: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. over-weight categories in relation to POP by BMI category reporting; World Health Organization criteria (yes, no)

A

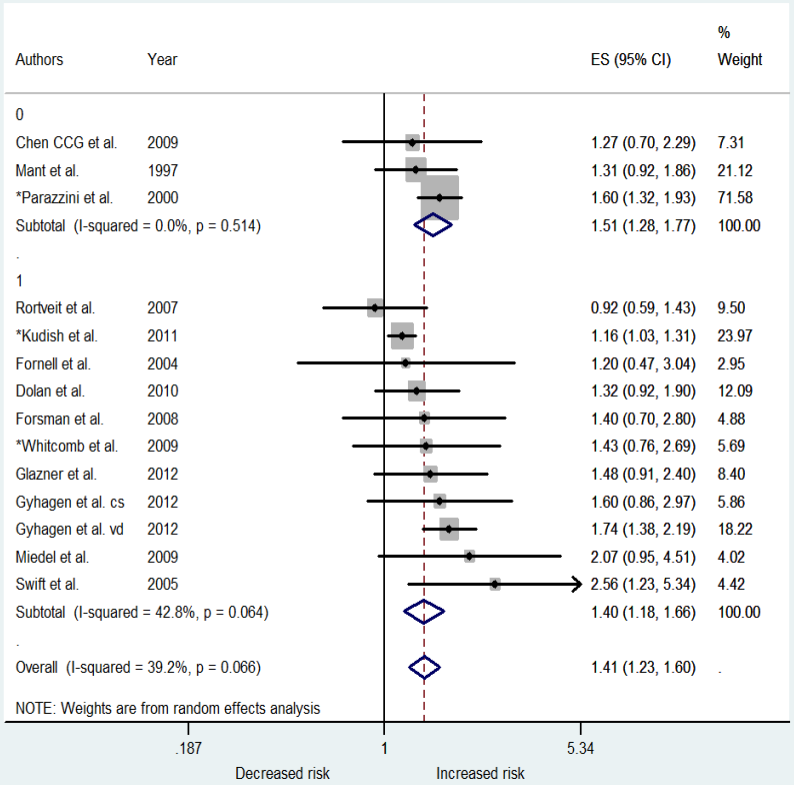
B



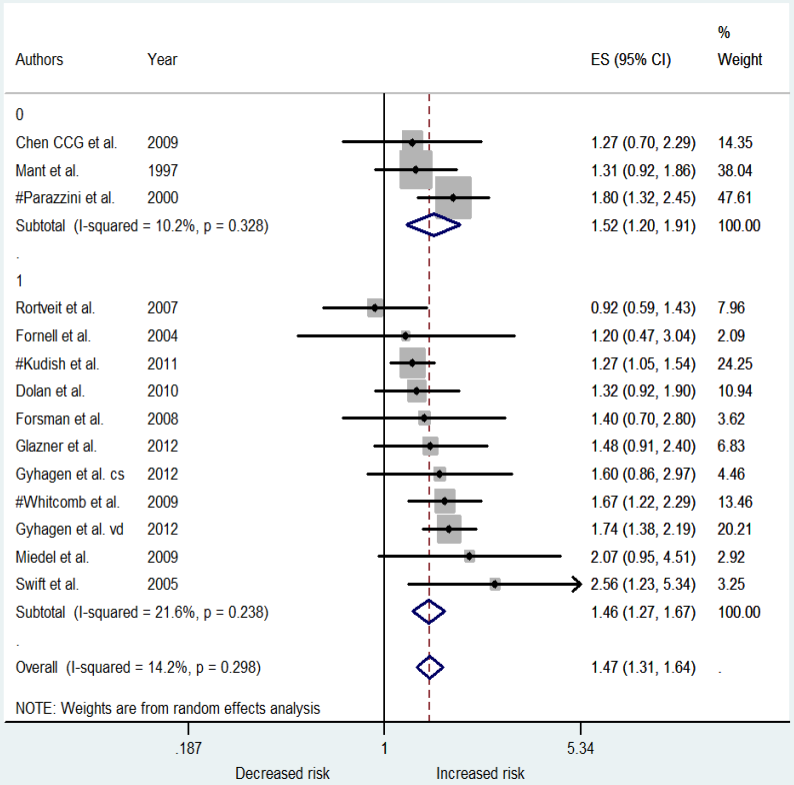
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-15: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by BMI category reporting; World Health Organization criteria (yes, no)

A



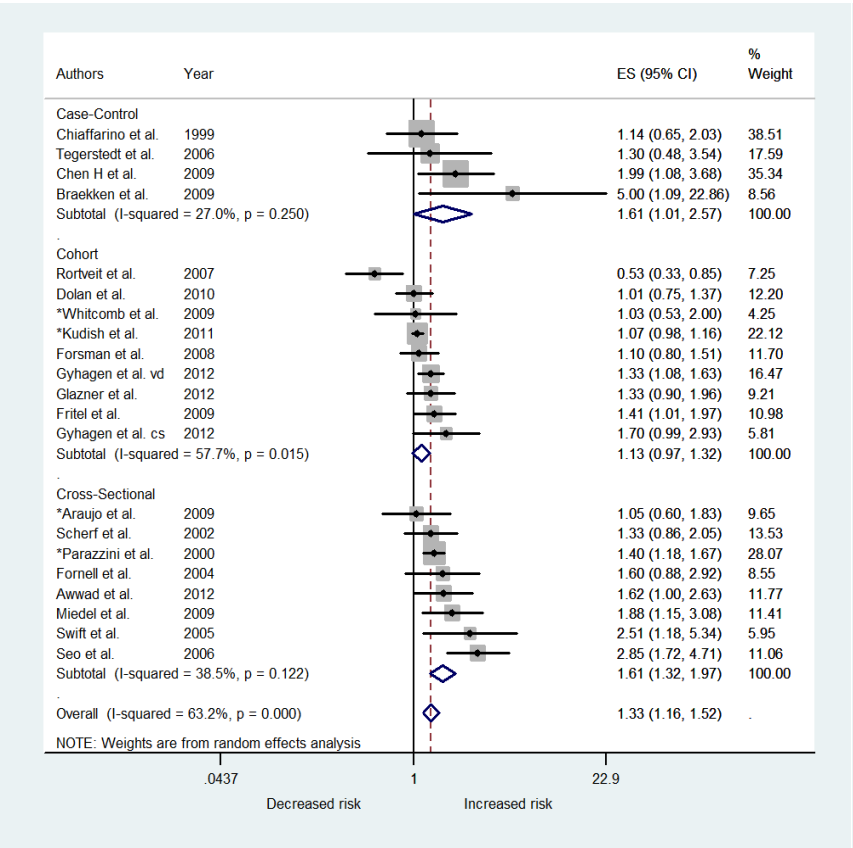
B



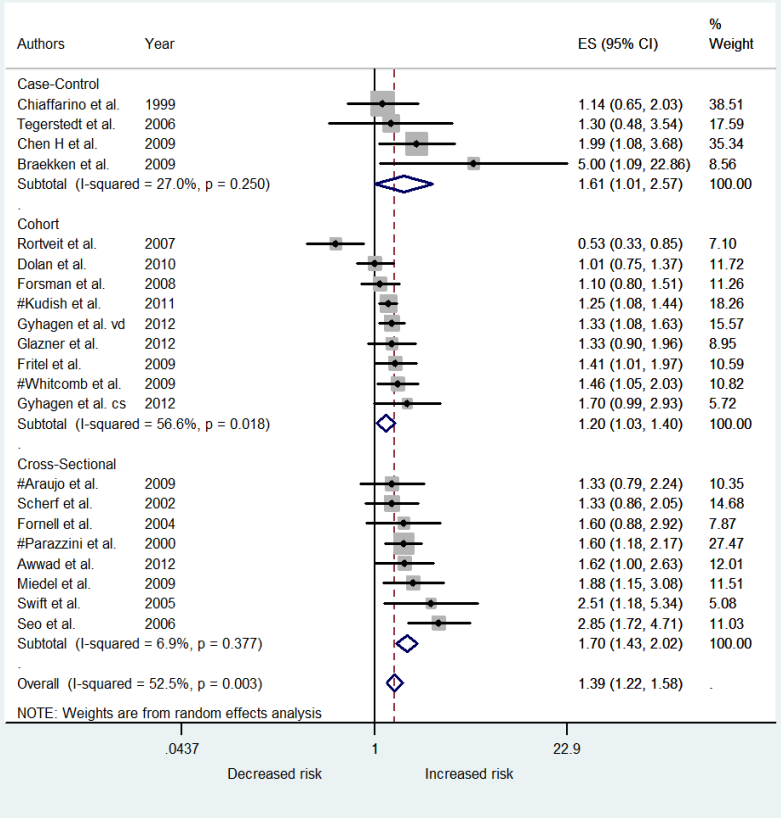
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-16: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. over-weight categories in relation to POP by reported study design

A



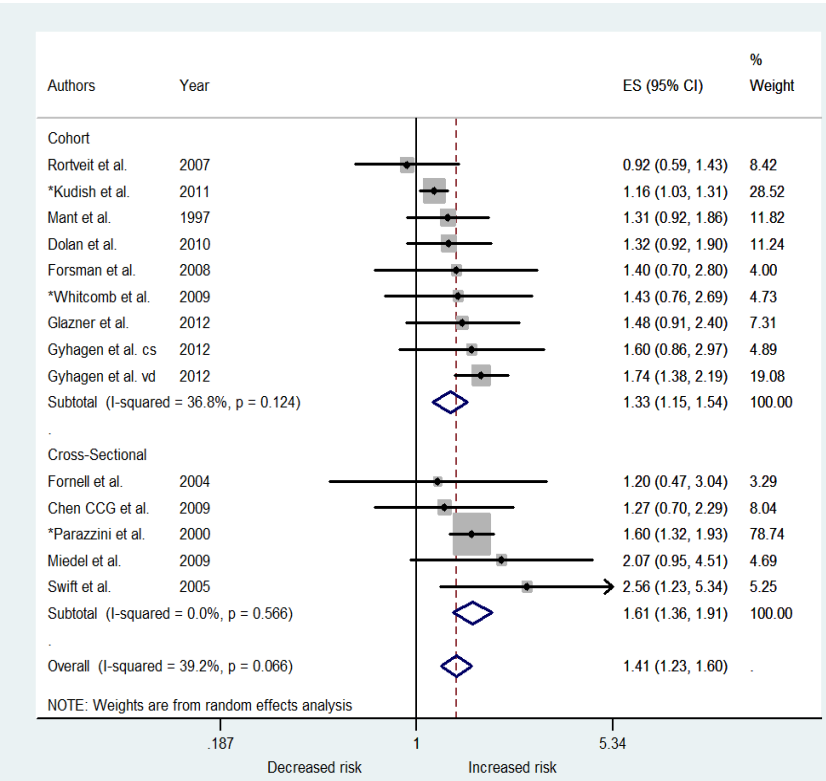
B



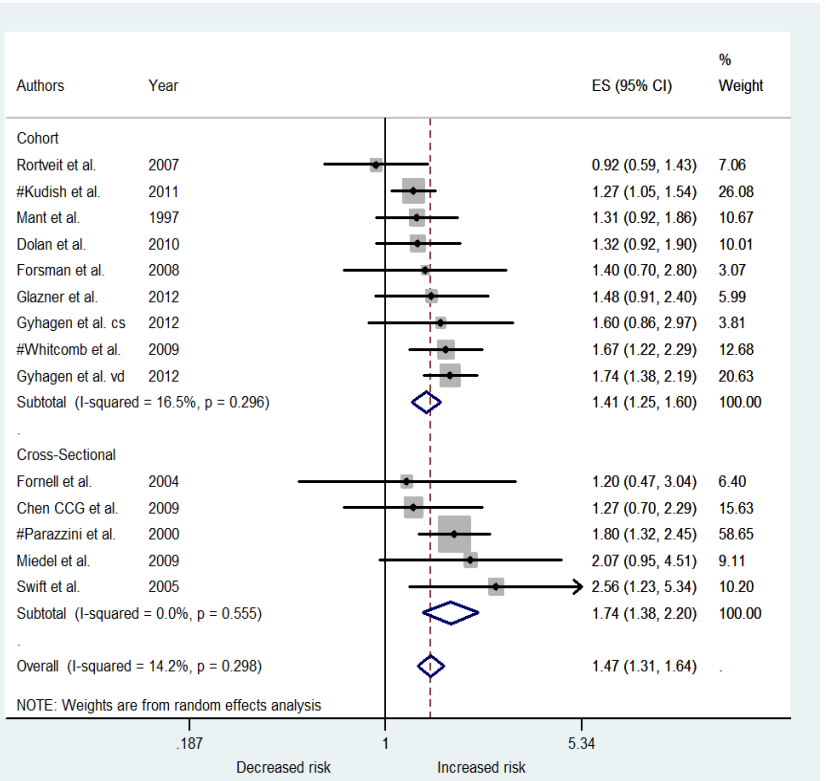
A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Figure 6-17: A-B. Subgroup-analyses: Forest plot for studies evaluating normal-weight vs. obese categories in relation to POP by study design

A



B



A: Minimum Scenario= represents the meta-analysis risk ratio when comparing the smallest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; B: Maximum Scenario= represents the meta-analysis risk ratio when comparing the largest effect estimates provided by a study in the scenario when a study reported two or more effect estimates; * represents the minimum estimate from studies that reported 2 or more estimates; # represents the maximum estimate from studies that reported 2 or more estimates; cs = estimates based on women who had caesarean section; vd= estimates based on women who had vaginal delivery

Discussion

We performed a systematic review of the medical literature published in English to evaluate analytic observational studies that reported the association between obesity measures and POP. Since BMI was the most widely reported obesity trait, we performed the first meta-analysis evaluating the association between categories of BMI in relation to POP. We show that among studies that evaluated a relative measure of risk (odds ratio, relative risk or hazard ratio), women in the overweight and obese categories were more likely to have POP than women in the normal-weight category.

In a recent systematic review of the risk factors for POP and recurrence, Vergeldt and colleagues performed a qualitative review of articles evaluating the association between measures of obesity and POP, among other risk factors [25]. The authors used extremely stringent criteria to only include studies that reported clinically-significant objectively measured POP in cross-sectional and cohort studies. While this strategy provided a potentially more homogeneous pool of high-quality studies to compare, it severely limited their sample size to only eight studies of which they reported that five studies reported statistically significant associations. Additionally, the authors did not perform a meta-analysis of the studies, most likely due to the small pool of studies. The authors concluded that BMI was a risk factor for POP on the basis that two or more studies reported statistically significant associations. They aptly stated that of the various suspected risk factors for POP, obesity was the only practically modifiable risk factor since genetic predisposition, aging and child birth can hardly be considered modifiable. Despite this most useful review, the field is still left with no consensus on the magnitude of association between measures of obesity and POP.

In this systematic review, we took a different approach by considering a less stringent eligibility criterion for study-inclusion into this meta-analysis. We placed no restriction on the age ranges of women which were evaluated in the study, or on the measurement criteria that studies utilized for assessing POP and additionally allowed studies with sample sizes as small as 40 POP cases to be included in the meta-analysis. This strategy was taken by design for the following reasons: 1) larger sample size provides higher statistical power for detecting associations, 2) an inclusive strategy allows for greater generalization of the study results, 3) including small and large studies allows for a more meaningful assessment of potential publication bias, and 4) larger sample size also allows for sub-group analyses to evaluate potential sources of heterogeneity by strata of study attribute. As we expected, there was a considerable amount of heterogeneity in effect-estimates (I^2 as high as 63%) attributed to factors other than random error in the overweight analysis category. Interestingly, there was negligible heterogeneity across studies for the obese category primary meta-analyses, most likely because the normal weight (referent) and obese categories were far enough apart for studies to consistently report larger effect estimates.

To identify potential sources of heterogeneity especially for the primary over-weight category meta-analysis, we conducted several sub-group meta-analyses. Some of the heterogeneity can most likely be attributed to the aggregation of the various methods of POP measurement. Sub-group analyses clearly showed that aggregation of effect estimates from studies reporting clinically significant objectively measured POP was much higher than aggregation of effect estimates from studies which reported symptomatic POP or any grade of objectively measured POP. We also found that studies which had higher proportions of post-menopausal women on average reported smaller effect estimates than studies which had a lower

proportion of post-menopausal women included in their population, suggesting yet another source of heterogeneity.

It is also of interest that studies that adjusted for key covariates tended to report on average stronger effect estimates than studies which did not adjust for key covariates. This was most apparent in the obese-category analyses. More so than identifying another potential source of heterogeneity, this difference illuminates the nature of scientific reporting where studies which had statistically significant or perhaps larger effect estimates were more likely to present analyses adjusted for key covariates than those studies which did not find a statistically significant association in univariate analyses. A greater tendency of publication bias is supported by the detection of possible publication bias evidenced in our overweight analyses, where smaller studies tended to present larger effect estimates; a majority of these smaller studies with larger estimates tended to be from case-control studies. This observation of potential small-study bias is further supported by the fact that a majority of the analytic studies which were not eligible in our meta-analysis because they reported only mean/median BMI by case-control status [18;95;99;157-161] or only reported effect estimate for BMI as a continuous variable [13;41;51;92;97;162;163] were case-control studies. Six out of the seven studies which used BMI as a continuous measure were case-control studies, only two of which reported a statistically significant association between BMI and POP. Similarly, six out of the eight studies which reported mean/median BMI by case-control status were case-control studies, none of which showed a statistically significant association between BMI and POP. A majority of these studies had relatively small sample sizes. However, this should not invalidate the results of our findings from cross-sectional/cohort studies, as these studies were more likely to utilize study samples that were representative of the general community being investigated. In our sub-group

analyses, even though we present separate effect estimates for cross-sectional and cohort design, it should be mentioned that majority of these studies performed a cross-sectional analyses where the assessment of BMI did not necessarily precede the assessment of POP.

This meta-analysis is limited in the level of causal inference that it can provide. Even though several studies stated that they had a cohort design, studies reported the association between current BMI and POP, where measurement of BMI did not necessarily precede POP measurement. Therefore, this meta-analysis can only conclude that among studies reported in the literature, individuals with higher BMI are more likely to have POP as opposed to stating that individuals with higher BMI are more likely to develop POP. Of the 22 studies included in the meta-analysis, only one study performed a prospective investigation of BMI in relation to POP and reported a positive association between BMI and POP [23]. Kudish and colleagues additionally performed a longitudinal investigation of POP progression in the same population (WHI-HT), which was not included in the meta-analysis due to overlapping populations [164]. In this study, they reported that the risk of rectocele, cystocele and uterine prolapse progression in overweight and obese women ranged from 32% to 69% (largest increase for uterine prolapse) compared with women with normal weight at baseline [164]. In their analysis they also reported that weight loss did not significantly reduce POP regression and suggested that damage to the pelvic floor associated with obesity may be irreversible. We note that there is a dearth of studies which prospectively evaluate the association between obesity measures and POP and the association between weight loss and POP progression. Our meta-analytic investigation of obesity measures and POP was limited to BMI as only two studies reported the association between waist-circumference and POP. If one of the mechanisms of obesity-induced POP is through elevated and sustained pressure to the pelvic support system, then it would follow that abdominal

obesity would be more relevant to the study of obesity and POP than BMI alone, which is a global measure of obesity.

There were several other studies which were ineligible for our meta-analysis but are worth mentioning. Based on the National Health and Nutrition Examination survey of 1,961 women across the US (with 58 POP cases), Nygaard and colleagues reported weighted prevalence rates for self-reported POP to be 1.7 (95% CI: 0.6, 2.9) in women with BMI <25 kg/m^2, 3.4 (95% CI: 1.2, 2.5) in women with BMI 25.0-29.9 kg/m^2, and 3.6 (95% CI: 2.0, 5.2) in women with BMI ≥30 kg/m2 [5]. Despite the increasing trend in prevalence rate by increasing category of BMI, the test for trend was not statistically significant. Three studies compared non-obese (BMI <30 kg/m^2) versus obese women (BMI ≥30 kg/m^2) and did not find any meaningful relationships between BMI and POP [165-167]. In a population-based cross-sectional assessment of middle-aged women in Michigan, Trowbridge and colleagues evaluated obese vs. non-obese women with POP-Q points as a continuous variable (while adjusting for age, race, parity, hysterectomy, estrogen use and stress urinary incontinence), and did not report any meaningful correlations [167]. It is possible that the authors may have over-adjusted their models, as we know from the literature that BMI is correlated with urinary incontinence, and adjusting for this variable may have removed any association between BMI and POP. Handa and colleagues [165] (N = 394, mean age = 47.6) and Washington and colleagues [168;169] (N = 1,011, median age = 39.5), did not find meaningful differences in the percent of women with clinically significant POP in non-obese (BMI <30 kg/m^2) versus obese women (BMI ≥ 30 kg/m^2). One possible explanation for disagreement with our conclusions could be that the reference group in these studies included overweight women, who we show in this meta-analysis have higher odds of POP than normal weight women. Additionally, the authors only presented percentages and did

not provide effect estimates adjusted for key confounders such as age, parity and race. Interestingly, Whitcomb and colleagues evaluated 1,155 (mean age = 56.4) obese women and reported that compared with women obese women (BMI 30-34.9 kg/m^2) women who were severely obese (BMI 35-35.9 kg/m^2) and morbidly obese (BMI $\geq$40 kg/m^2) had odds ratios of 1.55 (95% CI: 0.92, 2.60) and 2.09 (95% CI: 1.18, 3.68) for POP, respectively, in analyses adjusted for age, mode of delivery and parity [169].

In conclusion, our analytic review of the literature suggests that obesity as measured by BMI is positively associated with POP. The association between BMI and POP increases in magnitude with increasing categories of BMI and is larger for clinically significant POP. Obesity appears to be the only practically modifiable risk factor for POP. Given the dearth of studies which prospectively evaluate the association between obesity measures and POP and our lack of understanding of the underlying mechanisms involved in obesity and POP, there is a need for prospective and mechanistic investigations.

