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Can genes for mammographic density inform cancer etiology?

Linda E Kelemen¹, Thomas A Sellers², and Celine M Vachon³

¹Department of Population Health Research, Alberta Cancer Board, 1331 29th Street NW, Calgary, AB, T2N 4N2, Canada.

²H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA.

³Department of Health Sciences Research, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN, 55905 USA.

Preface

Mammographic density (MD) reflects variation in fat, stromal and epithelial tissues that is thought to be regulated by several genes. High MD is an established risk factor for breast cancer; therefore genes that regulate MD may indirectly influence breast cancer. These genes may also be fewer in number and easier to identify than those for breast cancer risk outside of inherited predisposition syndromes. In this Perspective, we review the limited genetic studies of MD and propose future directions.

Unraveling the genetics of complex diseases can be enhanced by focusing on underlying risk factors. For example, studies on lipoproteins and blood pressure have been shown to correlate well with the risk of vascular disease^{1, 2}. Insights into the pathogenesis of breast cancer may be informed in a similar way using MD because both may be determined by similar genetic variants. In fact, the number of genetic and environmental factors influencing an intermediate such as MD is likely smaller than that influencing the endpoint, namely breast cancer³. Consequently, studies to identify genes for MD could be more powerful and efficient than those for breast cancer.

MD reflects the proportion of stromal and epithelial tissue relative to fat on a mammogram. Dense breast tissue appears light or lucent on radiographs of breast images; in contrast, fat appears dark. Early measures of MD were qualitative, relying on visual scales of morphologic features^{4, 5}. Contemporary methods seek to provide a quantitative and continuous measure of the amount of dense tissue in the mammographic breast image^{6–9} (Box 2). Results from both measures show that MD is strongly associated with breast cancer risk¹⁰. Indeed, the ~30% 11 of women who have dense breast tissue in >50% of total breast area have a 3–5 fold increased risk of breast cancer compared to women with no measurably dense breast tissue^{10, 11}. Although the [0]inclusion of MD in breast cancer risk prediction models has minimally improved their overall discrimination among women of mammogram age,^{12–14} the identification of genes that regulate MD might further enhance the ability to identify women at risk of developing breast cancer. Improved risk prediction will be particularly important for young women, not yet of recommended mammogram screening age for whom prevention efforts will be most beneficial.

Evidence for a Genetic Influence

Evidence for a genetic influence on MD has come from studies on family history, familial aggregation, twin and segregation analyses.

Family History

If the genetic factors that underlie MD increase breast cancer risk, then MD should be associated with family history of breast cancer. Early investigations focused on whether unrelated women with a family history of breast cancer are more likely to have a high MD pattern than women without such a history. Both positive^{15–17} and negative^{18–20} results were found. More recently, two large studies of 35,019²¹ and 6,146²² women from population-based mammography registries have illustrated positive associations between family history of breast cancer and higher MD as determined by BI-RADS^{21, 22}. The odds of a woman having dense breast tissue is 17% greater for a first degree relative with a family history of breast cancer and 22% greater for women with breast cancer that was diagnosed at <50 years of age²¹.

Familial Aggregation and Twin Studies

Data on related individuals, although based on small sample sizes, show similar mammographic patterns among mother-daughter and sister pairs compared to unrelated pairs²³. Familial correlation of percent MD was estimated to be 22% among 275 sister pairs but attenuated to 16% after adjustment for body weight.

Twin studies demonstrated greater concordance of MD patterns among monozygotic compared to dizygotic twin pairs^{24–25}. A large multi-centered study of 571 monozygotic and 380 dizygotic twin pairs estimated that 60–67% of the variation in MD is attributable to common genetic factors^{26–27}. Estimates of heritability are stronger when twin pairs are of similar BMI (77% versus 28% when dissimilar on BMI)²⁸. This underscores the importance of accounting for BMI and possibly other covariates when searching for genetic influences on MD.

Segregation Analysis

The above analyses implicate a genetic influence on MD but not the possible mode (Mendelian pattern of inheritance, for example) of transmission of a putative gene(s). In the only reported segregation analysis, the distribution of MD among 1,370 females in 258 independent families was compatible with Mendelian dominant or codominant transmission of a major gene. This was only apparent after adjustment for covariates, including BMI and postmenopausal hormone (PMH) use²⁹. Under the dominant model, 12% of the female population could carry an allele for higher MD, and women carrying at least one dominant allele would have a mean MD that is 27% higher than the rest of the female population²⁹.

The Search for Mammographic Density Genes

Two approaches have been used to identify genes that influence MD: a hypothesis-dependent approach in which knowledge of breast biology guides the selection of candidate genes, and a hypothesis-free approach, in which markers positioned across the genome are evaluated with MD using linkage or genome wide association methodology.