CHAPTER VII

Findings for Specific Aim 2: Do genetic variants modify the association between obesity and pelvic organ prolapse, or between parity and pelvic organ prolapse in European American, African American and Hispanic women from the Women's Health Initiative

Abstract

Background and Motivation: There is evidence to suggest that genetic predisposition, parity and obesity are all associated pelvic organ prolapse (POP). However, there is very little understanding of how genetic variations interact with parity and obesity to influence POP risk. We evaluated whether SNPs from 96 candidate genes (which have been linked to POP, connective tissue disorders, or obesity) interact with parity and obesity in European American, African American, and Hispanic women from the WHI-HT trial.

Methods: POP was evaluated in the WHI-HT at baseline and in select follow-up visits for uterine prolapse, rectocele and cystocele using the WHI-POP Grading system. Cases were characterized using two definitions: 1) grade 1 or higher POP for any one of the three types of prolapse either at baseline or follow-up visits (any POP) and 2) grade 2 or higher POP for any one of the three types of prolapse at baseline or follow-up visits (moderate/severe POP). Controls were also characterized using two definitions: 1) absence of all forms of POP in at least one WHI-HT visit and no mention of POP (all controls) and 2) absence of all forms of POP in at least two WHI-HT visits and no mention of POP (stringent controls). Using multiple logistic regression models, we first evaluated interactions between BMI (continuous) and SNPs (dosage) and parity (discrete) and SNPs (dosage) while adjusting for key covariates including age at diagnosis, BMI, parity and continuous axes of genetic ancestry for each of the four WHI sub-

studies: WHI-MS (European American), WHI-GARNET (European American), WHI-SHARe (African American) and WHI-SHARe (Hispanic) individually. Interaction beta-estimates (continuously modeled) from WHI-MS (European American), WHI-GARNET (European American), WHI-SHARe (African American) and WHI-SHARe (Hispanic) sub-studies were then aggregated using random-effects meta-analysis for each one of the four case-control sets. SNPs with a p-value less than 2.16×10^{-4} were considered (2 log values higher than the statistically significant mark) were considered for stratified analyses by strata of SNP dosage cut off point (dosage < 0.5 and dosage ≥0.5). Only the WHI-MS and WHI-GARNET datasets were considered for stratum-specific analyses since sample sizes for the African American and Hispanic populations were too small. Formal evaluation for effect modification for the association between BMI and POP and between parity and POP by strata of SNP was conducted using the likelihood-ratio test.

Results: Meta-analysis of 12,614 individuals included 5,702 European American women from WHI-MS (3,679 any POP cases of whom 1,116 were moderate/severe POP cases, and 2,023 controls of whom 1,002 were stringent controls), 4,218 European American women from WHI-GARNET (2,491 any POP cases of whom 657 were moderate/severe POP cases, and 1,727 controls of whom 534 were stringent controls), 1,761 African American women from WHI-SHARe (805 any POP cases of whom 156 were moderate/severe POP cases, and 958 controls of whom 344 were stringent controls), and 931 Hispanic women from WHI-SHARe (621 any POP cases of whom 168 were moderate/severe POP cases, and 310 controls of whom 115 were stringent controls). While we did not find any statistically significant interactions (p-value threshold 2.16×10^{-6}), meta-analysis of continuous interaction estimates for BMI and SNPs showed SNPs from seven independent loci which were suggestive (p-value < 2.16×10^{-4}) in at

least one of the four case-control formulations. Four of the seven SNPs which interacted with BMI were located in genes previously associated with obesity measures: intron variants in the *NRXN3* gene (rs31404, p: $2.54x10^{-5}$), *TMEM160* gene (rs76002066, p: $4.28x10^{-5}$), *CADM2* gene (rs62263916, p: $7.11x10^{-5}$), and *FTO* gene (rs17820328, p: $1.82x10^{-4}$). Additionally, an upstream variant within two kilo-bases of the *ELN* gene (rs55675441, p: $4.39x10^{-5}$), an intron variant in the *COL11A1* gene (rs71664978, p: $1.91x10^{-4}$) and a mis-sense variant in the *ZDHHC24* gene which is also upstream of the *ACTN3* gene (rs2305534, p: $5.96x10^{-5}$) interacted with BMI. In stratified analyses, SNP rs71664978 had the largest interaction odds ratio magnitude (Interaction OR: 0.53, LRT p: 0.042) in the moderate/severe POP analysis with stringent controls. Odds ratio for moderate/severe POP was higher for overweight women with rs71664978 ≤0.5 (Pooled OR: 2.09; 95% CI: 1.77, 2.48) than for overweight women with rs71664978 >0.5 (Pooled OR: 1.13; 95% CI: 0.62, 2.07). Variants in three loci interacted with parity below the suggestive threshold: intron variants in the *ITPR2* gene (rs7962822, p: $5.54x10^{-5}$), and *CADM2* gene (rs6801271, p: $9.91x10^{-5}$) and a variant upstream of the *ETV5* gene (rs200780606, p: $1.74x10^{-4}$) interacted with parity below the suggestive significance threshold. In stratified analyses, SNP rs200780606 had the largest interaction odds ratio magnitude (Interaction OR: 0.74; LRT p: 0.0006) in the moderate/severe POP analysis with stringent controls. Odds ratio for moderate/severe POP was higher for each additional birth in women with rs200780606 ≤0.5 (Pooled OR: 1.54; 95% CI: 1.46, 1.63) than in women with rs200780606 >0.5 (Pooled OR: 1.16; 95% CI: 0.99, 1.36).

Conclusions: In this hypothesis-driven evaluation of interactions between SNPs and BMI and SNPs and parity in relation to POP, we show that there is some evidence to suggest that SNPs in several genes associated with measures of obesity modify the association between BMI and POP and the association between parity and POP. SNPs in the elastin gene and collagen type 11-A1

gene may also modify the association between BMI and POP. However, these findings require validation from sufficiently powered, independent samples before any firm conclusions can be drawn. Validation of SNPs interacting with parity may be especially important as these may help identify parous individuals who are most at risk for POP, for whom prophylactic interventions may be designed prior to POP onset.

Introduction

Pelvic organ prolapse (POP) is a common condition affecting up to 40% post-menopausal women [22]; yet is relatively under-studied due to its sensitive nature combined with the difficulty of ascertainment. In POP, the pelvic organs including the uterus, bladder, bowels, and/or rectum descend from their normal positions into the vaginal space [1;2]. While not all women with POP are symptomatic or require surgical correction, prolapse approaching or extending past the introitus of the vagina is thought to be clinically significant [29]. Defects in one or more components of the pelvic support system, which is a complex web of musculature and connective tissue matrices, lead to loss of anatomical support and results in prolapse of one or more of the pelvic organs [66;73]. Anatomically, this is the most immediate basis for POP; however, the reasons behind this loss of support are not fully understood. The etiology of POP is complex and likely multifactorial, involving a combination of predisposing, inciting and promoting factors combining to manifest POP [1;25].

Child birth [16;22], especially through vaginal route of delivery [15], is one of the most well-studied and well-understood risk factors for POP [25]. Compared with nulliparous women, parous women have been consistently shown to have higher risk for POP. The pelvic support system is subjected to a tremendous amount of insult during the labor process, which is thought to be an inciting risk factor for POP. Additionally, modifiable risk factors such as obesity may promote the development of POP [25]. In Specific Aim 1, we showed that overweight and obese women were more likely to have POP than women in the normal weight category. However, it is not clear how obesity impacts POP; circumspectly, it is possible that either sustained elevated strain/pressure to the pelvic area, or potentially biochemical alterations in pelvic floor maintenance associated with obesity may impact POP.

A growing body of evidence also supports the role of genetic predisposition in relation to POP. A meta-analysis of observational studies showed that family history of POP was associated with 2.58 (95% confidence interval [CI]: 2.12, 3.15)-fold increased odds of POP compared with women without a family history of POP [83]. A study of monozygotic and dizygotic female twins in Sweden suggested shared-genetic factors explained 40% of variability in relation to POP [55]. Recognizing the heritable aspect of POP, several candidate-gene studies have evaluated the expression of genes or germ-line variation in genes which may be key players involved in the maintenance of the pelvic support system [26]. Additionally, two genome-wide association studies, one in women of European descent [57], and the other in African American and Hispanic women have evaluated POP [170]; both studies suggested different loci of interest. While replication of genetic polymorphisms across studies has not always been consistent, these studies have provided the literature with a strong list of candidate genes for investigation.

The notion of POP as a heritable condition, combined with the knowledge that POP does not always accompany important risk factors such as parity and obesity, leaves room for the possibility that genetic factors may modify the association between parity and POP and obesity and POP. Therefore, we sought to evaluate whether select single nucleotide polymorphisms (SNPs) modify the association between body mass index (BMI) and POP and the association between parity and POP. We chose SNPs from three categories of genes: 1) SNPs from candidate genes which have previously been evaluated in relation to POP, 2) SNPs from genes which have been associated with a broad spectrum of connective tissue disorders and 3) SNPs from gene-regions which have previously been associated with obesity measures. We chose to evaluate parity and BMI since parity is the strongest risk factor for POP and BMI is most likely the only practically modifiable risk for POP. To our knowledge, no other study has attempted to

evaluate interactions between genetic factors and other important risk factors for POP, most likely due to the reality that standardized information on POP and genetic data on participants have rarely been simultaneously available in sufficiently large numbers to allow testing for interactions. To perform this study, we utilized data from the WHI-HT trial, which is a truly unique resource as it provides unprecedented access to over 12,000 multi-ethnic post-menopausal women (European American, African American and Hispanic) for whom GWAS data, validated information on POP, and standardized information on risk factors for POP are simultaneously available.

Methods

For detailed description of methods utilized for this Specific Aim please revisit Chapter V. Sub-sections:

Parent Study for Specific Aims 2 and 3 (page numbers: 73-84)

Methods for Specific Aim 2 (page numbers: 85-120)

Results

Characteristics for European American, African American and Hispanic WHI-HT participants used in this study are provided in Table 7-1. Considering all of the 12,614 individuals included in this study, average age at baseline was 65.72 (SD = 7.01). European American women were on average slightly older at baseline and at age at POP evaluation than African American and Hispanic women. On average, African American women had slightly higher BMI, were more likely to be current smokers, and were more likely to have had a hysterectomy at baseline than European American or Hispanic women.

In Table 7-2 we report the association between BMI and parity for four formulations of case- and control definition sets for each of the four datasets. Compared with women in the normal-weight category (BMI <25 kg/m^2), women in the overweight/obese category (BMI ≥25 kg/m^2) had odds ratios ranging from 1.19 (95% CI: 0.83, 1.69) in the WHI-SHARe (Hispanic) subset to 1.30 (95% CI: 1.15, 1.46) in the WHI-MS subset, when evaluating cases with any POP and controls with at least one confirmed visit without POP (Set 1). When we restricted choice of controls to those who had at least two confirmed visits without POP (Set 2), the association between overweight/obesity increased with odds ratios for any POP ranging from 1.45 (95% CI: 1.00, 2.09) in the WHI-SHARe (African American) subset to 1.75 (95% CI: 1.42, 2.16) in the WHI-MS dataset. Each unit increase in parity was associated increased odds of any POP, with odds ratios ranging from 1.16 (95% CI: 1.07, 1.27) in the WHI-SHARe (Hispanic) subset to 1.19 (95% CI: 1.15, 1.24) in the WHI-MS subset when we used controls with at least one confirmed visit without POP (Set 1). Again, restricting the choice of controls to individuals with at least two confirmed visits without POP (Set 2) showed higher odds ratio estimates for each unit in parity than those obtained from Set 1. Similar increases in estimates for BMI and parity were observed

with moderate/severe POP when we restricted the choice of controls to individuals who had at least two confirmed visits without POP (Set 4), than when using all controls (Set 3). These observations suggested that there was possibly a lesser degree of outcome misclassification when stringent criteria were used to define controls.

For our primary analyses, we first modeled the interaction terms for BMI x SNP, and parity x SNP as continuous variables in all four of the individual datasets and then performed inverse-variance weighted random-effects meta-analysis for all four case-control sets.

Table 7-1. Characteristics of cases (Any POP) and controls participating in the WHI-HT by strata of genotyping platform and self-reported ethnicity/race

	WHI-MS (European American)		WHI-GARNET (European American)		WHI-SHARe (African American)		WHI-SHARe (Hispanic)	
	Cases (3679)	**Controls (2023)**	**Cases (2491)**	**Controls (1727)**	**Cases (805)**	**Controls (958)**	**Cases (621)**	**Controls (310)**
Continuous Variables	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age at baseline (years)	68.02 (5.7)	68.13 (6.11)	65.73 (6.80)	65.66 (7.02)	61.85 (6.96)	61.13 (7.02)	59.62 (6.35)	59.65 (6.30)
Age at evaulation (years)	68.83 (5.83)	70.51 (6.45)	66.44 (6.83)	67.04 (7.37)	62.76 (7.03)	62.88 (7.23)	60.31 (6.39)	61.32 (6.70)
BMI (kg/m^2)	28.73 (5.48)	27.82 (5.58)	30.01 (5.94)	29.22 (6.07)	31.71 (6.19)	31.62 (6.39)	29.95 (5.24)	29.79 (5.88)
Categorical Variables	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
BMI								
<25	990 (26.91)	689 (34.06)	542 (21.76)	484 (28.03)	93 (11.55)	134 (13.99)	102 (16.43)	64 (20.65)
25-30	1359 (36.94)	736 (36.38)	795 (31.91)	568 (32.89)	264 (32.80)	284 (29.65)	241 (38.81)	116 (37.42)
≥30	1324 (35.99)	558 (29.07)	1149 (46.13)	668 (38.68)	446 (55.40)	535 (55.85)	277 (44.61)	128 (41.29)
Missing	6 (0.16)	10 (0.49)	5 (0.20)	7 (0.41)	2 (0.25)	5 (0.52)	1 (0.16)	2 (0.65)
Smoking status								
Never	1917 (52.13)	992 (49.06)	1298 (52.13)	808 (46.87)	392 (48.70)	431 (45.04)	396 (63.77)	184 (59.35)
Past	1481 (40.28)	829 (41.00)	913 (36.67)	695 (40.31)	298 (37.02)	359 (37.51)	162 (26.09)	98 (31.61)
Current	225 (6.12)	183 (9.05)	251 (10.08)	210 (12.18)	103 (12.80)	147 (15.36)	54 (8.70)	28 (9.03)
Missing	54 (1.47)	18 (0.89)	28 (1.12)	11 (0.64)	12 (1.49)	20 (2.09)	9 (1.45)	0 (0.00)
Hormone therapy use								
Never	2099 (57.05)	1004 (49.63)	1293 (51.91)	696 (40.30)	459 (57.02)	509 (53.13)	366 (58.94)	162 (52.26)
Past	1294 (35.17)	837 (41.37)	910 (36.53)	809 (46.84)	280 (34.78)	360 (37.58)	184 (29.63)	113 (36.45)
Current	241 (6.55)	167 (8.26)	152 (6.10)	160 (9.26)	51 (6.34)	85 (8.87)	54 (8.70)	34 (10.97)
Missing	45 (1.22)	15 (0.74)	136 (5.46)	62 (3.59)	15 (1.86)	4 (0.42)	17 (2.74)	1 (0.32)
Hysterectomy								
No	2960 (80.46)	1076 (53.19)	1542 (61.90)	512 (29.65)	463 (57.52)	286 (29.85)	452 (72.79)	100 (32.26)
Yes	719 (19.54)	947 (46.81)	949 (38.10)	1215 (70.35)	342 (42.48)	672 (70.15)	169 (27.21)	210 (67.74)

(Table Continued)

Categorical Variables	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Parity (full term births)								
0	245 (6.66)	290 (14.34)	155 (6.22)	186 (10.78)	81 (10.06)	160 (16.72)	39 (6.29)	31 (10.00)
1	226 (6.14)	161 (7.96)	144 (5.78)	152 (8.81)	91 (11.30)	148 (15.46)	44 (7.10)	32 (10.32)
2	697 (18.95)	423 (20.91)	498 (19.99)	365 (21.26)	181 (22.48)	210 (21.94)	115 (18.55)	63 (20.32)
3	956 (25.99)	466 (23.04)	557 (22.36)	418 (24.23)	143 (17.76)	170 (17.76)	109 (17.58)	66 (21.29)
4	710 (19.30)	342 (16.91)	496 (19.91)	297 (17.22)	107 (13.29)	99 (10.34)	119 (19.19)	43 (13.87)
≥5	822 (22.34)	328 (16.21)	629 (25.25)	301 (17.45)	191 (23.73)	162 (16.93)	184 (29.68)	72 (23.23)
Missing	23 (0.63)	13 (0.64)	12 (0.48)	6 (0.35)	11 (1.37)	8 (0.84)	10 (1.61)	3 (0.97)

Cases = grade 1-3 POP; Controls = grade 0 POP in at least one WHI-visit; SD = standard deviation

Table 7-2. Associations for BMI and POP, and parity and POP for varying definitions of case-control sets

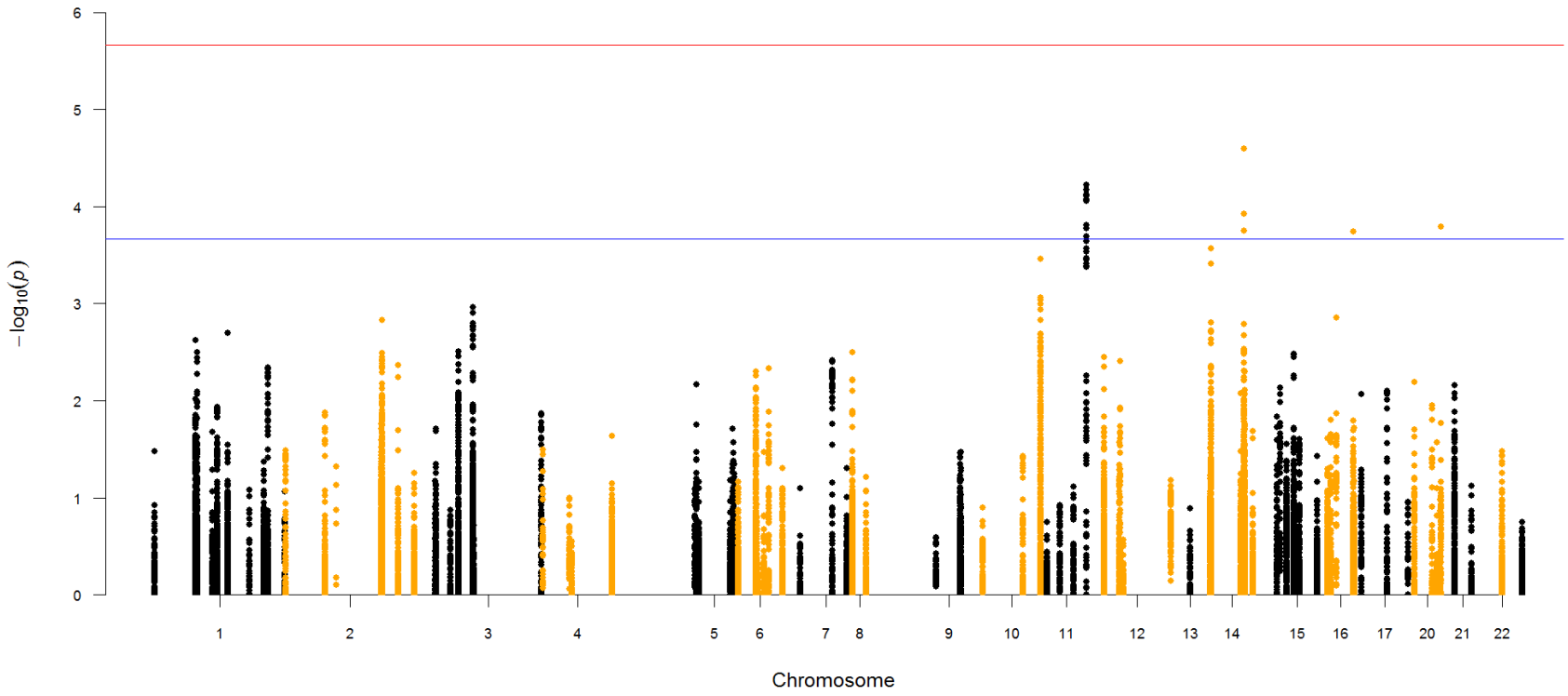
		WHI-MS (European American)		WHI-GARNET (European American)		WHI-SHARe (African American)		WHI-SHARe (Hispanic)	
Case-control sets	**Variable**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Set 1: Any POP vs. All Controls	BMI ≥25kg/m^2 vs. <25 kg/m^2	1.30	(1.15, 1.46)	1.34	(1.16, 1.55)	1.26	(0.94, 1.68)	1.19	(0.83, 1.69)
	Parity (continuous)	1.19	(1.15, 1.24)	1.18	(1.13, 1.23)	1.18	(1.11, 1.25)	1.16	(1.07, 1.27)
Set 2: Any POP vs. Stringent Controls	BMI ≥25kg/m^2 vs. <25 kg/m^2	1.47	(1.26, 1.72)	1.75	(1.42, 2.16)	1.45	(1.00, 2.09)	1.48	(0.90, 2.44)
	Parity (continuous)	1.26	(1.20, 1.33)	1.28	(1.20, 1.37)	1.26	(1.16, 1.36)	1.34	(1.18, 1.53)
Set 3: Mod/Sev POP vs. All Controls	BMI ≥25kg/m^2 vs. <25 kg/m^2	1.64	(1.38, 1.95)	1.75	(1.39, 2.21)	1.17	(0.69, 1.96)	1.20	(0.70, 2.06)
	Parity (continuous)	1.39	(1.32, 1.47)	1.34	(1.25, 1.43)	1.37	(1.23, 1.53)	1.47	(1.28, 1.69)
Set 4: Mod/Sev POP vs. Stringent Controls	BMI ≥25kg/m^2 vs. <25 kg/m^2	1.92	(1.57, 2.35)	2.21	(1.67, 2.93)	1.26	(0.71, 2.24)	1.56	(0.78, 3.10)
	Parity (continuous)	1.49	(1.40, 1.59)	1.49	(1.37, 1.63)	1.48	(1.30, 1.68)	1.75	(1.45, 2.11)

OR = odds ratio

BMI x SNP Interactions

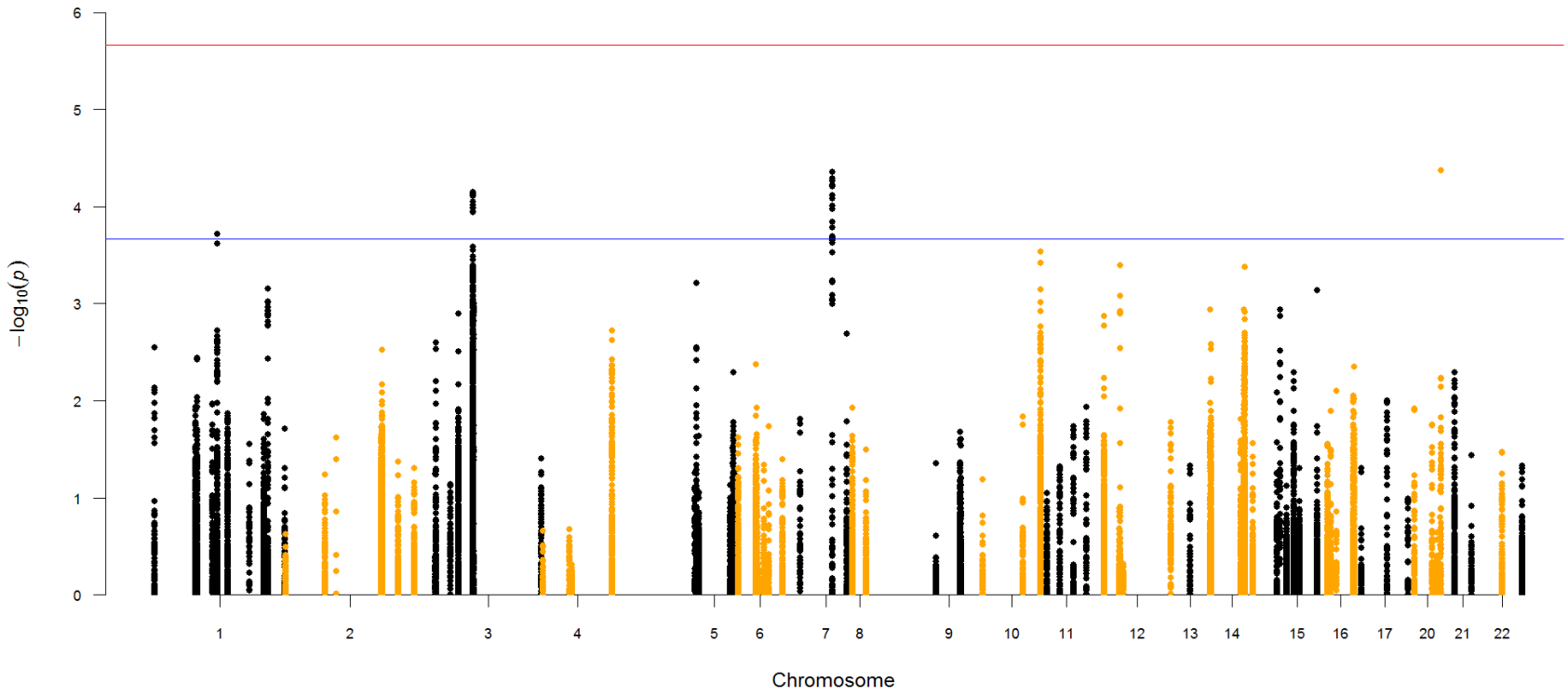
Manhattan plots for meta-analysis p-value estimates for BMI x SNP for all four case-control sets are shown in Figure 7-1 to 6-4. An examination of negative $\log_{10}$p-values alone from the Manhattan plots did not show consistent patterns across the four meta-analysis sets. Meta-analysis of interaction beta-estimates (BMI x SNP) and negative $\log_{10}$p-values for SNPs which had p-values less than 2.16×10^{-4} originating from any of the four case-control sets are shown in Figure 7-5. Analyses considering any POP vs. all controls (Set 1) and any POP vs. stringent controls (Set 2) showed smaller p-value above the suggestive statistical significance threshold, primarily attributed to large sample size. However, the interaction beta-estimates for BMI x SNP were almost always larger for analyses utilizing stringent controls than for case-control sets utilizing all-controls.

Figure 7-1. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of BMI x SNP interaction estimates (modeled continuously) for Any POP vs. all controls



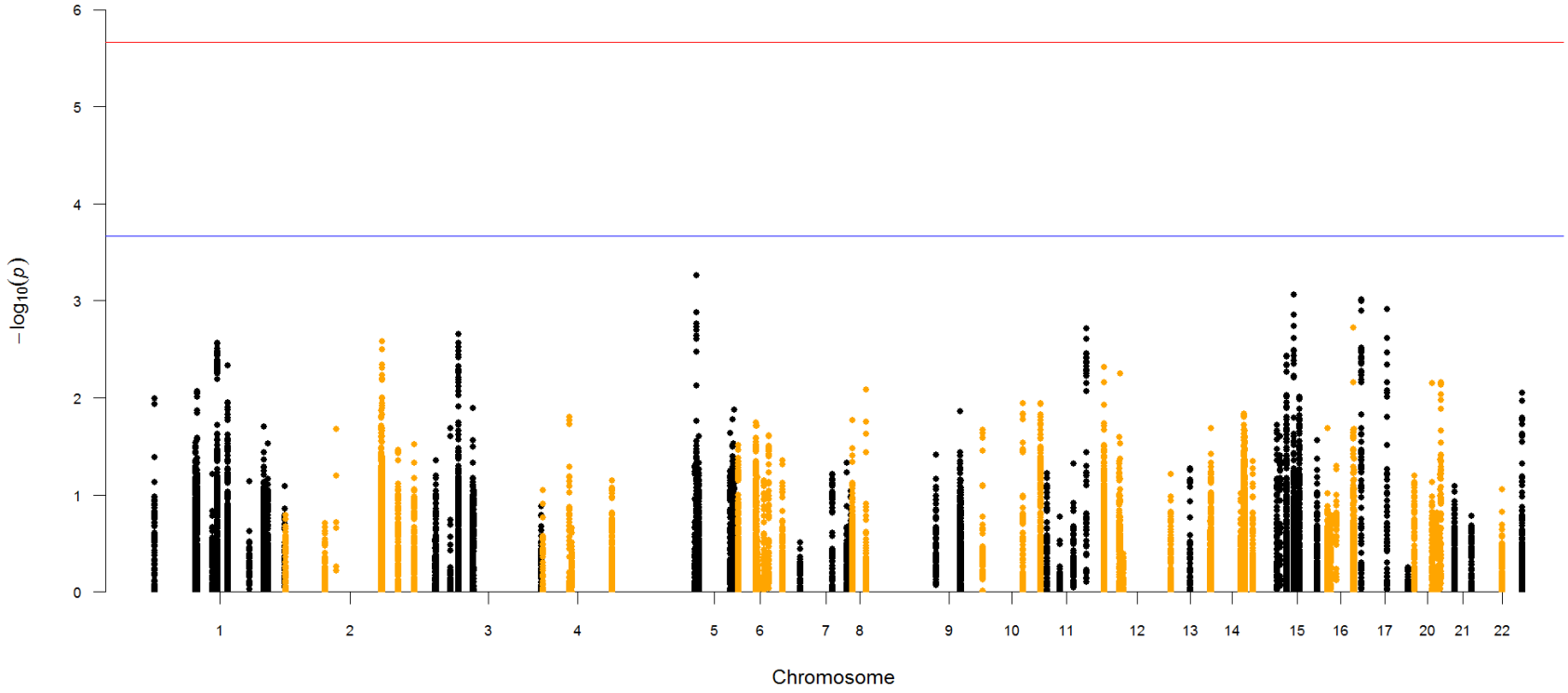
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-2. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of BMI x SNP interaction estimates (modeled continuously) for Any POP vs. stringent controls



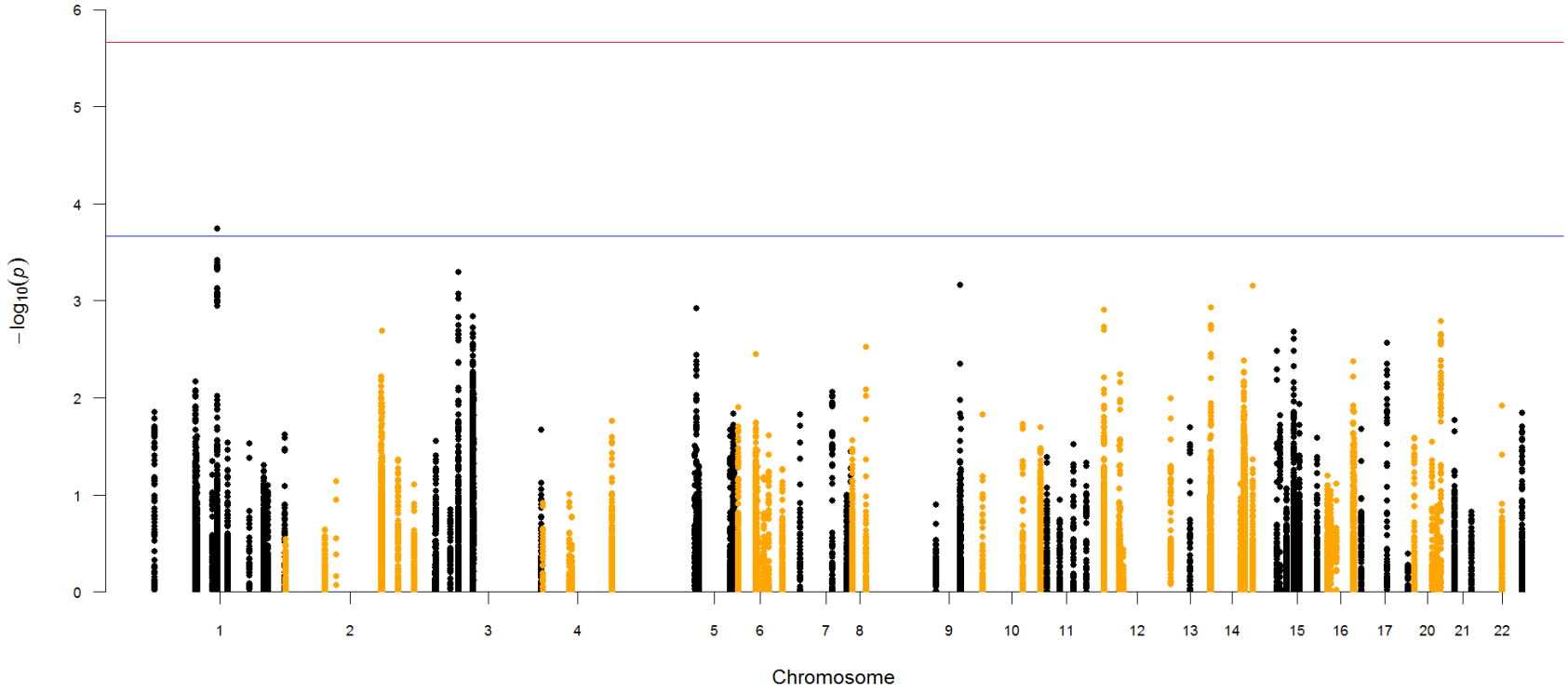
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-3. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of BMI x SNP interaction estimates (modeled continuously) for moderate/severe POP vs. all controls



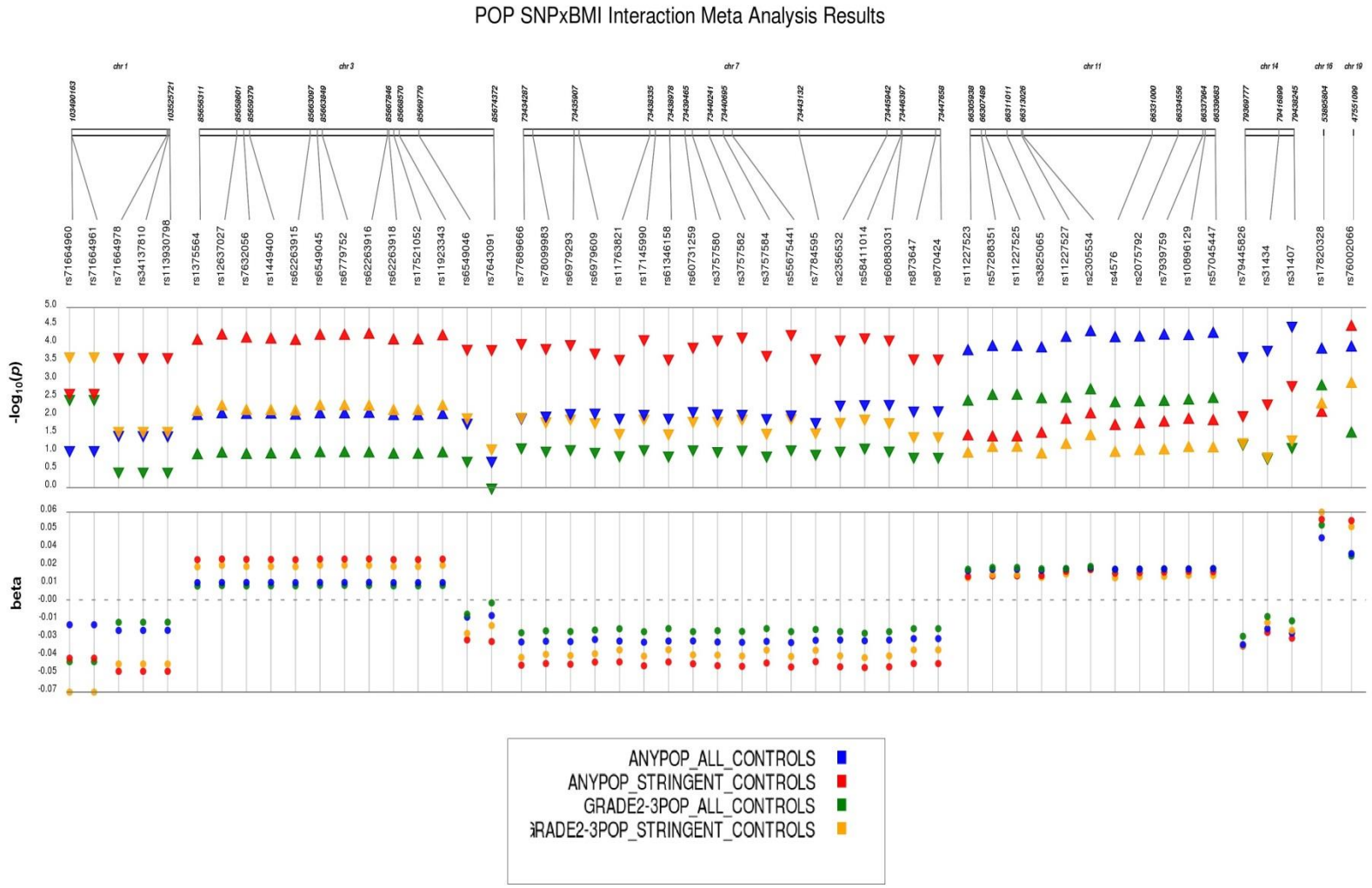
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-4. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of BMI x SNP interaction estimates (modeled continuously) for moderate/severe POP vs. stringent controls



Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

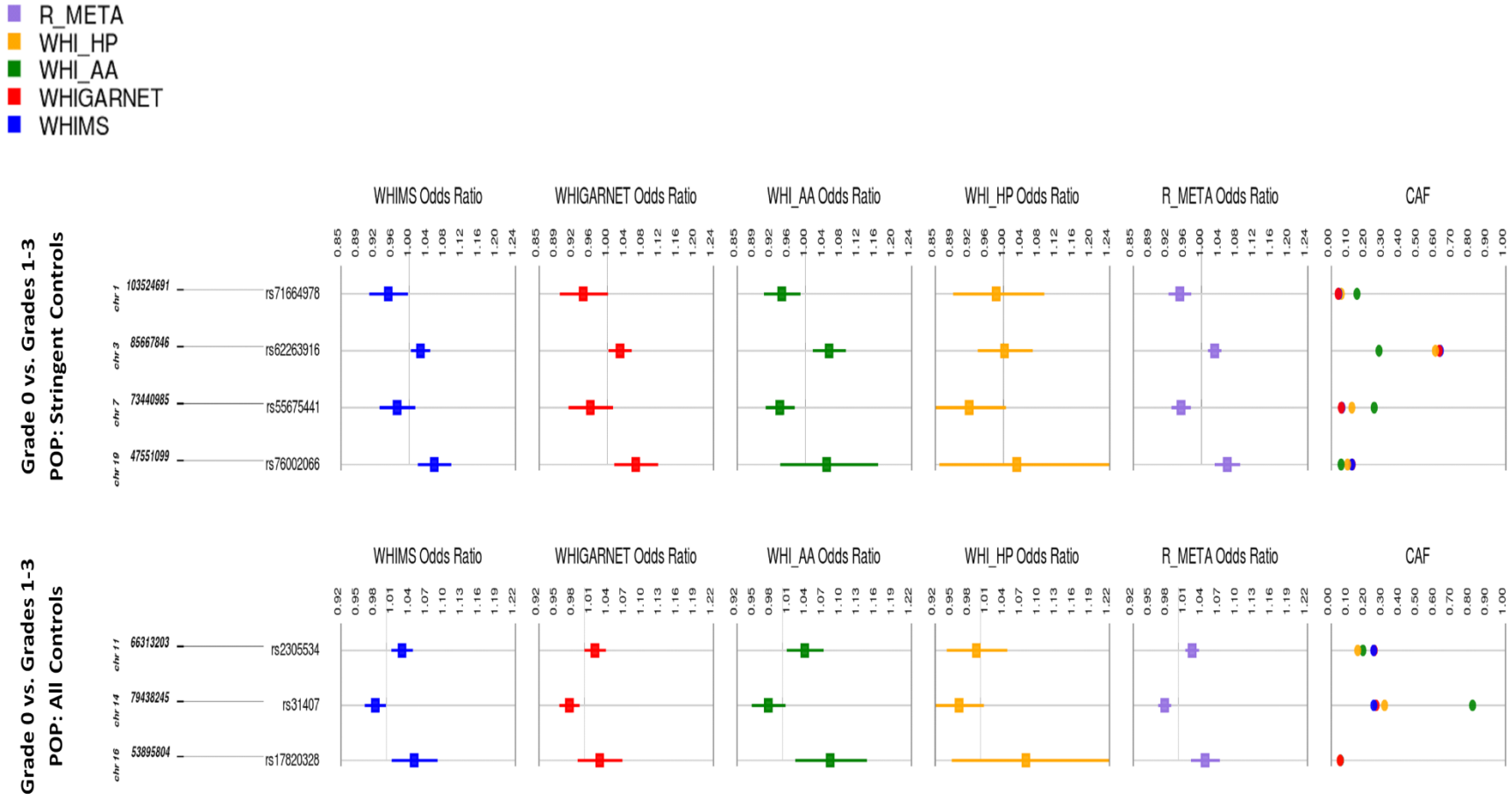
Figure 7-5. Plot summarizing beta-estimates and negative log_{10} p-values for top suggestive loci from meta-analysis of BMI x SNP interaction estimates (modeled continuously) for all four datasets



Meta-analysis: Any POP vs. all controls (blue); Any POP vs. stringent controls (red); moderate/severe POP vs. all controls (green); moderate/severe POP vs. stringent controls (yellow)

Additionally, meta-analysis interaction beta-estimates were always in the same direction for all four models for any given SNP (either consistently negative or consistently positive). Estimates of interaction odds ratios/beta-estimates for the most-statistically significant SNPs (meta-analysis $p < 2.16x10^{-4}$) in seven independent loci for each dataset and their meta-analysis estimates are provided in Figure 7-6 and Table 7-3. Three of the seven independent loci were identified in analyses using all controls whereas the remaining four loci were identified in analyses using stringent controls. Four of the seven SNPs which interacted with BMI were located in genes previously associated with obesity measures with $p <2.16x10^{-4}$: intron variants in the *NRXN3* gene (rs31404, p-value: $2.54x10^{-5}$), *TMEM160* gene (rs76002066, p-value: $4.28x10^{-5}$), *CADM2* gene (rs62263916, p: $7.11x10^{-5}$), and *FTO* gene (rs17820328, p: $1.82x10^{-4}$). Additionally, an upstream variant within 2 kilo-bases of the *ELN* gene (rs55675441, p: $4.39x10^{-5}$), an intron variant in the *COL11A1* gene (rs71664978, p: $1.91x10^{-4}$) and a mis-sense variant in the *ZDHHC24* gene which is also upstream of the *ACTN3* gene (rs2305534, p: $5.96x10^{-5}$) interacted with BMI below the threshold value.