Candidate Gene Analysis

This approach estimates the extent that polymorphisms in a biologically plausible candidate gene influence MD, usually in unrelated individuals. However, our limited understanding of the biology of MD makes selection of candidate genes challenging. Furthermore, MD is a dynamic trait, decreasing with older age and menopause, and even varying transiently during the menstrual cycle. Higher densities are observed among women who are premenopausal,

nulliparous, of low BMI or taking combination PMH, and lower densities among those who are postmenopausal, multiparous, of high BMI or on tamoxifen therapy. Because of these hormonal influences the majority of candidate gene studies have focused on evaluation of the pathways that regulate steroid hormone synthesis and metabolism, hormone receptors and proliferative pathways including the insulin-like growth factor (IGF) pathway. However, some studies have focused on genes previously noted to be strongly (*BRCA1/BRCA2*) or hypothetically (*XPB*, *XRCC3*, and *ERBB2*) associated with breast cancer risk. The results from all of these candidate gene association studies are reviewed in the online Table and some are highlighted below. It should be noted that the size of these studies vary greatly, as do the aims of the studies, the characteristics of the women analyzed and the method of assessing MD, which makes interpretation challenging.

Characteristics of Studies—The majority of studies compared a difference in MD among women with 1 or 2 copies of a polymorphism to women with 0 copies and also estimated tests for trend of a dose-response of 0, 1 or 2 copies. Of 25 identified publications (Table 1), three studies examined several polymorphisms representing genetic variation throughout the entire gene and used haplotype approaches for analyses^{30–32} while the remainder largely evaluated candidate polymorphisms with putative functional effects that were previously examined with breast cancer. Six studies (24%) had fewer than 300 women^{33–38}, 14 (56%) had between 300–650 women^{31, 39–51} and five (20%) had between 1,100–2,000 women^{30, 32, 52–54}. All studies except four investigations of *BRCA1/2* mutation carriers^{33–36} and one cross-sectional study⁵³ estimated MD using the computer-assisted thresholding method.

Most studies adjusted statistically for age and BMI, and also adjusted or stratified by menopausal status^{30, 31, 38, 39, 41–48, 50, 55} or PMH use^{31, 38–48, 55} or restricted analyses to women who did^{37, 52} or did not^{47, 53} take PMH, or women who were premenopausal^{32, 54}. Although stratification produces homogeneous subsets of women to examine associations between genetic factors and MD, the resulting sample sizes can be too small to produce any meaningful conclusions. The populations also varied and included women from screening mammogram populations who were^{42–44} or were not^{45, 46, 54} selected for extreme MD phenotypes, control subjects in case-control studies of breast cancer^{30, 31, 41, 55}, healthy women participating in cohort studies^{32, 47, 48, 52, 53}, breast cancer cases with pre-diagnosis mammograms^{39, 40, 51}, and a twin study⁵⁰. The highly-selected sample from a cardiovascular-focused clinical trial³⁷ will not be discussed in the remainder of this Perspective.

Below, we focus our discussion on those polymorphisms evaluated in >1 study of percent MD with similar sample characteristics, and we seek to explain similar or disparate findings. The differences in absolute dense and non-dense area in relation to the polymorphisms will not be discussed.

***CYP17A1* +27T>C:** This polymorphism is located in the cytochrome P450 17A1 promoter region but its effect on steroid hormone biosynthesis is inconclusive⁵⁶ (Figure 1). Inappreciable differences in percent MD across genotypes were observed in four studies restricted to 94 to 268 premenopausal women and regardless of age (< 50 years versus > 50 years), study design (mammograms from prospectively enrolled healthy women versus pre-diagnosis mammograms from breast cancer cases), and ethnicity^{39, 41, 42, 46}. Non-significant associations were also observed among samples of 171 to 1,260 postmenopausal women irrespective of current^{39, 41} or past or never^{39, 53} PMH use. The data, therefore, provide little support for a role of this polymorphism in MD or in breast cancer⁵⁷.

***COMT* Val¹⁵⁸Met:** The COMT enzyme inactivates the bioactive 2- and 4- hydroxy catechol estrogens by methylation⁵⁸ (Figure 1). The Val¹⁵⁸Met polymorphism decreases enzyme activity⁵⁸ and is the most widely studied polymorphism with MD. Among healthy

premenopausal women^{41, 42, 46}, carriers of two copies of the Met allele had higher MD in one study of Caucasians participating in a case-control study of breast cancer⁴¹, and lower MD in two studies of women enrolled from a screening mammography clinic^{42, 46}. The latter two studies comprised mixed ethnicities including Asians and, although adjustment was made for ethnicity, this and the younger age distribution compared to the study by Haiman et al⁴¹ may account for the differences among relatively healthy premenopausal women. The remaining studies of *COMT* Val¹⁵⁸Met among premenopausal women included breast cancer patients whose screening mammograms were retrieved within the five years preceding diagnosis³⁹ or whose mammograms were performed after breast cancer diagnosis but prior to treatment³⁸ showed only minor differences in MD across *COMT* genotypes, and the study by Takata et al³⁸ did not evaluate healthy controls separately from cases by menopausal status.