Figure 7-6. Plot summarizing beta-estimates of BMI x SNP interaction estimates (modeled continuously) and effect-allele frequency for top SNPs from seven independent loci for each of the four datasets



BMI-SNP interaction odds ratios (exponentiated): WHI-MS (blue), WHI-GARNET (red) WHI-SHARe African American (green), WHI-SHARe Hispanic (yellow) and Meta-analysis odds ratio (purple)

Table 7-3. Meta-analysis of interaction beta-estimates for BMI (continuous) x SNP (continuous) interaction analyses in relation to POP in the WHI-HT

					WHI-MS		WHI-GARNET		WHI-SHARe (African American)		WHI-SHARe (Hispanic)		Meta-analysis		
Any POP (Original Controls)															
RSID	**CHR**	**POS**	**EA/RA**	**EAF***	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**P-value**
rs2305534	11	66313203	C/T	0.24/0.25/0.18/0.15	0.03	0.01	0.02	0.01	0.04	0.02	-0.01	0.03	0.02	0.01	$5.96x10^{-05}$
rs31407	14	79438245	C/G	0.24/0.26/0.81/0.31	-0.02	0.01	-0.03	0.01	-0.02	0.01	-0.04	0.02	-0.02	0.01	$2.54x10^{-05}$
rs17820328	16	53895804	G/A	0.05/0.05/0.05/0.05	0.05	0.02	0.03	0.02	0.08	0.03	0.07	0.06	0.04	0.01	$1.82x10^{-04}$
Any POP (Stringent Controls)															
RSID	**CHR**	**POS**	**EA/RA**	**EAF***	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**Int-Beta**	**Int-SE**	**P-value**
rs71664978	1	103524691	C/T	0.04/0.04/0.15/0.06	-0.05	0.02	-0.06	0.03	-0.05	0.02	-0.02	0.05	-0.05	0.01	$1.91x10^{-04}$
rs62263916	3	85667846	T/G	0.62/0.62/0.27/0.60	0.02	0.01	0.03	0.01	0.05	0.02	0.00	0.03	0.03	0.01	$7.11x10^{-05}$
rs55675441	7	73440985	C/T	0.06/0.06/0.24/0.12	-0.03	0.02	-0.04	0.03	-0.06	0.02	-0.08	0.04	-0.05	0.01	$4.39x10^{-05}$
rs76002066	19	47551099	A/G	0.12/0.12/0.06/0.09	0.05	0.02	0.06	0.02	0.05	0.05	0.03	0.09	0.06	0.01	$4.28x10^{-05}$

EA= Effect allele; RA=Reference allele; *Effect allele frequency for WHI-MS/WHI-GARNET/WHI-SHARe (African American)/WHI-SHARe (Hispanic)

Meta-analysis and pooled odds ratios for European American women (WHI-MS and WHI-GARNET) by strata of SNP (dosage: ≤ 0.5 and >0.5) for BMI (≥25 kg/m^2 compared with BMI <25 kg/m^2) are shown in Table 7-4 for any POP vs. stringent controls, and in Table 7-5 for moderate/severe POP vs. stringent controls. With the exception of rs17820328, for which, evidence for effect modification was minimal, based on the likelihood ratio test (LRT) p = 0.05 threshold, all other SNPs showed evidence for interaction in either the any POP or the moderate/severe POP analyses. The direction of interaction was consistent for all SNPs across both models (any POP and moderate/severe POP). Evidence for effect modification (LRT p < 0.05) for the association between BMI and POP was consistently noted in both models (any POP and moderate-severe POP analyses) for rs71664978, rs62263916 and rs31407. The odds ratio for any POP was higher for overweight women with rs71664978 ≤ 0.5 (Pooled OR: 1.63; 95% CI: 1.43, 1.85) than for overweight women with rs71664978 >0.5 (Pooled OR: 0.94; 95% CI: 0.60, 1.48); interaction p = 0.022) (Table 7-4). Similarly, the odds ratio for any POP was higher for overweight women with rs31407 ≤0.5 (Pooled OR: 1.75; 95% CI: 1.49, 2.07) than for overweight women with rs31407 >0.5 (Pooled OR: 1.32; 95% CI: 1.09, 1.60); interaction p = 0.021). Odds ratio for any POP was lower for overweight women with rs62263916 ≤0.5 (Pooled OR: 1.07; 95% CI: 0.77, 1.51) than for overweight women with rs62263916 >0.5 (Pooled OR: 1.65; 95% CI: 1.45, 1.89); interaction p = 0.021). With the exception of rs55675441, rs17820328 and rs76002066, the interaction odds ratios for all other SNPs were stronger (away from the null) in the moderate/severe analyses (Table 7-5) than for the any POP analyses (Table 7-4).

Table 7-4. Stratum specific interaction analyses for BMI and SNP in relation to Any POP in the WHI-MS and WHI-GARNET datasets: BMI <25 kg/m^2 (ref) vs. BMI ≥25 kg/m^2 by allele strata

				WHI-MS		WHI-GARNET		Meta-Analysis		Pooled Interaction			
SNP	**EA/RA**	**EA dosage**	**Gene**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**P-value**
rs71664978	C/T	≤0.5 (TT)	*COL11A1*	1.55	(1.32, 1.83)	1.81	(1.46, 2.25)	1.62	(1.31, 2.01)	1.63	(1.43, 1.85)	0.59	0.022
		>0.5 (CT/CC)		0.86	(0.50, 1.48)	1.10	(0.47, 2.57)	0.90	(0.59, 1.37)	0.94	(0.60, 1.48)		
rs62263916	T/G	≤0.5 (GG)	*CADM2*	0.95	(0.62, 1.44)	1.39	(0.79, 2.47)	1.03	(0.72, 1.46)	1.07	(0.77, 1.51)	1.51	0.021
		>0.5 (GT/TT)		1.58	(1.34, 1.87)	1.82	(1.45, 2.28)	1.65	(1.32, 2.06)	1.65	(1.45, 1.89)		
rs55675441	C/T	≤0.5 (TT)	*ELN*	1.50	(1.27, 1.77)	1.98	(1.59, 2.47)	1.61	(1.30, 2.00)	1.65	(1.44, 1.88)	0.63	0.022
		>0.5 (CT/CC)		1.31	(0.82, 2.09)	0.57	(0.27, 1.22)	0.75	(0.53, 1.07)	1.01	(0.68, 1.49)		
rs2305534	A/G	≤0.5 (GG)	*ZDHHC24/ACTN3*	1.30	(1.06, 1.61)	1.57	(1.18, 2.08)	1.37	(1.09, 1.71)	1.38	(1.17, 1.64)	1.25	0.076
		>0.5 (AG/AA)		1.73	(1.37, 2.19)	2.01	(1.46, 2.77)	1.81	(1.28, 2.54)	1.79	(1.49, 2.16)		
rs31407	C/T	≤0.5 (TT)	*NRXN3*	1.63	(1.33, 2.00)	2.05	(1.55, 2.72)	1.73	(1.30, 2.30)	1.75	(1.49, 2.07)	0.74	0.021
		>0.5 (CT/CC)		1.26	(0.99, 1.62)	1.48	(1.08, 2.04)	1.33	(1.03, 1.72)	1.32	(1.09, 1.60)		
rs17820328	C/G	≤0.5 (GG)	*FTO*	1.45	(1.23, 1.71)	1.7	(1.36, 2.13)	1.52	(1.24, 1.85)	1.52	(1.34, 1.74)	1.18	0.422
		>0.5 (CG/CC)		1.74	(1.06, 2.85)	2.33	(1.20, 4.49)	1.86	(0.88, 3.94)	1.89	(1.28, 2.78)		
rs76002066	G/A	≤0.5 (AA)	*TMEM160*	1.32	(1.11, 1.58)	1.59	(1.25, 2.02)	1.39	(1.14, 1.70)	1.40	(1.22, 1.62)	1.58	0.003
		>0.5 (AG/GG)		2.13	(1.54, 2.96)	2.57	(1.63, 4.06)	2.24	(1.23, 4.08)	2.26	(1.73, 2.94)		

Analyses utilized stringent controls; EA= Effect allele; RA=Reference allele; OR = odds ratio; P-value based on likelihood ratio test comparing models with and without interaction term

Table 7-5. Stratum specific interaction analyses for BMI and SNP in relation to Moderate/Severe POP in the WHI-MS and WHI-GARNET datasets: BMI <25 kg/m^2 (ref) vs. BMI ≥25 kg/m^2 by allele strata

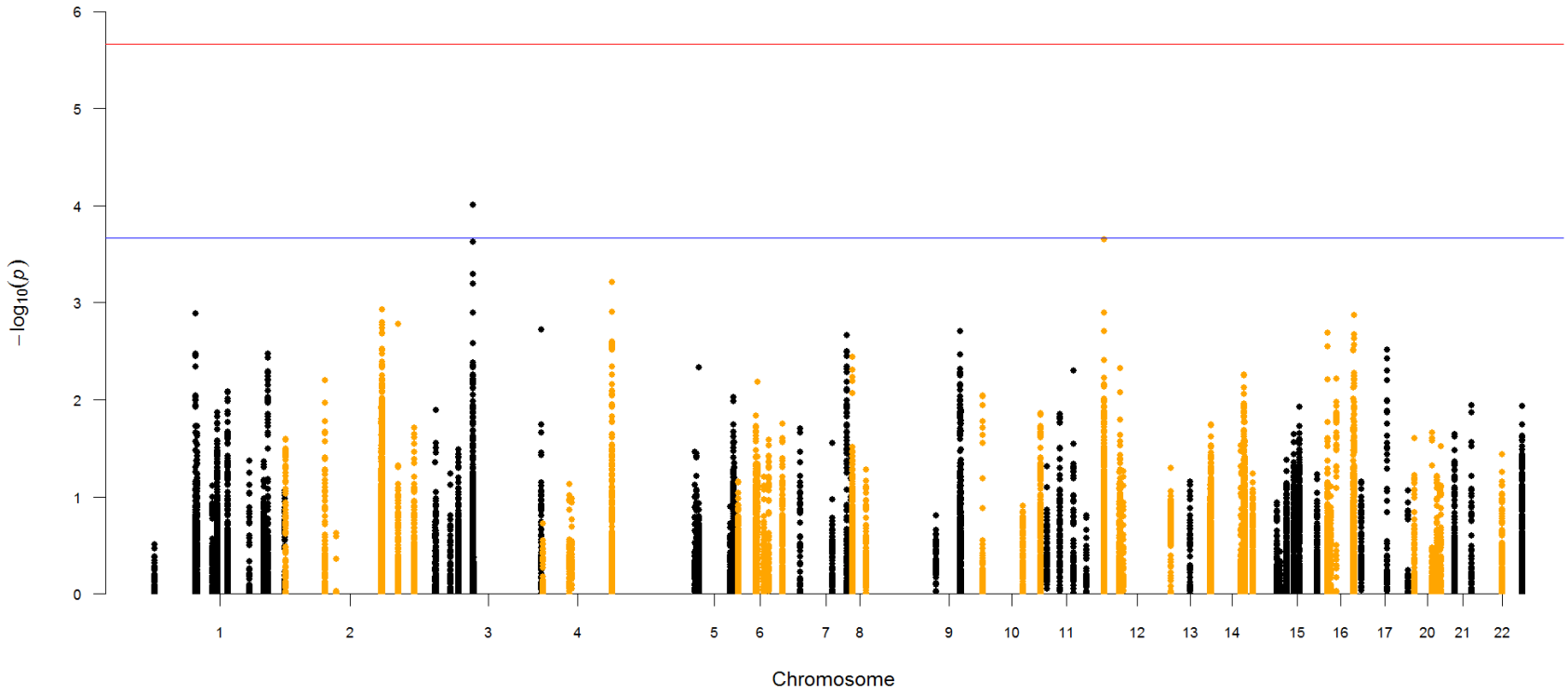
				WHI-MS		WHI-GARNET		Meta-Analysis		Pooled Interaction			
SNP	**EA/RA**	**EA dosage**	**Gene**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**P-value**
rs71664978	C/T	≤0.5 (TT)	*COL11A1*	2.03	(1.64, 2.50)	2.32	(1.73, 3.10)	2.11	(1.46, 3.03)	2.09	(1.77, 2.48)	0.53	0.042
		>0.5 (CT/CC)		1.15	(0.56, 2.36)	0.99	(0.30, 3.19)	1.09	(0.56, 2.14)	1.13	(0.62, 2.07)		
rs62263916	T/G	≤0.5 (GG)	*CADM2*	1.16	(0.68, 1.97)	1.72	(0.78, 3.79)	1.24	(0.71, 2.17)	1.31	(0.86, 2.02)	1.61	0.041
		>0.5 (GT/TT)		2.09	(1.68, 2.61)	2.29	(1.69, 3.10)	2.15	(1.46, 3.17)	2.14	(1.79, 2.55)		
rs55675441	C/T	≤0.5 (TT)	*ELN*	2.00	(1.61, 2.48)	2.38	(1.77, 3.20)	2.1	(1.45, 3.03)	2.09	(1.76, 2.49)	0.66	0.126
		>0.5 (CT/CC)		1.34	(0.72, 2.50)	1.68	(0.57, 5.01)	1.39	(0.65, 2.98)	1.37	(0.81, 2.33)		
rs2305534	A/G	≤0.5 (GG)	*ZDHHC24/ACTN3*	1.63	(1.25, 2.14)	1.78	(1.22, 2.58)	1.68	(1.16, 2.42)	1.67	(1.35, 2.08)	1.46	0.024
		>0.5 (AG/AA)		2.44	(1.78, 3.34)	2.79	(1.80, 4.32)	2.54	(1.32, 4.86)	2.49	(1.94, 3.21)		
rs31407	C/T	≤0.5 (TT)	*NRXN3*	2.31	(1.77, 3.02)	2.7	(1.83, 3.97)	2.41	(1.41, 4.11)	2.39	(1.92, 2.97)	0.65	0.009
		>0.5 (CT/CC)		1.48	(1.08, 2.04)	1.76	(1.16, 2.67)	1.56	(1.05, 2.31)	1.56	(1.22, 2.00)		
rs17820328	C/G	≤0.5 (GG)	*FTO*	1.91	(1.54, 2.37)	2.15	(1.59, 2.89)	1.98	(1.40, 2.79)	1.97	(1.65, 2.34)	1.11	0.708
		>0.5 (CG/CC)		1.99	(1.02, 3.87)	3.14	(1.23, 8.02)	2.15	(0.64, 7.18)	2.25	(1.32, 3.84)		
rs76002066	G/A	≤0.5 (AA)	*TMEM160*	1.75	(1.39, 2.21)	2.05	(1.49, 2.83)	1.83	(1.29, 2.59)	1.83	(1.52, 2.21)	1.44	0.063
		>0.5 (AG/GG)		2.71	(1.78, 4.13)	2.87	(1.56, 5.27)	2.76	(1.06, 7.17)	2.72	(1.93, 3.83)		

Analyses utilized stringent controls; EA= Effect allele; RA=Reference allele; OR = odds ratio; P-value based on likelihood ratio test comparing models with and without interaction term

Parity x SNP Interactions

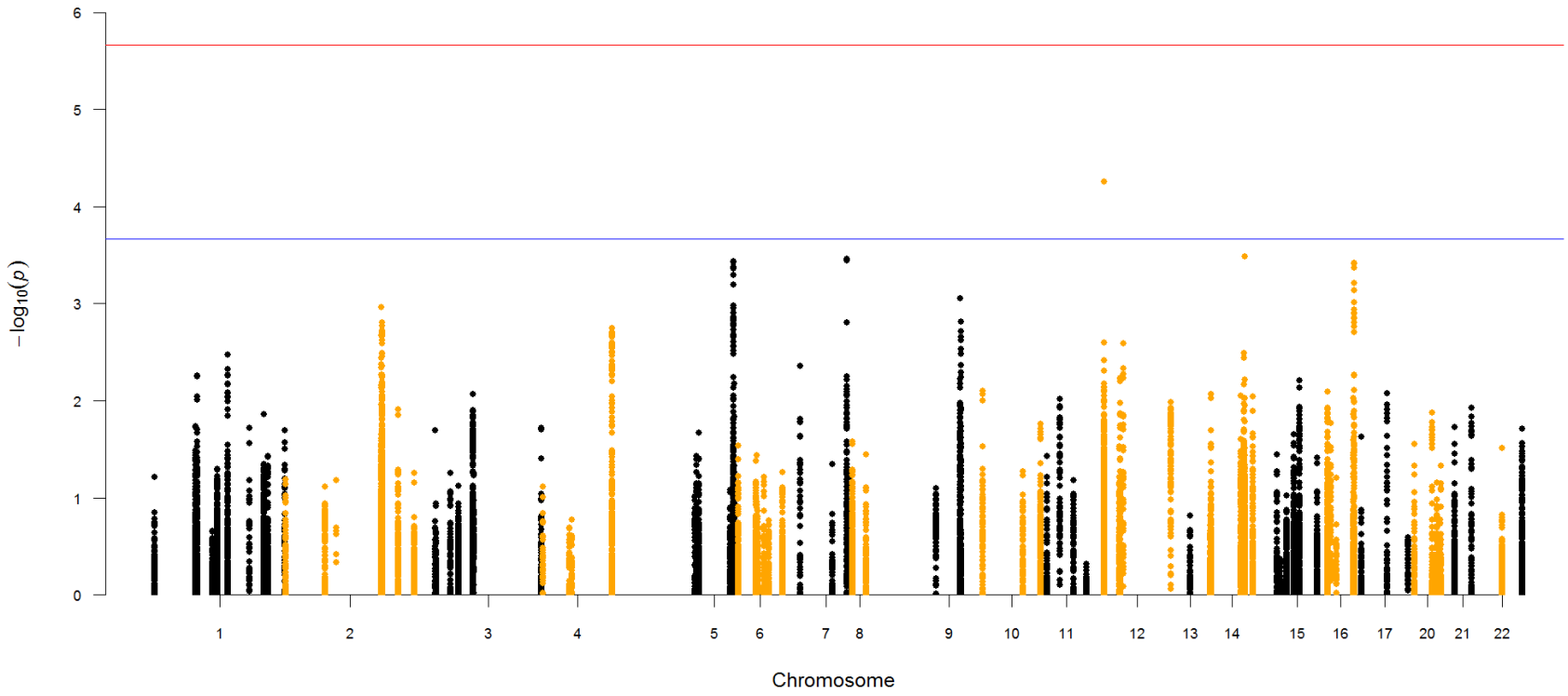
Manhattan plots for meta-analysis estimates for parity x SNP interactions for all four case-control sets are shown in Figure 7-7 to Figure 7-10. An examination of negative $\log_{10}$p-values from the Manhattan plots identified three loci, two in chromosome 3 and one in chromosome 12 which were associated with POP below the suggestive p-value threshold of 2.16×10^{-4}. Meta-analysis of interaction beta-estimates (parity x SNP) and negative $\log_{10}$p-values for SNPs which had p-values less than 2.16×10^{-4} originating from any of the four case-control sets are shown in Figure 7-11. As was the case in the BMI x SNP interaction analyses, interaction-beta estimates were strongest (away from the null) for analyses utilizing stringent controls (Figure 7-11).

Figure 7-7. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of parity x SNP interaction estimates (modeled continuously) for Any POP vs. all controls



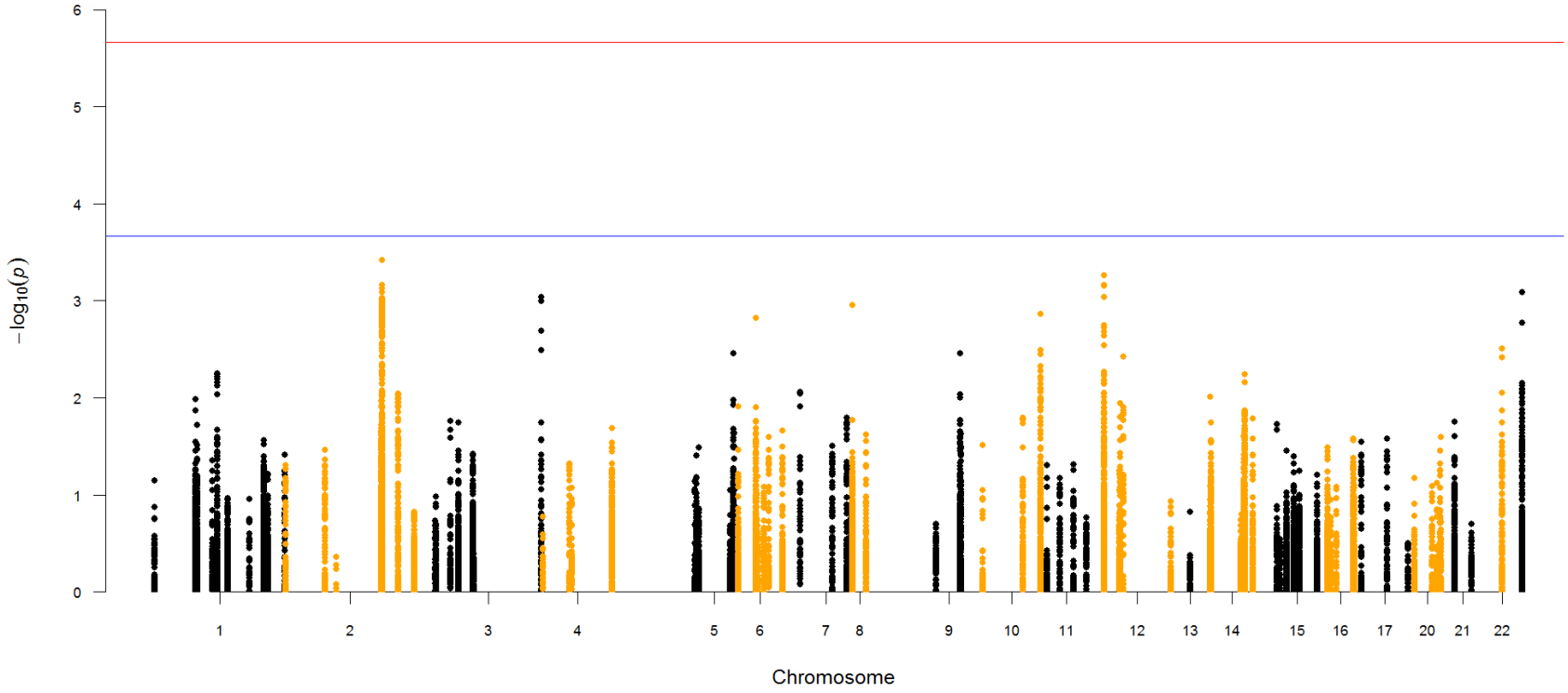
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-8. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of parity x SNP interaction estimates (modeled continuously) for Any POP vs. stringent controls



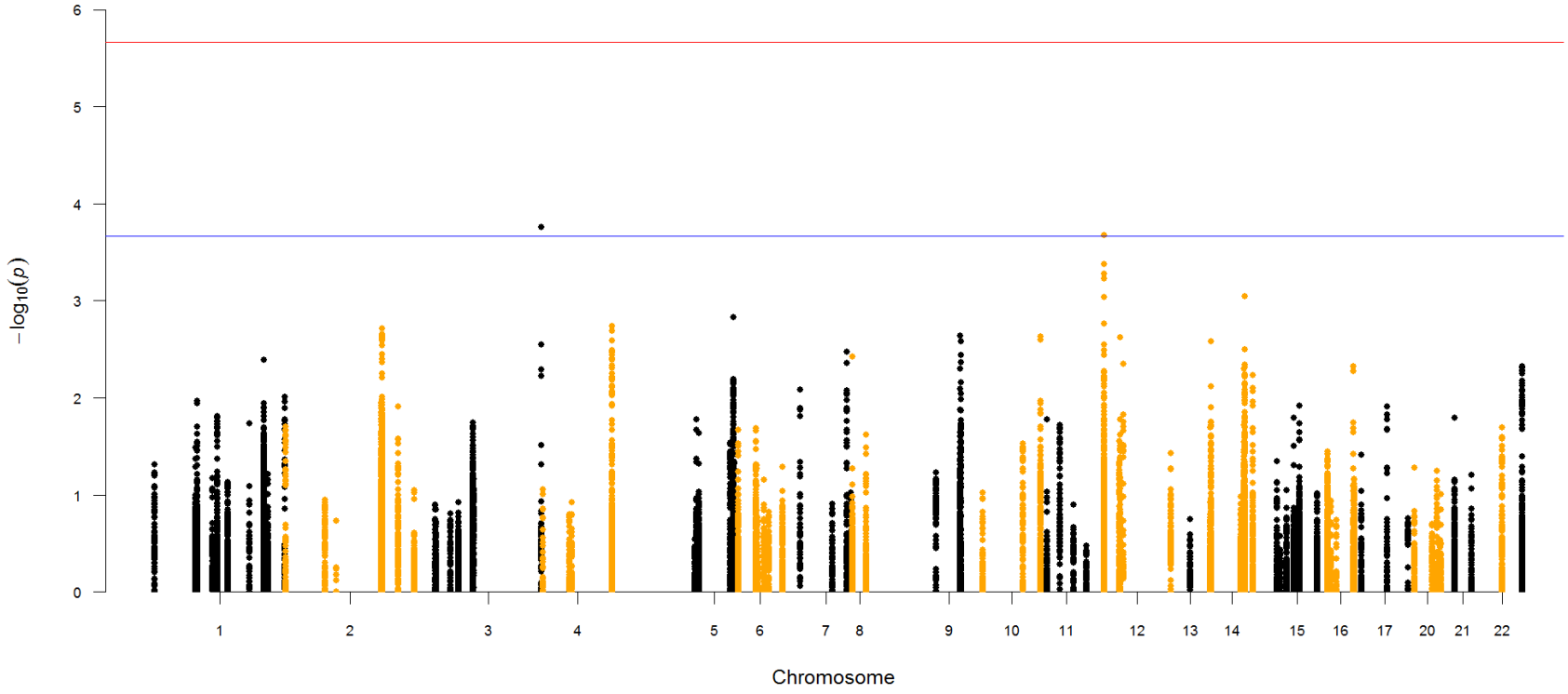
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-9. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of parity x SNP interaction estimates (modeled continuously) for Moderate/Severe POP vs. all controls



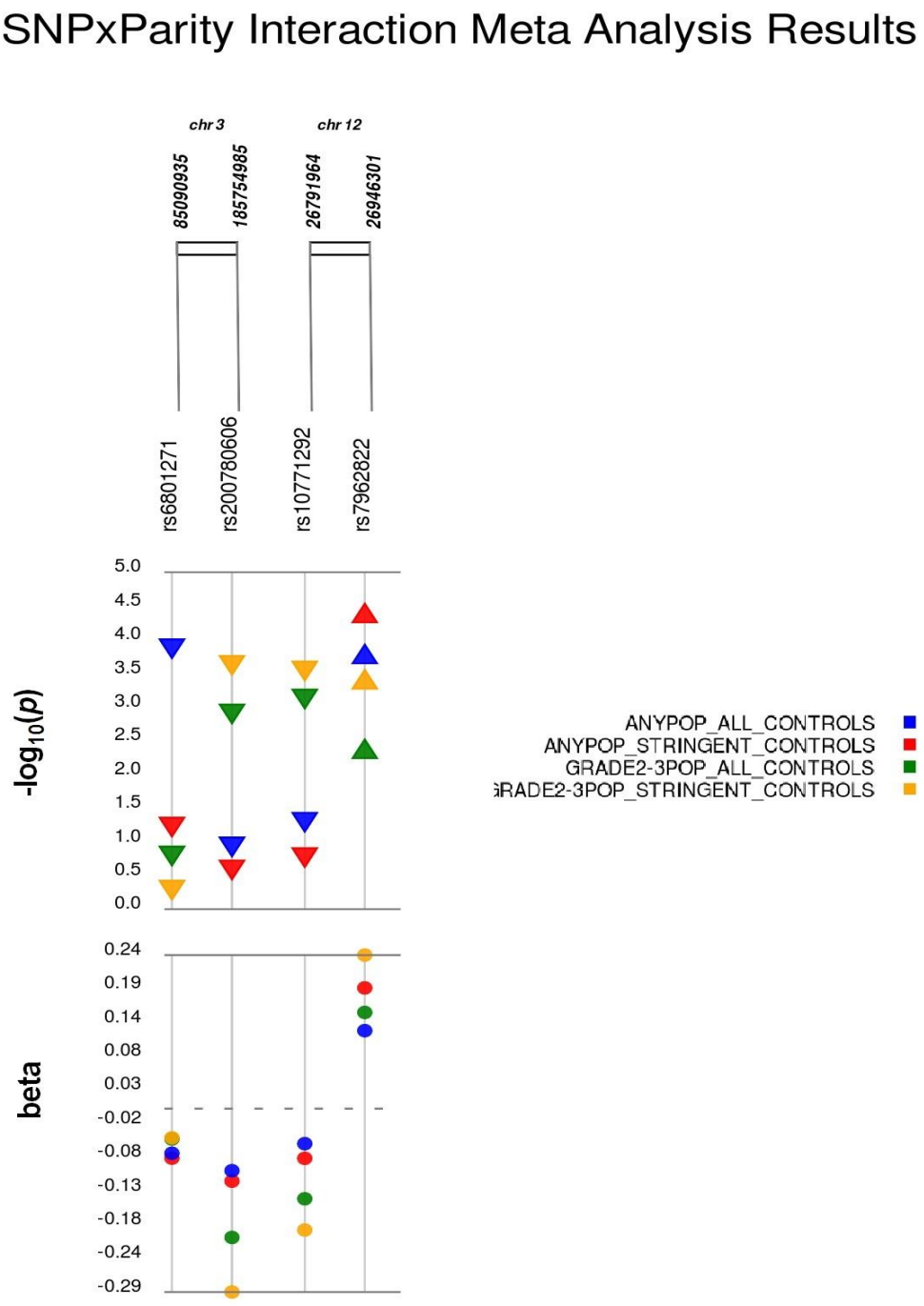
Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

Figure 7-10. Manhattan plot negative $\log_{10}$ p-values for meta-analysis of parity x SNP interaction estimates (modeled continuously) for Moderate/Severe POP vs. stringent controls



Horizontal red line: Statistical significance threshold; Horizontal blue line: Suggestive threshold

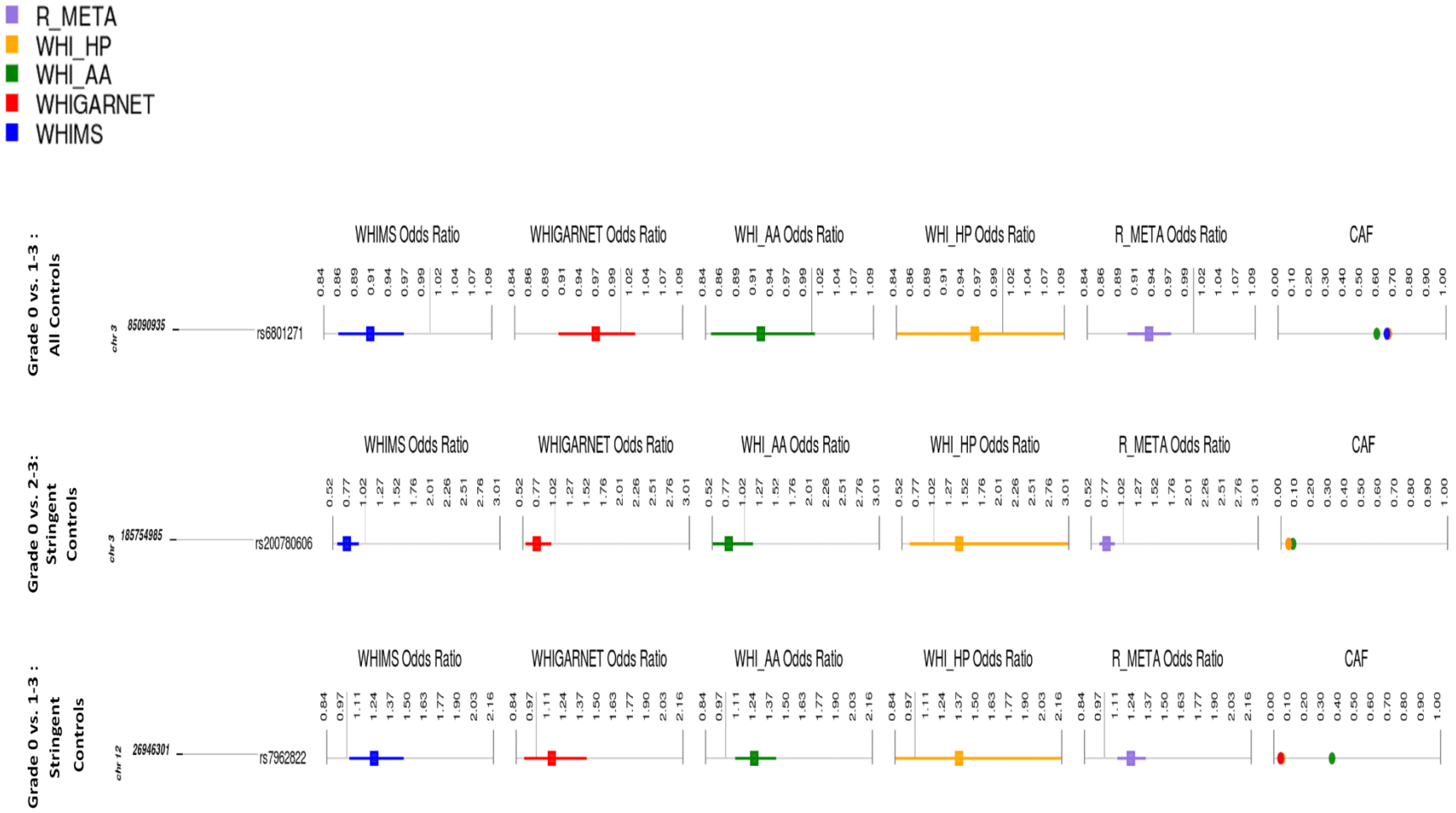
Figure 7-11. Plot summarizing beta-estimates and negative log_{10} p-values for top suggestive-loci from meta-analysis of parity x SNP interaction estimates (modeled continuously) for all four datasets



Meta-analysis: Any POP vs. all controls (blue); Any POP vs. stringent controls (red); moderate/severe POP vs. all controls (green); moderate/severe POP vs. stringent controls (yellow)

Estimates of interaction odds ratios/beta-estimates for the most-statistically significant SNPs (meta-analysis p: < 2.16×10^{-4}) in three independent loci for each dataset and their meta-analysis estimates are provided in Figure 7-12 and Table 7-6. For the most part, interaction odds ratios across the four WHI datasets for a given SNP were in the same general direction (either negative or positive), with the exception of SNP rs200780606, for which the interaction odds ratio in WHI-SHARe (Hispanic) dataset was in the positive direction, while it was in the negative direction for all other datasets (Figure 7-12). All three loci which interacted with parity were located in genes previously associated with obesity measures with p-values below the 2.16×10^{-4} threshold: intron variants in the *ITPR2* gene (rs7962822, p: 5.54×10^{-5}), and *CADM2* gene (rs6801271, p: 9.91×10^{-5}) and a variant upstream of the *ETV5* gene (rs200780606, p: 1.74×10^{-4}) interacted with parity below the suggestive significance threshold (Table 7-6).

Figure 7-12. Plot summarizing beta-estimates of parity x SNP interaction estimates (modeled continuously) and effect-allele frequency for top SNPs from three independent loci for each of the four datasets



Parity-SNP interaction odds ratios (exponentiated): WHI-MS (blue), WHI-GARNET (red) WHI-SHARe African American (green), WHI-SHARe Hispanic (yellow) and Meta-analysis odds ratio (purple)

Table 7-6. Meta-analysis of interaction beta-estimates for parity (continuous) x SNP (continuous) interaction analyses in relation to POP in the WHI-HT

					WHI-MS		WHI-GARNET		WHI-SHARe (African American)		WHI-SHARe (Hispanic)		Meta-analysis		
RSID	**CHR**	**POS**	**EA/ RA**	**EAF***	**Int-Beta**	**Int-Se**	**Int-Beta**	**Int-Se**	**Int-Beta**	**Int-Se**	**Int-Beta**	**Int-Se**	**Int-Beta**	**Int-Se**	**P-value**
Any POP (Original Controls)															
rs6801271	3	85090935	G/A	0.65/0.66/ 0.59/0.66	-0.10	0.03	-0.04	0.03	-0.08	0.04	-0.04	0.07	-0.07	0.02	$9.91x10^{-05}$
Moderate/Severe POP (Stringent Controls)															
rs200780606	3	185754985	A/TA	0.05/0.05/ 0.07/0.05	-0.32	0.11	-0.32	0.13	-0.27	0.20	0.32	0.40	-0.29	0.08	$1.74x10^{-04}$
Any POP (Stringent Controls)															
rs7962822	12	26946301	C/A	0.04/0.04/ 0.35/0.05	0.20	0.09	0.12	0.11	0.21	0.07	0.30	0.24	0.19	0.05	$5.54x10^{-05}$

EA= Effect allele; RA=Reference allele; *Effect allele frequency for WHI-MS/WHI-GARNET/WHI-SHARe (African American)/WHI-SHARe (Hispanic)

Meta-analysis- and pooled- odds ratios for European American women (WHI-MS and WHI-GARNET) by strata of SNP (dosage: ≤ 0.5 and >0.5) for each additional birth (each unit increase in parity) are shown in Table 7-7 for any POP vs. stringent controls analyses, and Table 7-8 for moderate/severe POP vs. stringent controls analyses. There was consistent evidence for effect modification for top SNPs at all three loci in both analyses (any POP and moderate/severe POP); p-values from likelihood ratio tests were <0.05. Each additional birth was associated with higher odds of POP in women with rs6801271≤0.5 (Pooled OR: 1.49, 95%CI: 1.33, 1.67) compared with women with rs6801271 >0.5 (Pooled OR: 1.25, 95% CI: 1.20, 1.30); with interaction odds ratio of 0.84 and LRT p of 0.003. Similarly, each additional birth was associated with higher odds of POP in women with rs200780606 ≤ 0.5 (Pooled OR: 1.29, 95%CI: 1.24, 1.35) compared with women with rs200780606 > 0.5 (Pooled OR: 1.09, 95% CI: 0.95, 1.25); with interaction odds ratio of 0.84 and LRT p of 0.015. Each additional birth was associated with lower odds of POP in women with rs7962822 ≤ 0.5 (Pooled OR: 1.26, 95%CI: 1.21, 1.31) compared with women with rs7962822 > 0.5 (Pooled OR: 1.48, 95% CI: 1.30, 1.69); with interaction odds ratio of 1.18 and LRT p of 0.018. Interaction odds ratios were of equal strength or stronger (away from the null) when evaluating moderate/severe POP (Table 7-8) than when evaluating any POP (Table 7-7).

In sensitivity analyses, changing the cut-point for SNP dichotomization from 0.5 to 0.8 or 0.2 did not appreciably change the effect estimates for the BMI x SNP interactions or the parity x SNP interactions (data not shown). Additional adjustment for smoking or hysterectomy similarly did not change results appreciably (data not shown).

Table 7-7. Stratum specific interaction analyses for parity (continuous) and SNP in relation to Any POP in the WHI-MS and WHI-GARNET datasets by allele strata

				WHI-MS		WHI-GARNET		Meta-Analysis		Pooled Interaction			
SNP	**EA/RA**	**EA dosage**	**Gene**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**P-value**
rs6801271	G/A	≤0.5 (AA)	*CADM2*	1.62	(1.40, 1.88)	1.31	(1.08, 1.59)	1.47	(1.23, 1.75)	1.49	(1.33, 1.67)	0.84	0.003
		>0.5 (AG/GG)		1.23	(1.17, 1.29)	1.28	(1.20, 1.37)	1.25	(1.19, 1.31)	1.25	(1.20, 1.30)		
rs200780606	A/TA	≤0.5 (TA)	*ETV5*	1.29	(1.23, 1.36)	1.3	(1.21, 1.39)	1.29	(1.23, 1.36)	1.29	(1.24, 1.35)	0.84	0.015
		>0.5 (TA,A/AA)		1.04	(0.87, 1.25)	1.19	(0.96, 1.48)	1.09	(0.94, 1.27)	1.09	(0.95, 1.25)		
rs7962822	C/A	≤0.5 (AA)	*ITPR2*	1.25	(1.19, 1.31)	1.27	(1.19, 1.36)	1.26	(1.20, 1.32)	1.26	(1.21, 1.31)	1.18	0.018
		>0.5 (AC/CC)		1.52	(1.28, 1.80)	1.4	(1.13, 1.74)	1.47	(1.20, 1.79)	1.48	(1.30, 1.69)		

Analyses utilized stringent controls; EA= Effect allele; RA=Reference allele; OR = odds ratio; P-value based on likelihood ratio test comparing models with and without interaction term

Table 7-8. Stratum specific interaction analyses for parity (continuous) and SNP in relation to Moderate/Severe POP in the WHI-MS and WHI-GARNET datasets by allele strata

				WHI-MS		WHI-GARNET		Meta-Analysis		Pooled Interaction			
SNP	**EA/RA**	**EA dosage**	**Gene**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**P-value**
rs6801271	G/A	≤0.5 (AA)	*CADM2*	1.94	(1.57, 2.40)	1.49	(1.13, 1.96)	1.70	(1.27, 2.27)	1.79	(1.52, 2.10)	0.84	0.03
		>0.5 (AG/GG)		1.44	(1.35, 1.54)	1.52	(1.39, 1.67)	1.47	(1.35, 1.59)	1.47	(1.39, 1.55)		
rs200780606	A/TA	≤0.5 (TA)	*ETV5*	1.53	(1.43, 1.64)	1.56	(1.42, 1.71)	1.54	(1.42, 1.68)	1.54	(1.46, 1.63)	0.74	0.0006
		>0.5 (TA,A/AA)		1.14	(0.92, 1.42)	1.20	(0.93, 1.54)	1.17	(0.96, 1.41)	1.16	(0.99, 1.36)		
rs7962822	C/A	≤0.5 (AA)	*ITPR2*	1.46	(1.37, 1.56)	1.50	(1.37, 1.64)	1.47	(1.36, 1.59)	1.47	(1.40, 1.55)	1.24	0.022
		>0.5 (AC/CC)		1.95	(1.52, 2.51)	1.60	(1.20, 2.16)	1.77	(1.25, 2.49)	1.81	(1.51, 2.18)		

Analyses utilized stringent controls; EA= Effect allele; RA=Reference allele; OR = odds ratio; P-value based on likelihood ratio test comparing models with and without interaction term

Discussion

In this study we evaluated if associations between BMI and POP and between parity and POP are modified by SNPs from select genes which have either been hypothesized to be associated with POP, have been implicated with other connective tissue disorders or have been previously associated by measures of obesity. Even though the interactions we observed were not statistically significant after considering multiple comparisons, as the first large scale study to evaluate SNP-environment interactions for POP, we provide nominal evidence for SNPs in several interesting genes which may modify the associations between parity and BMI and POP.

In our evaluation of interaction between SNPs and BMI, four out of the seven independent loci that we observed to have suggestive evidence for POP were SNPs from gene regions which were previously associated with obesity measures such as body mass index and waist-circumference. These included SNPs in *CADM2*, *FTO* and *TMEM160*, gene loci for which have been previously associated with BMI [124], and *NRXN3* which has previously been associated with waist-circumference [125]. Of these genes, evidence for effect modification was strongest for SNPs in the *NRXN3* region: for which the magnitude of interaction increased substantially in the moderate/severe POP analyses. *NRXN3* belongs to a family of genes which encode proteins that serve as receptors in the nervous system [171]. Alpha-isoforms of the *NRXN3* gene products have laminin-G binding domains [171]. The laminins are a class of glycoproteins that are thought to be a major non-collagenous component in extracellular matrices [11;73]. A linkage study suggested an autosomal dominant mode of transmission of the rs10911193 SNP located in the promoter region of the *LAMC-1* gene [103]. However, attempts to replicate SNPs in this locus have not been successful in studies with larger sample sizes [100]. In addition to its connection with extra-cellular matrices and its associations with obesity,

NRXN3 has been shown to be associated with several behavioral disorders including smoking [172;173]. In our dataset, additional adjustment for smoking did not materially alter observed effect modification.

SNPs from the elastin (*ELN*) gene, collagen-11a1 (*COL11A1*) gene and *ACTN3* gene which interacted with BMI may have more direct relationships with regard to the maintenance of the pelvic floor support system. Moon and colleagues reported increased elastin metabolism due to increased expression of elastolytic protease activity in uterosacral ligaments of women with POP compared with women without POP [174]. Zong and colleagues reported a 432% and 55% higher concentration of tropoelastin and mature elastin protein, respectively, in vaginal tissues of women with POP compared with vaginal tissues of women without POP [175]. Along the same lines, one study reported that turnover of elastin protein was found to be significantly higher in obese hypertensive children compared with children with healthy normal weight children [176]. However, contrary to this evidence another study evaluated the association between obesity and elastin levels of obese vs. lean subjects and found that elastin levels were lower and there were shorter elastin fibrils in sub-cutaneous abdominal adipose tissue from obese patients than lean subjects [177]. Although speculative at this point, obesity induced elastin metabolism could disrupt the pelvic support system over time, eventually leading to POP.

Alpha-actinin-3 (*ACTN3*) is a cross-linking protein which along with *ACTN2* works to stabilize and anchor myofibrillar actin filaments in muscle cells. A nonsense mutation in the *ACTN3* gene which leads to *ACTN3* deficiency is found to have positive selection with Africans having the lowest frequency compared with Europeans and East Asian populations which have the highest frequency and it additionally is positively correlated with geographical areas with lower latitudes and elevated environmental temperatures [178]. This gene has been suggested to

be related to energy expenditure, as studies have found higher prevalence *ACTN3* deficiency in elite endurance athletes and lower prevalence in elite sprinters [179]. Variation in expression of the *ACTN3* gene could potentially influence contractile properties of the skeletal muscles involved in the pelvic support system, especially in the event of damage due to child birth. Direct evidence tying *ACTN3* to POP is limited, with the exception of one small gene-expression study which found two-fold decreased expression of *ACTN3* gene tissue samples from pubococcogeus muscles from women with POP compared with women without POP [123]. It is of interest that in our analyses interaction odds ratios of SNPs associated with the *ACTN3* gene were largest in the moderate/severe analyses and smaller in the any POP analyses.

Collagen type 11 a1 (*COL11A1*) gene produces the type XI collagen proteins which are primarily found in cartilage, but are also expressed in muscle, and joints [180-182]. There is also evidence to suggest that type V and type XI collagens are important coordinators in collagen fibril nucleation in developing tendons [183;184]. Currently in the literature, no direct ties between *COL11A1* and POP have been reported and the association remains unclear. One of the major components of the pelvic support system is the complex web of extra-cellular matrix which are composed of ligaments such as the uterosacral ligaments and tendinous tissue such as the arcus tendinous [73]. A study evaluating Achilles tendinopathy found significant interactions between *COL11A1* and *COL5A1* polymorphisms [185]. It was suggested that alteration in type V and type XI collagen expression may lead to the formation of larger, less densely paced collagen fibrils which are weaker than smaller more densely packed fibrils. It is of interest that a study evaluating the diameters of collagen fibrils from uterosacral and cardinal ligaments found significantly larger collagen fibril diameters in tissues of women with POP compared with women without POP [186]. Although there is no direct evidence that suggests the role of

COL11A1 gene in healing tendon and ligament tissues, it can be postulated that considering the constant elevated intra-pelvic pressure associated with obesity, variations in SNPs which alter expression of *COL11A1* gene may lead to altered collagen fiber strength subsequently leading to POP.

We observed SNPs in three loci which nominally interacted with parity in influencing POP risk: Cell adhesion molecule 2 (*CADM2*), ETS related variant 5 (*ETV5*) and Inositol 1,4,5-triphosphate receptor, type 2 (*ITPR2*). Interestingly, all three loci have been previously associated with measures of obesity [124;125]. With the exception to its link with obesity, mechanistically it is not clear how these genes may interact with parity to manifest POP; it requires further investigation. *CADM2* is a member of the synaptic cell adhesion molecule 1 (Syncam) family of genes which is involved in crosslinking spectrin which interacts with the actin cytoskeleton to determine cell shape and cell morphology. *ETV5* is an ETS-related transcription factor belonging to a larger family of transcription factors which are highly conserved. ETS-related genes are primarily involved in mammalian development. Skorupski and colleagues postulated that insertion of an extra guanine basepair at position -1607/-1608 upstream of the MMP-1 gene could create a binding site for the Ets family of transcription factors, thereby increasing MMP-1 transcription, which acts on degrading collagen proteins [99]. *ITPR2* is part of a broader family of receptors which are involved in regulating calcium release channels. *ITPR2* mediates a variety of functions including, cell migration, smooth muscle contraction and neuronal signaling. A genomewide study identified *ITPR2* as a susceptibility gene for sporadic amyotrophic lateral sclerosis, a degenerative neuromuscular disorder [187].

To our knowledge this is the first study that has evaluated potential interactions between important risk factors for POP (BMI and parity) and genetic factors. Although the gene regions

that we evaluated were likely not exhaustive, our study identified SNPs in/around several genes which may interact with environmental factors to lead to POP. Despite having a sizable sample size of >12,000 participants none of the SNPs evaluated for interaction in our study were statistically significant after considering multiple comparisons. In part, our inability to find statistically significant interactions may have been due to the difficulty of defining what a POP case is. In the best case-scenario we had over 7,000 POP cases; however these cases included individuals who had grade 1 POP, which is not considered to be clinically meaningful. When we evaluated POP that may be considered clinically meaningful (grade 2 or higher POP), which would be predicted to have stronger genetic associations, our case-sample size was severely reduced. However, it is reassuring that for the majority of the top hits that we evaluated, the magnitude of the interaction odds ratio were largest in analyses evaluating moderate/severe POP. Another difficulty in conducting studies with POP has to do with defining proper controls. We showed that using a stringent definition for controls (two or more confirmed pelvic exams without POP) markedly increased the interaction odds ratios that we observed likely due to reduced misclassification of possible future cases as controls. From a statistical standpoint the balance between choosing a larger sample size of cases (any POP cases) and a less misclassified, yet smaller sample size of controls yielded the smallest p-values. It was also reassuring that despite fluctuation in p-values between case-control definition sets, interaction odds ratios for a set of SNPs at a given loci were always in the same direction. Additionally, interaction associations in individual datasets (WHIMS, WHI-GARNET, WHI-SHARe (African American) and WHI-SHARe (Hispanic)) prior to meta-analysis were also for the most part in the same direction. For analyses by strata of SNP we had to limit our investigation to samples from European American women from the WHI-HT (WHIMS and WHI-GARNET), as only these

samples were large enough to provide reliable effect estimates. The interaction odds ratios for dichotomously modeled SNPs were also in the same direction as when modeled continuously.

In an attempt to further understand the underlying factors which are involved in POP etiology, our study suggests that inciting risk factors such as parity and promoting risk factors such as BMI may interact with genetic variation in the population to influence POP. However, there is a need for functional studies to verify the associations observed in this study and for mechanistic studies which can shed light on potential mechanisms for signals observed in this study.

CHAPTER VIII

Findings for Specific Aim 3: Associations between global and local ancestry in relation to pelvic organ prolapse in African American women from the WHI-HT trial

Abstract

Background and Motivation: POP, a common in women after menopause (up to 41%), is the leading indication for hysterectomy in post-menopausal women in the US. Evidence from epidemiologic studies suggests two- to five-fold increased odds of having POP in European American women when compared with African American women. This information combined with the evidence that up to 40% of POP may be heritable and that physiological differences in pelvic floor attributes exist between European American and African American women makes this phenotype a strong candidate for an admixture mapping study. Therefore, we used GWAS data from the WHI-HT study to evaluate the association between genetic ancestry (global and local ancestry) and POP in African American women.

Methods: POP was measured using a standardized grading system in all participants at baseline and select follow-up visits. Women with grade 1 or higher classification for uterine prolapse, cystocele and rectocole were considered to have any POP. Women with grade 2 or higher classification for any of the three types of prolapse were considered to have moderate/severe (clinically significant) POP. Women were considered controls for this study if they had at least two pelvic exams in the WHI with confirmed absence of POP (grade 0 POP) in at least two visits for all three types of prolapse. We performed standard genotype QC measures. Inference of

global and local ancestry was made using LAMP-ANC software. We first evaluated the association between global ancestry and POP using multivariable logistic regression while adjusting for age, BMI and parity. We then performed a case-only admixture mapping study to compute Z-scores, and case-control admixture-mapping analyses using multivariable logistic regression adjusted for age, BMI, parity and continuous axes of genetic ancestry. The threshold for statistical significance was established with 10,000 permutation tests at 1.82×10^{-5}.

Results: Global ancestry (per 10% increase in European ancestry) was not associated with either measure of POP. Case-only and case-control local ancestry analyses revealed the presence of two ancestry-specific loci which were associated with POP. For one locus (Chr 1:q42.1-q42.3), each unit increase in European ancestry was associated with increased POP odds (Odds ratio (OR): 1.69; 95% confidence interval [CI]: 1.28, 2.22; p-value 1.93×10^{-4}). This broad region harbors several genes which may be relevant to actin including *ACTN2*, *TBCE* and *ACTA1*. For the second locus (Chr 15:q26.2), each unit increase in European ancestry was associated with decreased POP odds (OR: 0.35; 95% CI: 0.30, 0.57; p-value 1.48×10^{-5}). This region harbors the gene *RGMA*, which is a potent regulator of the BMP family of genes, which in turn regulate collagen expression and turnover. We imputed regions under the admixture mapping peaks for both loci. In multivariable logistic regression models assessing local ancestry and POP, adjustment for the most-significant SNPs under the peak attenuated the admixture mapping peaks.

Conclusions: As the first admixture mapping study for POP, we show evidence of two ancestry-specific loci which showed opposing associations between European ancestry and POP suggesting that women of African descent and European descent may have unique non-overlapping susceptibility loci which increase POP risk.

Introduction

Pelvic organ prolapse (POP) is a common condition in women with up to 40% of women having some form of prolapse after menopause. While not all POP requires surgical intervention or is symptomatic, it is one of the most common indications for gynecologic surgery in the US after uterine fibroids and endometriosis, and the most common indication for hysterectomy in post-menopausal women [33].

Several factors such as aging, family history for POP, genetic predisposition, increasing parity, and increasing BMI have been associated with increased risk for POP [1;10;25]. Additionally, race/ethnic status has also been postulated to be associated with POP, with African Americans having lower risk for POP than European American and Hispanic women. However, it is not clear if this apparent disparity is due to biological/genetic differences or due to factors such as varying access to medical care and varying care-seeking behaviors between races/ethnicities. Early reports of racial disparity in POP between African American and European women were based on evaluation of surgical rates for POP using data from the National Hospital Discharge Survey (NHDS). Using NHDS data for 1997, Brown and colleagues reported that self-identified whites were three times more likely to have surgery for POP than self-identified blacks [188]. Based on the 2003 NHDS data, Shah and colleagues reported similarly lower rates for POP surgery in self-identified blacks than for self-identified whites [28]. However, disparity in surgical rates for POP may not necessarily be due to biological differences between whites and blacks but could also be reflective of cultural differences in electing to undergo surgery for POP. If one were to postulate biological differences as the underlying cause for racial differences, then racial differences in POP prevalence would provide a stronger line of evidence than surgical rates.

The Reproductive Risks for Incontinence Study at Kaiser 2, a population based cohort study showed that compared to African American women, white women were 5.35 (95% CI: 1.89, 15.12) times more likely to have POP when considering self-reported symptomatic POP, and 1.40 times more likely to have POP when considering objectively measured POP at or below the hymen [20]. Similarly, baseline evaluation of POP prevalence rates from the WHI-HT trial showed that African American women were 0.65 to 0.55 times less likely to have uterine prolapse, rectocele or cystocele, compared with European American women [22]. The estimates from the WHI-HT study are particularly noteworthy as this is the largest multi-ethnic study conducted to-date which consistently measured objective POP in women of all ethnicities at baseline and therefore provides a less biased assessment of POP prevalence rates across ethnicities. In a more granular and prospective assessment of POP using the WHI-HT data, Kudish and colleagues showed that African American women were 0.7 times less likely to develop any grade of POP and 0.53 times less likely to develop moderate/severe POP [23]. Biologic evidence for racial disparity in POP between African American women and white women are limited. One study reported that African American women had higher pelvic muscle mass and increased pelvic muscle strength when compared with European American women [189]. Another study compared characteristics of the female pelvis between African American and European American nulliparous women and found that African American women had on average a smaller angle in the pelvic arch due to closer attachment of the puborectalis muscle than European American women [110].

These data taken together suggest the possibility that racial differences in POP between European American and African American women could in part be due to genetic differences related to ancestry. Additionally, for complex polygenic and multifactorial diseases such as POP,

it is possible that both populations may have unique ancestry susceptibility disease loci given that the rates are common in both populations albeit higher in one population versus the other. Therefore, postulating that genetic ancestry may play a role in POP, we evaluate the association between individual level genetic ancestry (% European) and local genetic ancestry in relation to POP in African American women from the WHI-HT.

Methods

For detailed description of methods utilized for this Specific Aim please revisit Chapter V. Sub-sections:

Parent Study for Specific Aims 2 and 3 (page numbers: 73-84)

Methods for Specific Aim 3 (page numbers: 121-135)

Results

Characteristics of study participants are summarized in Table 8-1. Women with any POP and especially women with moderate/severe POP were more likely to have higher parity on average, compared with controls. At the WHI baseline visit, women without POP were slightly younger (mean age: 60.11) than women with any POP (mean age: 61.77) and women with moderate/severe POP (mean age: 62.79). However, controls were more likely to be older when considering age at ascertainment than cases since women were only considered controls in this sub-study because we recorded their age at last visit without POP prior to being lost to follow up or study's end. Of the 805 any POP cases, 292 women developed POP during follow-up visits, whereas the rest of the women already had POP at baseline. Of the 156 women who had moderate/severe POP in our study, 92 women developed moderate/severe POP during follow-up visits.

Table 8-1. Characteristics of African American POP cases and controls from the WHI-HT Trial

Variable	Controls (N=344)	Any POP (N=805)	Grade 2-3 POP (N=156)
	Mean (SD)	Mean (SD)	Mean (SD)
Age at baseline	60.11 (6.68)	61.77 (6.96)	62.79 (6.77)
Age at ascertainment	65.20 (6.80)	62.75 (7.02)	64.85 (6.98)
Body mass index (kg/m^2)	31.19 (6.59)	31.71 (6.19)	31.71 (6.07)
Parity (# child births)	2.26 (1.59)	2.85 (1.64)	3.28 (1.60)
Hysterectomy at baseline (%)	23.20%	42.20%	34.80%
European Genetic Ancestry (%)	24.30%	23.15%	23.05%

We evaluated the association between global ancestry (% European ancestry) in relation to any POP and moderate severe POP using multivariable logistic regression models (Table 8-2). We did not observe any meaningful associations between global ancestry and POP.

Multivariable adjusted odds ratio (OR) considering any POP for every 10% increase in European ancestry was 0.98 (95% CI; 0.89, 1.08); similar estimates were observed for moderate/severe POP.

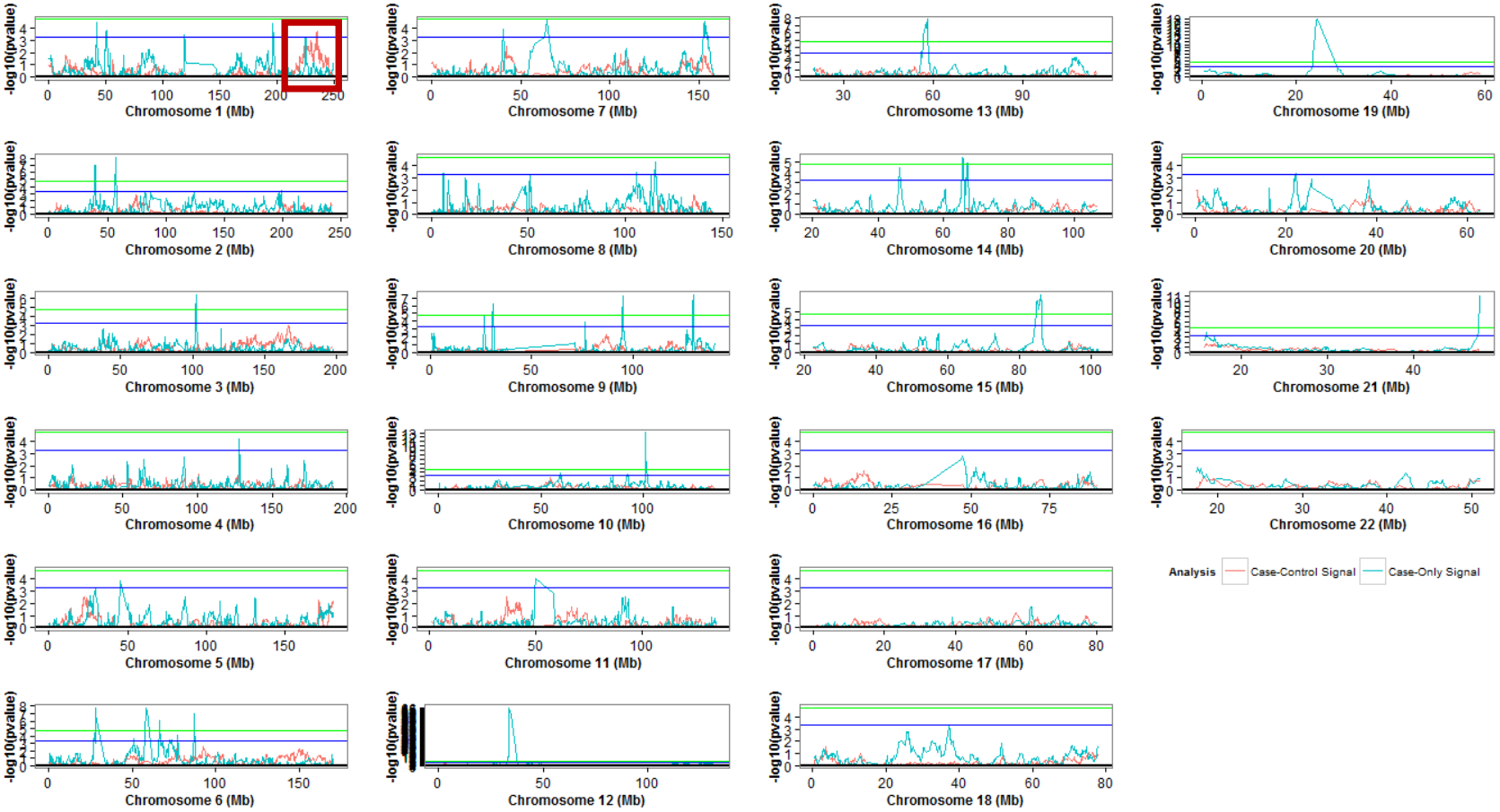
Table 8-2. Association between European-ancestry percent in relation to POP in African American women from the WHI-HT Trial

Model	N-controls/N-cases	OR	(95% CI)	P
Grade 0 vs. Grade 1-3 POP	341/794			
10% increase European Ancestry		0.98	(0.89, 1.08)	0.71
Grade 0 vs Grade 2-3 POP	341/155			
10% increase European Ancestry		0.98	(0.85, 1.13)	0.77

Models were adjusted for age at ascertainment, body mass index (continuous) and parity (continuous)

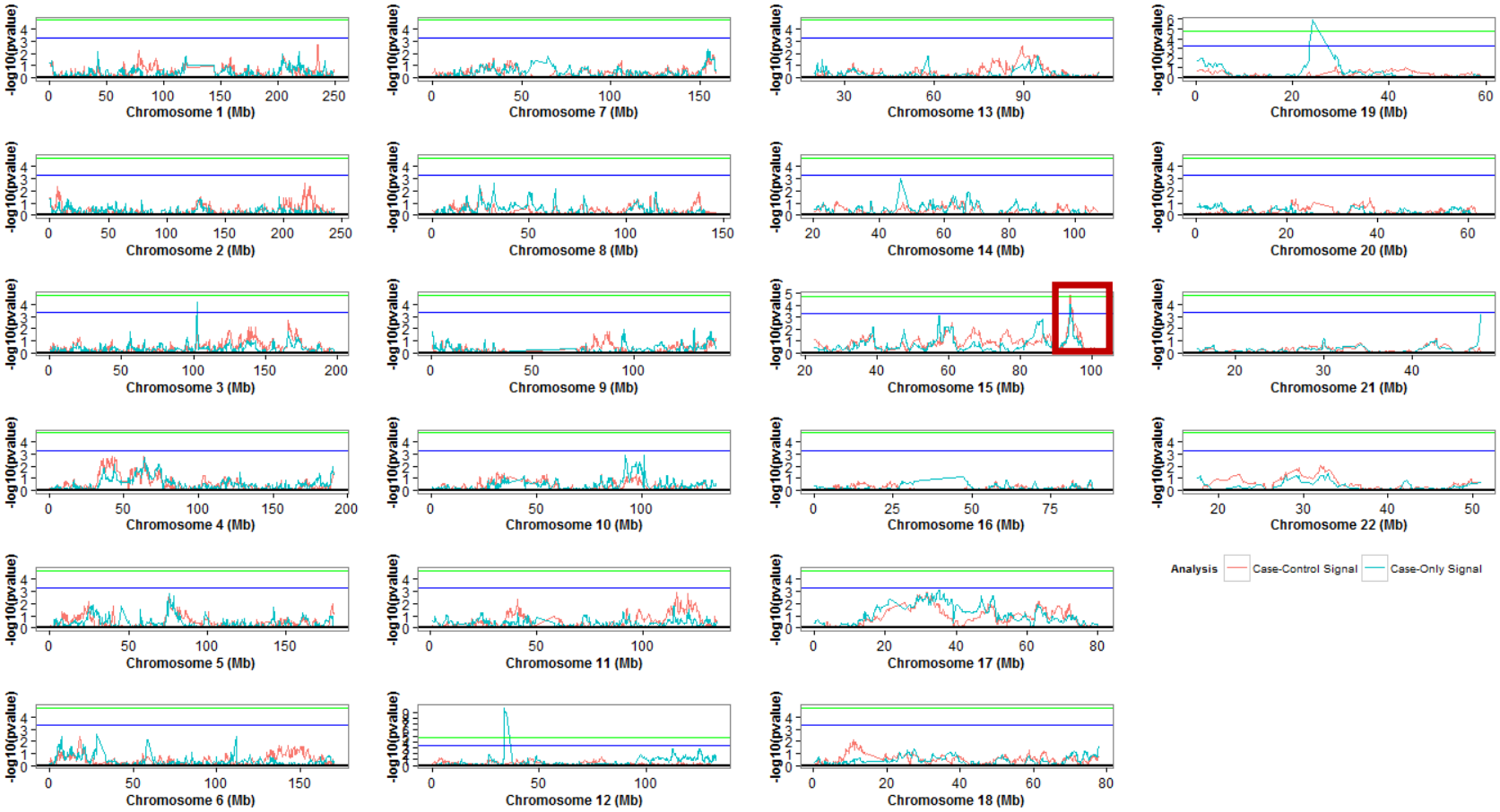
We then evaluated the association between local ancestry (inferred ancestry at the SNP level for 0, 1 or 2 copies of European ancestry) in relation to any POP and moderate/severe POP using a case-only design, and a case-control design. Admixture mapping peaks (negative $\log_{10}$p-values) for chromosomes 1-22 for both designs are presented in Figure 8-1 for any POP and in Figure 8-2 for moderate/severe POP. The case-only design was highly sensitive to deviations from the genome-wide average especially in analyses considering any POP (n= 805 cases). However, only a few case-only signals consistently overlapped with case-control signals suggesting that many of these peaks were false-positive signals. Overlapping suggestive and statistically significant signals (p-values less than 5.0×10^{-4}) for case-only and case-control designs with were observed in chromosome 1 (220-240 mega-bases (Mb)) for the any POP analyses (Figure 8-1) and in chromosome 15 (90 to 100 Mb) for the moderate/severe POP analyses (Figure 8-2).

Figure 8-1. Case-only and case-control admixture mapping peaks for autosomal chromosomes using any POP cases and controls



Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold; Red box on chromosome 1: shows overlapping signals between case-only and case-control mapping strategies

Figure 8-2. Case-only and case-control admixture mapping peaks for autosomal chromosomes using moderate/severe POP cases and controls

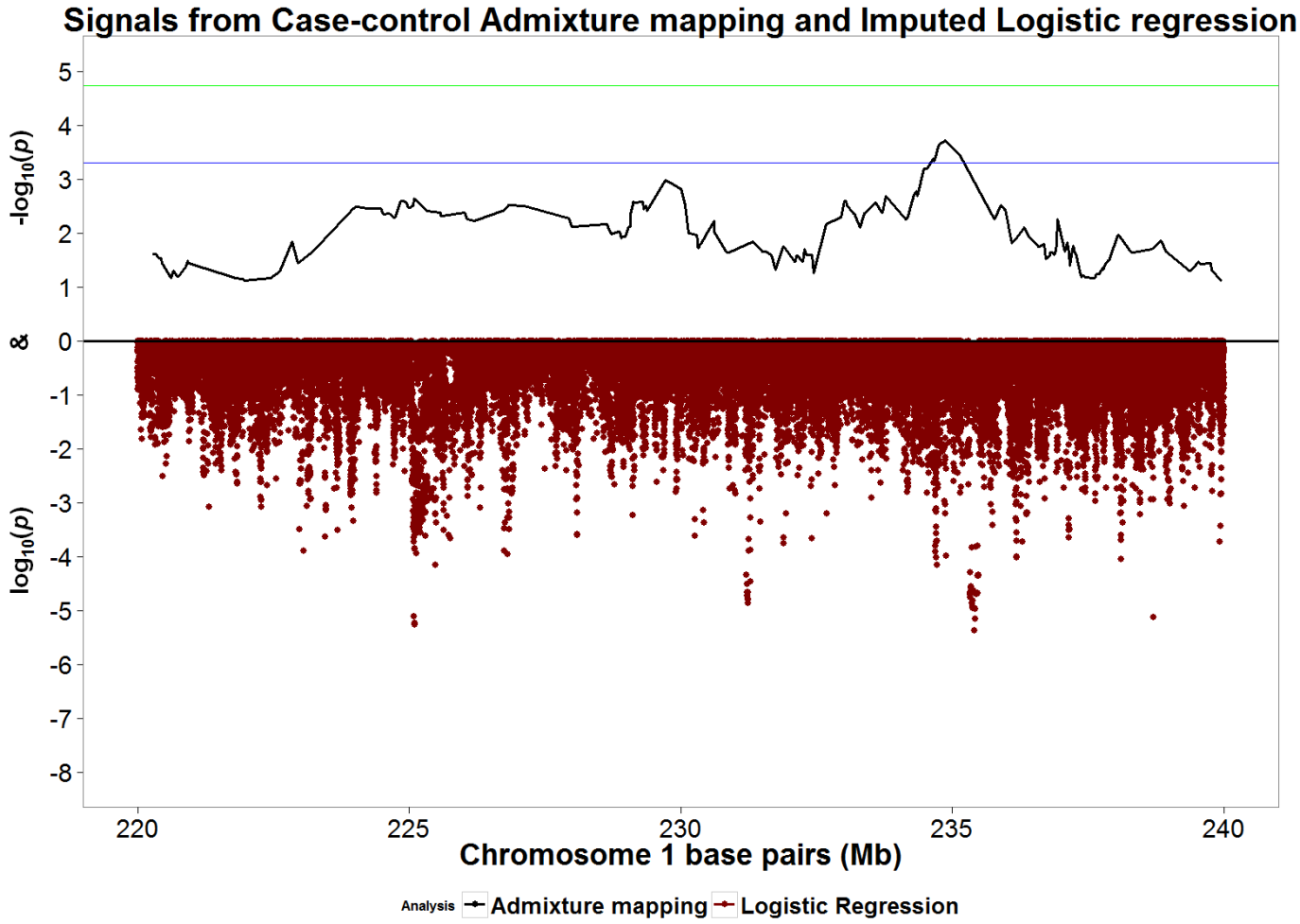


Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold; Red box on chromosome 15: highlights overlapping signals between case-only and case-control mapping strategies

In multivariable adjusted case-control analysis, the strongest admixture mapping signal observed in the chromosome 1: q42.1-42.3 region was associated with increased odds of any POP (OR: 1.69; 95% CI: 1.28, 1.22; p: $1.93x10^{-4}$) for each unit increase in European ancestry (Table 8-3). We then evaluated the association between imputed SNPs in the region below the admixture mapping peak in relation to any POP to identify potential SNPs which may be associated with POP. Admixture mapping peaks ($-\log_{10}$(p-values)) from case-control analyses and SNP associations ($\log_{10}$(p-values)) are juxtaposed in Figure 8-3 for chromosome 1. SNP rs78992478 was situated directly below the admixture mapping peak and compared with the reference allele T, the effect allele C was associated with increased risk for any POP (OR: 3.15; 95% CI: 1.93, 5.14; p: $4.23x10^{-6}$) (Table 8-3). Comparing the allele frequency for the effect allele for this SNP across HAPMAP populations it was found that the SNP was fixed in the CEU population (allele-frequency 1.0) but not in the African populations (YRI allele-frequency: 0.98 and ASW allele frequency 0.94).

The second most significant SNP in this broad admixture mapping peak in chromosome 1 was rs2501094, for which effect allele C compared with reference allele A was associated with increased risk for POP (OR: 1.63; 95% CI: 1.32, 2.02; p: $5.47x10^{-6}$) (Table 8-3 and Figure 8-3). Interestingly, once again comparing effect allele frequencies across HAPMAP reference populations we found that this allele was highly common in the CEU reference population (effect-allele frequency 99%), and least common in the YRI population (effect-allele frequency 50%). In the model evaluating the association between local ancestry and any POP, additionally adjusting for SNP rs78992478 and/or rs2501094, decreased the admixture mapping signal (Figure 8-4) and also decreased the odds ratio for ancestry from 1.69 to 1.37 (Table 8-3). The largest drop in admixture mapping signal was seen with adjustment for rs2501094.

Figure 8-3. Signals from any POP case-control admixture mapping and imputed SNPs for chromosome 1 q42.1-42.3 region



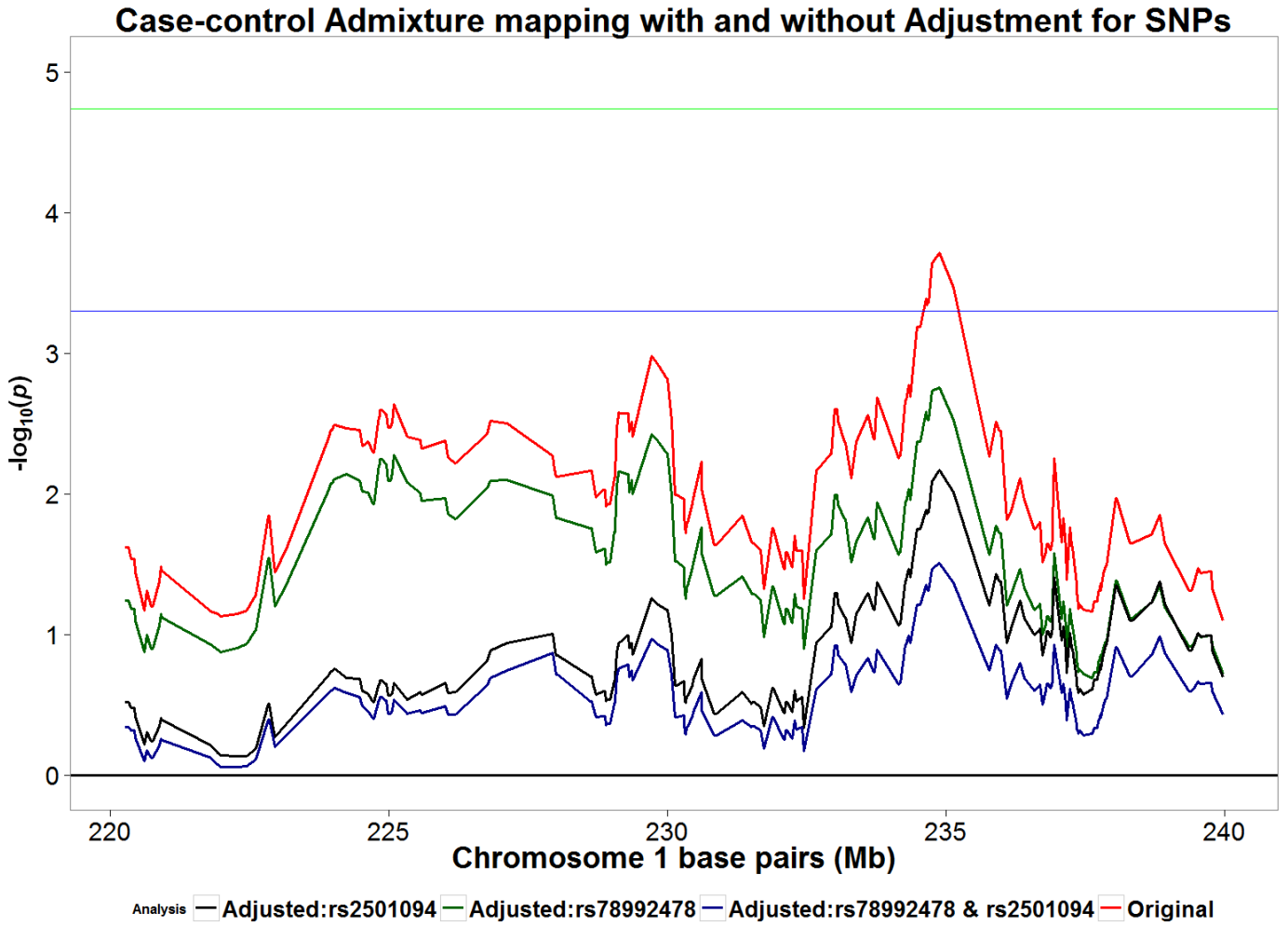
Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold

Table 8-3. Admixture mapping and imputed SNP signals for chromosome 1 and 15

CHR	Nearby Genes	Region/Locus	Model	Ancestral OR (95% CI)	SNP OR (95% CI)	P	EA/ RA	EAF	EAF-YRI/ASW/CEU
1	*ARID4B, TBCE, ACTN2*	q42.1-42.3	Model 1a	1.69 (1.28, 2.22)	-	$1.93x10^{-4}$	-	-	-
			Model 1b	1.56 (1.18, 2.06)	-	$1.75x10^{-3}$			
			Model 1c	1.48 (1.11, 1.97)		$6.80x10^{-3}$			
			Model 1d	1.37 (1.03, 1.32)	-	0.03			
		rs78992478	Model 1a	-	3.15 (1.93, 5.14)	$4.23x10^{-6}$	C/T	0.92	0.98/0.94/1.00
			Model 1b	-	2.53 (1.60, 4.00)	$7.34x10^{-5}$			
			Model 1c	-	-	-			
			Model 1d	-	-	-			
	DNAH14, PARP1, ACTA1,	rs2501094	Model 1a	-	1.63 (1.32, 2.02)	$5.47x10^{-6}$	C/A	0.55	0.50/0.63/0.99
			Model 1b	-	-	-			
			Model 1c	-	1.48 (1.18, 1.84)	$5.36x10^{-4}$			
			Model 1d	-	-	-			
15	*RGMA, CHD2*	q26.2	Model 2a	0.35 (0.22, 0.57)	-	$1.48x10^{-5}$	-	-	-
			Model 2b	0.50 (0.30, 0.85)	-	0.01			
		rs4777810	Model 2a	-	0.37 (0.23, 0.50)	$5.58x10^{-5}$	G/A	0.19	0.03/0.17/0.57
			Model 2b	-	0.46 (0.26, 0.84)	0.01			

Models 1a: grades 1-3 POP modeled against local European ancestry (0, 1 or 2 copies) or SNPs adjusted for age at ascertainment, BMI (continuous), parity (continuous) and continuous axes of MDS components; Models 1b: Model 1a + rs78992478; Models 1c: Model 1a + rs2501094; Model 1d: Model 1a + rs78992478 and rs2501094
Models 2a: grades 2-3 POP modeled against local European ancestry (0, 1 or 2 copies) or SNPs adjusted for age at ascertainment, BMI (continuous), parity (continuous) and continuous axes of MDS components; Models 2b: Models 2a + rs4477810

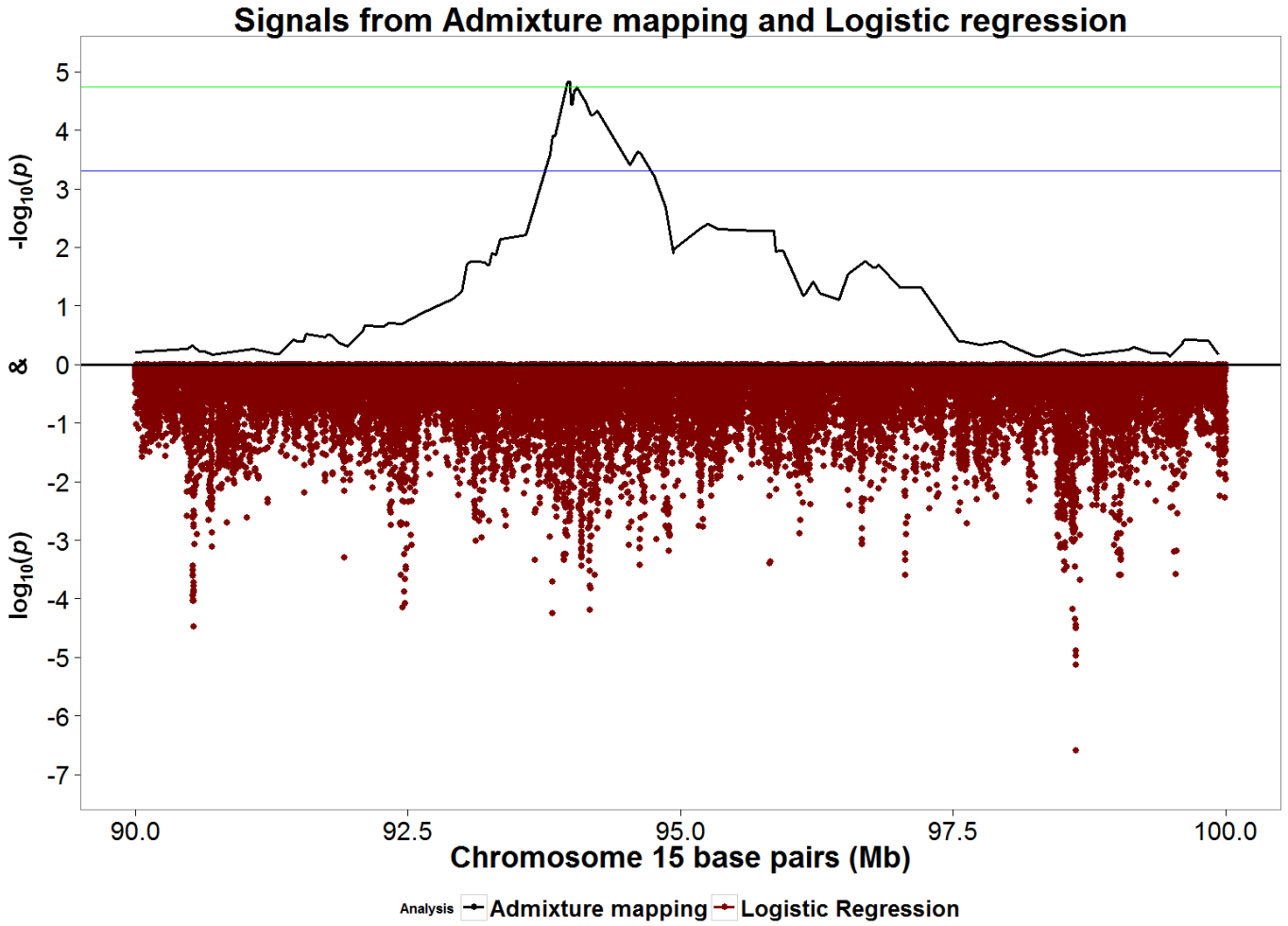
Figure 8-4. Any POP Chromosome 1 peaks with and without adjustment for most significant imputed SNPs in the region



Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold

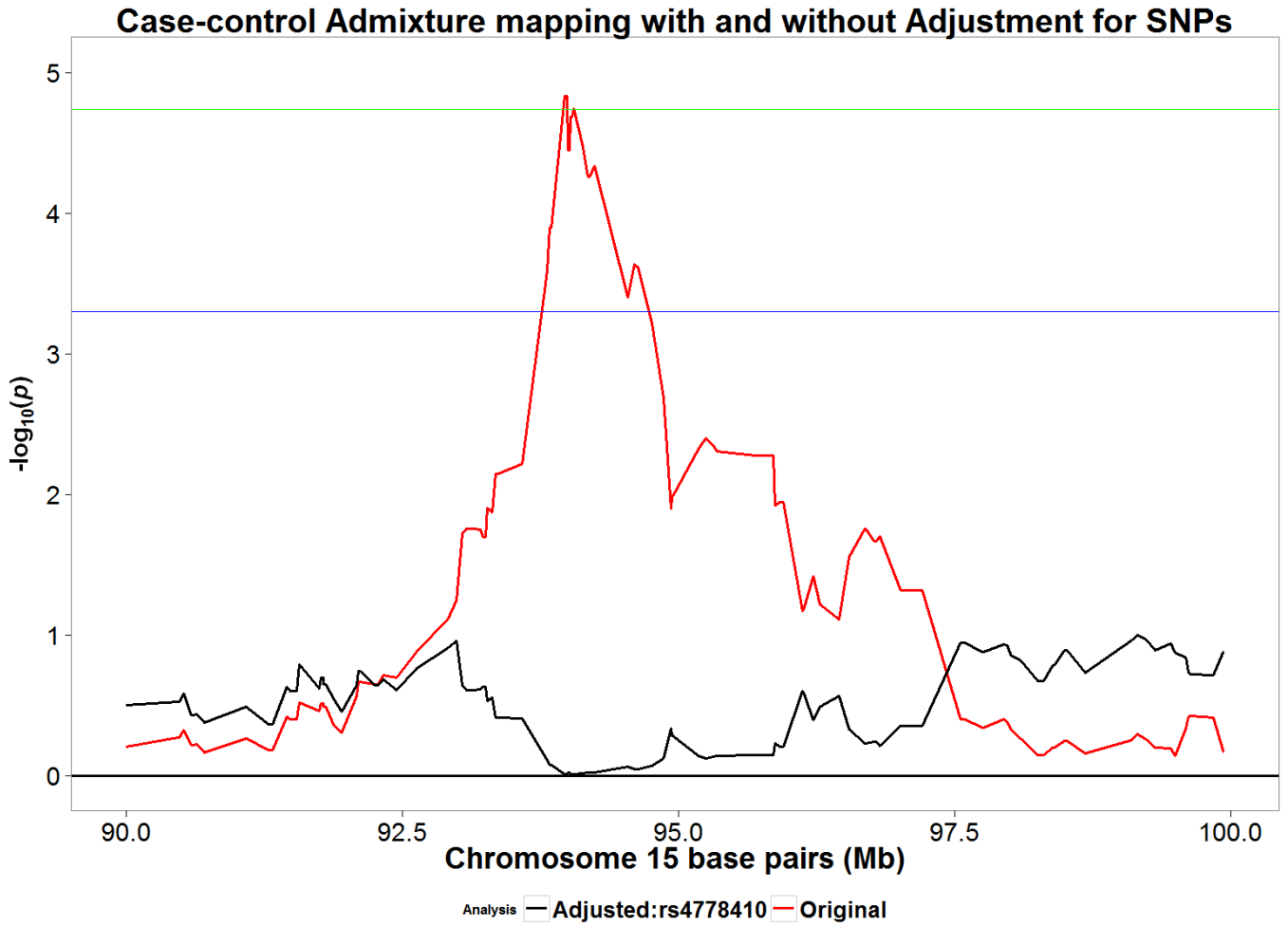
Interestingly, for each unit increase in European ancestry, the strongest admixture mapping peak observed in the chromosome 15:q26.2 region was associated with decreased odds of moderate/severe POP (OR: 0.35; 95% CI: 0.23, 0.57; p: 1.48x10-5) (Table 8-3). Imputed SNP rs4777810 was the most statistically significant SNP directly below the peak (Figure 8-5). Compared with the reference allele (A), the effect allele (G) was associated with decreased POP risk (OR: 0.37; 95% CI: 0.23, 0.50; p: $5.58x10^{-5}$) and this allele was rare in the YRI population (allele-frequency: 0.03) but common in the CEU population (effect-allele frequency: 57%) (Table 8-3). Additional adjustment for rs4777810 severely blunted the admixture mapping signal (Figure 8-6) and decreased the magnitude of ancestral odds ratio from 0.35 to 0.50).

Figure 8-5. Signals from moderate/severe case-control admixture mapping and imputed SNPs for chromosome 15 q26.2 region



Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold

Figure 8-6. Moderate/severe Chromosome 15 peak with and without adjustment for most significant imputed SNP in the region



Green horizontal line: statistical significance threshold; Blue horizontal line: suggestive threshold

Discussion

As the first study to evaluate the association between genetic ancestry and POP, our results suggest the presence of two unique ancestry-specific susceptibility loci, where in one region European ancestry was associated with increased risk for POP, and in another region African ancestry was associated with increased risk for POP. Contrary to epidemiological evidence which show higher prevalence of POP in European Americans than in African Americans, our evaluation of genetically inferred European ancestry proportion per person was not associated with increased risk for POP. While European American women in the WHI were more likely to have POP at baseline than African American women, POP prevalence was still common in both populations (40.2% in European American vs. 29.4% in African American) [22]. For such a highly prevalent complex condition, it is plausible that women from European or African ancestries may have shared susceptibility loci and additionally there may be distinct ancestry-specific susceptibility loci which are associated with increased risk for POP as has been shown for prostate cancer [190]. The presence of shared and/or opposing effect-estimates in ancestry-specific loci could have potentially diluted the association between global ancestry and POP as it is merely a representation of average ancestry along the genome.

The chromosomal region 1q42.1-q42.3 showed a greater presence of excess European ancestry in POP cases than in controls. Although the signal for this region was not statistically significant (p: 1.93×10^{-4}) after considering multiple comparisons (p-value threshold: 1.8×10^{-5}), several pieces of other evidence collectively point to the plausibility that the signal from this region may be of interest in relation to POP. This broad 20 mega-base region harbors several genes which may be related to maintenance of components of the pelvic support system and

evidently may be related to POP, including tubulin binding cofactor E (*TBCE*), actinin alpha-2 (*ACTN2*), and alpha-actin-1 (*ACTA1*) gene.

TBCE is a peripheral associated membrane protein which is plays an essential role in polymerizing microtubules [191]. This gene has been suggested to play a major role in forming neuromuscular junctions [192] and mutations in the *TBCE* gene have shown to cause loss of microtubule formation in distal ends of the axon [193;194]. This gene has been associated with amyolateral sclerosis in mouse models [195].

Denervation of major muscles involved in the pelvic support system due to stress-related insult during pregnancy and labor has been hypothesized as an important contributor to POP. It is plausible that altered expression of the *TBCE* gene may impact proper repair of denervation sites. The *ACTA1* gene is a globular protein which is important in thin microfilament formation including F-actin and G-actin filaments and plays an essential role in muscular contraction [196]. A recent study evaluated F-actin expression in vaginal fibroblasts acquired from women with and without POP and found that relative F-actin expression was higher in fibroblasts from women with POP than in fibroblasts from women without POP [197]. *ACTN2* is an actin-binding protein which is widely expressed in smooth, skeletal and striated muscles [198].

In addition to finding a chromosomal region with biologically relevant genes in chromosome 1, we showed that adjustment for the two most significant SNPs blunted the admixture mapping peak, especially with adjustment for SNP rs20501094. The effect allele for this SNP (C) was found in higher frequency in the HAPMAP European (CEU) reference population (effect-allele frequency: 0.99) than in African (YRI) reference population (effect-allele-frequency: 0.50). Furthermore, consistent with the ancestry odds ratio for this region,

where European ancestry was associated with increased risk for POP, the effect allele (C) was also associated with increased risk for POP.

The most-statistically significant case-control admixture mapping peak, which remained significant after considering multiple comparisons through permutation testing, is located in the chromosomal region 15:q26.2. Interestingly, here European ancestry was inversely associated with moderate/severe POP. This region harbors the repulsive guidance molecule family member a (*RGMA*) gene, which is a glycosylphospatidylinositol-anchored glycoprotein. *RGMA* was initially discovered for its role as an axon guidance protein in the central nervous system [199;200]. The RGM family of genes including *RGMA* has been shown to be important regulators of the bone morphogenic protein (BMP) family including the *BMP-1* gene [201;202]. The *BMP-1* gene is involved in activation of the lysl oxidase family of genes and plays a crucial role in maturation of procollagen chains [203]. A small study evaluating the association between POP cases and controls showed decreased expression of the *BMP-1* gene in POP cases compared with controls [61]. It can be postulated that *RGMA* may be an upstream regulator for pathways which are responsible for maintenance of the complex extracellular matrix, yet another major component of the pelvic support system. The association between *RGMA* and POP may also be related through increased adiposity. A recent clinical report showed three people with micro-deletions in the chromosome 15:q26.2 region which included the *CHD2* and *RGMA* genes were observed to have truncal obesity and suggested *RGMA* as the potential gene of interest [204]. Although not much can be inferred though case-studies, if *RGMA* is indeed associated with central adiposity, then it would suggest yet another mechanism through which it may be influencing POP. Additional evidence for the *RGMA* gene comes from the observation that SNP rs4777810, located within 135 kilo-bases upstream of the *RGMA* gene was found to be the most-

statistically significant imputed SNP in the region. The effect allele (G) for this SNP is less abundant in the African (YRI) reference population (effect allele frequency: 3%) and is more abundant in the European (CEU) reference population (effect-allele frequency: 57%). Additionally, consistent with the admixture mapping peak, where European ancestry was inversely associated with POP, the effect allele (G) was also found to be inversely associated with POP and adjusting for this SNP severely attenuated the admixture mapping peak which we observed for this region.

Despite identifying ancestry-specific susceptibility regions with biological plausibility, this is study is not without its limitations. *A priori*, assuming a case-sample size of 800 cases and a p-value threshold of 5×10^{-5} (approximately 1000 independent tests), we had estimated approximately 80% power to detect an ancestry odds ratio of 1.8. Even though we had 805 cases considering any severity of POP, our study only had a limited number of African American women (n=156) who had POP which is considered closer to clinically significant POP. To compensate for the small sample size in our study we only evaluated markers which were highly differentiated in the ancestral reference populations, for which we would have sufficient power to detect associations considering the multiple testing burden. Despite of our small sample size, we observed a statistically significant association in this study, the level of statistical significance for which was determined through permutation testing. However, it should be stated that we may not have been able to detect more subtle associations between ancestry and POP due to our small sample size.

Genetic studies of POP, especially in admixed populations such as African Americans in our study are difficult to conduct for three main reasons. Firstly, to date there are very few data resources that are readily available for studying POP since ascertainment of POP needs to be

confirmed through specialized pelvic exams which are not always routine. Secondly, as difficult it is to identify POP cases, it is equally tricky to identify proper POP controls since POP prevalence rates are high and prevalence rates increase with age (until age 80, after which POP prevalence stabilizes). Therefore, one needs to either identify older women who have not had POP in the past or women being considered as potential controls should be monitored over time to ensure they do not develop POP. Thirdly, availability of genetic data with validated POP status is scarce. The WHI-HT data provided us with a unique opportunity for addressing these issues. Since women in the WHI-HT had standardized pelvic exams at baseline and multiple follow-up visits, we were able to identify individuals who did not have POP at baseline but developed POP during the follow-up visits. Additionally, we chose stringent criteria for defining controls where women were only considered controls if they were confirmed to have no prolapse for uterine prolapse, cystocele and rectocele in at least two WHI visits. In the baseline WHI-HT dataset, women with hysterectomy were less likely to have POP than women with an intact uterus and it was postulated that some of these women may have had corrective surgery for POP prior to study enrollment. Since women without a uterus could still potentially have other forms of prolapse such as cystocele and rectocele we included these women in our study. Using our stringent definition for controls, the inverse association between hysterectomy at baseline and POP was in fact reversed in our sub-study. In our case-control admixture mapping models, additional adjustment for hysterectomy did not noticeably alter the associations we observed. It should also be noted that for the most-statistically significant hit an almost identical peak was noted for our case-only admixture mapping analyses as for the case-control analyses, thereby alleviating any concern that our results may suffer from bias introduced by hysterectomy status in controls.

In conclusion, the results from our study suggest that POP is a complex and likely polygenic condition with distinct susceptibility loci that are unique to both European and African ancestry. Replication of ancestry-specific loci and fine-mapping studies in larger African American populations are needed to confirm findings from this exploratory study.

CHAPTER IX

SYNOPSIS AND FUTURE DIRECTIONS

Overall study conclusions

By conducting a systematic review and meta-analysis of the literature evaluating the association between obesity and POP, we were able to show that being over-weight and obese increased risk for having POP compared with women with normal-weight. We were also able to demonstrate that the heterogeneous risk ratio estimates reported in the literature regarding the relationship between BMI and POP could in part be attributed to study characteristics such as varying methods of POP measurement, the age distribution of women enrolled in the studies and the study design itself. Studies reporting a definition of POP which most resembled clinically significant POP tended to have larger effect estimates for both over-weight and obese categories than studies considering any grade of clinically measured POP or self-reported symptomatic POP. It is also of interest that studies with a higher proportion of younger women tended to report larger effect estimates in the relationship between obesity and POP. Obesity is likely the only practically modifiable risk factor for POP. If the notion that the impact of obesity on POP progression is greater in earlier stages in life (before menopause) is true, highlighting the relationship between obesity and POP may be a plausible strategy to target both health conditions together.

It is also interesting that in our gene-environment interaction analyses (between SNPs from candidate genes and BMI, and parity) in relation to POP, we found suggestive evidence for SNPs in several genes, which have previously been associated with measures of obesity. Four

out of the top seven loci with suggestive signals in the SNP-BMI interactions have previously been associated with obesity including *CADM2, NRXN3, FTO* and *TMEM160*. Additionally, all three of the top suggestive signals identified in the SNP-parity interactions also have been previously associated with obesity (*CADM2*, *ETV5*, and *ITPR2*). These data add another layer of evidence which binds the association between obesity and POP. SNPs from three other genes were also found to interact with BMI (*COL11A1*, *ACTN3*, and *ELN*) in relation to POP.

Finally, we performed the first ever admixture mapping study for POP in African American women to understand if there is a biologic basis to reports of racial disparity in prevalence of POP. Contrary to epidemiological evidence which shows higher prevalence of POP in European American women than in African American women, increasing proportion of European ancestry in African American women was not associated with POP. Admixture mapping analysis using markers which are highly differentiated between African and European ancestries suggested that both European and African ancestries may carry risk increasing alleles for POP that is unique to each ancestry. Compared with African ancestry, one 10-Mb region in chromosome 15 showed a peak which corresponded to decreased POP risk for European ancestry, and another 20-Mb region in chromosome 1 showed a peak which corresponded to increased POP risk for European ancestry, both compared with African ancestry.

The work here demonstrates that POP is a complex trait which is influenced by modifiable and non-modifiable factors acting in concert. A clearer understanding of the causal agents behind the noted associations and a deeper understanding of the underlying mechanisms are needed.

Considerations

Utilizing the WHI-HT study provided several advantages which not only allowed us to improve on previous studies evaluating risk factors for POP, but also allowed us to evaluate hypotheses that have previously only been mentioned in the literature. Previous studies evaluating genetic and non-genetic risk factors for POP have been limited by sample size, thereby limiting the pursuit of hypotheses which allow for evaluation of gene-environment interactions. The WHI-HT is a well characterized cohort of ethnically diverse post-menopausal women who were uniformly assessed for three types of prolapse at baseline using a standardized procedure which recorded varying stages of prolapse severity. Additionally, to our knowledge this is the largest available resource which had information on over 12,000 individuals for whom GWAS data is available in addition to information on POP. Access to such a well-validated resource of unprecedented size with respect to genotype and phenotype (in relation to POP) allowed us to evaluate gene-environment interactions using SNPs from 96 candidate genes and two of the most important risk factors for POP: parity and BMI.

Studies previously evaluating POP have been limited to one measurement of POP mostly due to the rigor of the exam required to validate POP, or because self-report is the most-convenient and cost-limiting approach to conduct a well-powered study. An additional advantage of using the WHI-HT study is that it provides data for pelvic exams for multiple assessments of POP done during follow-up. Approximately 62% of the participants in the cohort were assessed for POP on two or more occasions including baseline and yearly follow-up visits. Having multiple assessments for POP allowed us to improve on study designs from previous studies evaluating POP in the following ways. Firstly, this allowed us to reduce the amount of outcome misclassification in our analysis dataset. Women who were classified as controls at baseline, but

later developed POP during follow-up were properly classified as cases in this study. Secondly, having multiple assessments for POP allowed us to perform several sensitivity analyses especially regarding the selection of controls. We were able to utilize two definitions of controls, one which allowed individuals with at least one confirmed visit with no POP, and another definition which allowed only individuals with two or more confirmed visits as controls to serve as controls in this sub-study. This allowed us to confirm our suspicion that misclassification of outcome was greatly reduced in analyses which utilized stringent controls as shown by larger (away from the null) main-effect and interaction effect estimates for case-control sets with stringent controls when compared with loosely defined controls. However, while this is a marked improvement in POP verification compared with other studies, it is important to acknowledge that a large proportion of individuals classified as controls only had one assessment for POP. Therefore, outcome misclassification still remains a concern for this study, the effect of which can be presumed to have led to an underestimate of the associations evaluated in this study.

However, despite having a large sample size, it should be noted that the largest case-sample size was available when POP cases were defined as having any POP (Grade I or higher). When POP was classified as moderate/severe POP (Grade II or higher), which is closer to what is considered clinically relevant POP, the case-sample size was greatly reduced by two-thirds. Even though our analyses demonstrated that utilizing clinically relevant POP cases and stringent controls provided the highest degree of case-control specification (largest effect estimates for BMI, parity and interactions), the statistical power to detect associations for moderate/severe POP analyses was greatly reduced considering the multiple-testing burden.

The WHI study collected detailed information on reproductive history at baseline including number of pregnancies, number of live/still births. One drawback of the WHI study is

that information on mode of delivery was not collected. This is important because women who give birth vaginally on at least one occasion have been shown to have higher risk for POP than women who elected to have C-sections. Therefore, the effect estimates associated with increasing parity are likely underestimated in our study and by extension so would the interaction effect estimates relating to parity. This in part, provides another explanation for why the study only observed marginally statistically significant results in the interaction analyses.

Considering that all participants were post-menopausal study, that information regarding reproductive history was collected as standard procedure from all participants, that it was collected prior to officially being recruited for the WHI-HT study it is relatively safe to assume that recall bias related to the primary outcome of the WHI-HT study or the outcome of this sub-analysis is less probable. Additionally, the possibility of reverse causality due to temporal issues regarding SNP-parity interactions is also less likely in this study as birth events likely happened at least 10 or more years prior to WHI enrollment. However, the possibility of reverse causality in our assessment of SNP-BMI interactions cannot be completely ruled out as a large proportion of women already had some degree of POP during baseline assessment. Therefore, for these individuals it is not possible to say if higher BMI led to increased risk in POP or that having POP to some degree led to higher BMI perhaps due to reduced mobility. The option of utilizing a prospective method of evaluation was considered. However, a large proportion of cases already had POP (any POP or moderate/severe POP) at baseline and approximately 38% of individuals had only one assessment for POP. Utilizing this method would have resulted in a severely underpowered study.

One of the greatest advantages of utilizing the WHI-HT population was that we were able to utilize data for ethnically diverse (Hispanic and African American) women in addition to

European American women. Majority of the studies evaluating POP have been limited to women of European descent. This is of importance because studies have shown that African American women have lower prevalence of POP and Hispanic women have a higher prevalence of POP compared with European American women. Considering that the distributions for one of the key risk factors for POP (BMI, as identified in Aim 1), is different across race/ethnicities in the US, and that there are important genetic differences relating to continental ancestry across ethnicities, the results from our interaction analyses have a greater opportunity to generalize results to all three sub-populations. It is also reassuring that our top suggestive interaction associations were in the same general direction across ethnicity-specific datasets. However, it should be clarified that majority of the power for the interaction meta-analysis was likely provided by the European American population given its several-fold larger sample size compared with the Hispanic and African American populations.

Another unique aspect about this study is that availability of GWAS data and POP data on African American women allowed us the opportunity to conduct the first admixture mapping study in this population in relation to POP. Although the number of POP cases in the African American population in WHI was only 805, considering any POP, the effective number of cases for moderate/severe POP was only 156 women. Despite having a small sample size we were able to find a statistically significant association (after considering multiple comparisons) by limiting the number of analyses to ancestry informative markers with large allele frequency differences. Although data from Hispanic women were available in the WHI-HT, an admixture mapping study was not performed because Hispanic sample size was even lower than the African American sample size. This is of importance especially because the Hispanic population in the US has an even more complex genetic architecture with recent ancestral contributions from

European American, African and Native American sub-populations, and would require a larger sample size to perform analytically rigorous and sound admixture mapping study. An admixture mapping study in the Hispanic population is especially warranted considering the complex genetic architecture and the highest prevalence of POP in this population.

Future directions

In Specific Aim 1 we used meta-analytic approaches to show that BMI was positively associated with POP in the literature. In Specific Aim 2 we showed that several loci previously associated with obesity measures modified the association between BMI and POP and between parity and POP. The next step would be to try to identify the mechanisms by which obesity may be influencing POP. Currently, the only explanation that is posited for the relationship between obesity and POP relates to the extra pressure that is added to the pelvic floor due to obesity. Therefore, it may be of interest to evaluate if SNPs associated with BMI or waist to hip ratio as reported in the current literature collectively contribute to POP, independent of obesity measures. Given that the GIANT and CHARGE consortiums have identified over 100 independent loci related to obesity measures [205;206]; genetic risk scores for BMI and waist-to-hip ratio could be constructed. POP status could then be regressed onto the genetic risk score while adjusting for other risk factors with or without BMI/waist-to-hip ratio. The presence of an association with the genetic risk score while adjusting for BMI would suggest pleiotropic effects of these SNPs that not only simultaneously affect POP through increased BMI but also through other mechanisms.

With respect to expanding on Specific Aims 2 and 3, independent studies need to be conducted to validate the signals obtained from these aims. However, currently we are not aware of many promising resources that have available information on POP, its risk factors, and genetic data. One potential source of acquiring reliable information on POP is the synthetic derivative

electronic medical record system at Vanderbilt University [207]. Our group is currently in the process of validating algorithms to identify POP cases and control selection with a high degree of sensitivity and specificity. Using preliminary algorithms we have identified a total of 1,000 individuals in the BioVU for whom information on POP status could be validated. Upon further refinement of the algorithm this resource could then be used for validating potential signals observed in Specific Aim 2.

Once the algorithm is finalized, the results from the admixture mapping study could potentially be validated by querying resources such as the Electronic Medical Records and Genomics (eMERGE) Network, a national consortium organized by NHGRI to combine EMR and genetic data from 9 institutions throughout the US; for which Vanderbilt University is the coordinating center [208]. Although the EMR records at Vanderbilt have limited information on African Americans with regard to POP, the algorithm developed here could be used to query the eMERGE network to identify African American women needed for a validation study for admixture mapping.

APPENDIX

Appendix 1. Systematic review search strategy in PubMed

(((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) NOT (((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND newspaper article[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND letter[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND comment[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND case reports[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND practice guideline[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND news[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND editorial[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND legal cases[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND in vitro[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND meta-analysis[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND interactive tutorial[pt]) OR ((((((("Pelvic Organ Prolapse"[Major] OR "Prolapse"[Major] OR "Prolapse"[tiab]) AND ("Risk Factors"[Mesh] OR "Risk"[tiab]))) AND (humans[mh]))) AND review[pt]))

Appendix 2. Women's Health Initiative Pelvic Exam form measuring the various types and grades of pelvic organ prolapse

WHI **Form 81 - Pelvic Exam** **Ver. 6.1**

9. Cervix:

☐0 Absent

☐1 Present →

	No	Yes, probably benign	Yes, possibly malignant
9.1. Flush with vaginal vault	☐0	☐1	☐2
9.2. Friable with contact	☐0	☐1	☐2
9.3. Surface lesion/ growth (other than ectopy, Nabothian cyst)	☐0	☐1	☐2
9.4. Polyp	☐0	☐1	☐2

10. Uterus:

☐0 Absent *(Go to Question 11.)*

☐1 Present →

☐9 Unable to palpate *(Go to Question 11.)*

10.1. Prolapse:	10.2. Uterine size:	10.3. Enlarged since last exam:
☐0 None	⌊⌊ weeks	☐0 No
☐1 Grade 1 (in vagina)		☐1 Yes
☐2 Grade 2 (to introitus)		
☐3 Grade 3 (outside vagina)		

11. Adnexae:

☐0 Normal

☐1 Mass present → 11.1. ☐1 Right ☐2 Left ☐3 Both

☐9 Unable to palpate/absent

External genitalia:

PAP SMEAR

12. Was Pap smear obtained?

☐0 No, not done

☐1 No, send for outside report

☐2 Yes, vaginal smear → Initiate *Form 92 - Pap Smear*

☐3 Yes, Pap smear

Follow-up

13. Was a referral made for follow-up care?

☐0 No

☐1 Yes →

13.1. Referred by: ⌊⌊⌊ _____

13.2. Date of referral: ⌊⌊ ⌊⌊ ⌊⌊ (M/D/Y)

13.3. Referred to:

MD/Clinic: _____

Address: _____

_____

Phone: _____

13.4. Pelvic follow-up results:

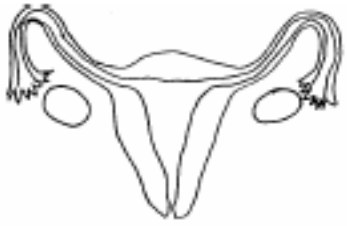
☐0 Normal

☐1 Benign changes

☐2 Possibly malignant

Cervix/vagina:

Ovaries/uterus:



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