Two studies which enrolled 67³⁹ and 1,260⁵³ postmenopausal women who never used PMH or used PMH in the past showed no particular pattern of percent MD across *COMT* genotypes. Investigations of postmenopausal women that did not differentiate among hormonal regimens but which adjusted for PMH use reported either no significant differences of MD across *COMT* genotypes^{42, 50} or significantly lower MD among carriers of both Met alleles⁴¹. The two studies^{41, 53} closest in comparability due to similar age distributions and study populations (healthy women participating in a nested case-control or prospective cohort study) had disparate findings, which could be attributable to the three fold difference in sample sizes, the statistical adjustment for PMH use⁴¹ versus sample restriction to past or never PMH use⁵³, or to method of mammogram assessments (computer assisted^{39, 41} versus visual assessment by three radiologists using the Boyd six-category scale⁵³). Statistical adjustment for PMH use or menopausal status without evaluation of the assumption that the effect is constant across strata of these variables results in pooled estimates that can conceal important stratum-specific effects. Also, although both MD assessment methods are shown to predict risk of breast cancer¹⁰, classification of MD using the Boyd categorical 6-point scale can overestimate risk of breast cancer among women aged >50 years compared to the computer-assisted threshold assessment method⁷. Thus, an ideal comparison of results by genotype necessarily relies on similar methodology. In summary, although the *COMT* Val¹⁵⁸Met polymorphism does not appear to be a strong risk factor for breast cancer⁵⁹ regardless of menopausal status⁵⁷, the data suggest it may influence premenopausal MD.

ESR1 XbaI and PvuII: Estrogen receptor alpha is a nuclear receptor that mediates estrogen or other steroid hormone action by regulating gene transcription (Figure 1). The two polymorphisms in intron 1 of *ESR1* (known as *XbaI* and *PvuII*) have no known functional effect on gene expression^{57, 60}, although intronic polymorphisms may affect the post transcriptional processing of gene products and affect receptor binding affinity.

Three studies examined the *XbaI* polymorphism. The European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts analyzed cross-sectional estimates of MD across *XbaI* genotypes from 620 postmenopausal women in the Netherlands (EPIC-Prospect) who never used PMH⁴⁷ and 1,286 postmenopausal women in the United Kingdom (EPIC-Norfolk) who were past or never users of PMH⁵³. While the former study⁴⁷ reported a significantly higher MD with increasing copies of the x variant allele, the latter study⁵³ reported no appreciable difference in percent MD across genotypes. The greater sample size in the latter study⁵³ is a strength, although MD assessments were subjective and differed from the computer-assisted method used in the EPIC-Prospect study. In a third study⁵², the computer-assisted method was used for all MD readings among mostly (~70%) postmenopausal women participating in the combined EPIC Prospect and Norfolk cohorts. In this study, the densities of 781 women who never used PMH were compared at baseline and at 5 years of follow-up across *XbaI* genotypes, thereby reflecting a natural change in MD during this time period. In addition, mammographic densities were compared across *XbaI* genotypes among 795 women who never used PMH at

baseline but who subsequently initiated PMH use during the 5 years of follow-up, thereby reflecting the influence of PMH use during this period. The results suggested that the women who carried at least one *x* allele had significantly attenuated declines in MD if they took PMH⁵². The *PvuII* polymorphism, which is in linkage disequilibrium with the *XbaI* polymorphism, was also studied in the two aforementioned investigations and shows similar results^{47, 52}. Confirmation of these findings by independent investigators would suggest that PMH use interacts with the *XbaI* and *PvuII* polymorphisms to moderate the natural decline in MD associated with the postmenopausal years, possibly to influence breast cancer risk, but this remains to be determined. The *PvuII* *p* allele and the *XbaI* *x* allele have each been associated with breast cancer^{61–63}.

***PGR* *PROGINS* complex:** Two (Val⁶⁶⁰Leu and His⁷⁷⁰His) of three polymorphisms in the progesterone receptor (*PGR*) gene are in complete linkage disequilibrium and together all three are called the *PROGINS* complex⁶⁴ (Figure 1): they may affect ligand binding and gene transcription⁶⁵. In the EPIC-Prospect⁴⁷ and EPIC-Norfolk⁵³ studies, neither Val⁶⁶⁰Leu or His⁷⁷⁰His were associated with cross-sectional estimates of MD among postmenopausal women who had not used or who were past users of PMH⁵³, nor with longitudinal change in MD among women who used PMH⁵². Each copy of the Val⁶⁶⁰Leu allele was reported to increase breast cancer risk by a modest 8% ($P = 0.05$)⁶⁶.

***IGFBP-3* –202C>A:** This polymorphism is located in the promoter region of the gene and may increase serum IGFBP-3 levels⁶⁷. It has been investigated by five groups. Examination of 215 healthy premenopausal women participating in a prospective study of breast cancer in the United Kingdom⁴⁸ and 741 healthy premenopausal women attending a mammography clinic in Quebec, Canada⁵⁴ showed no influence of the genotype on MD. A third study among 206 healthy premenopausal women attending an outpatient clinic in Toronto, Canada showed a significantly higher MD among women with two copies of the A allele⁴⁵. Each study used a computer-assisted method to assess MD. However, none of the studies defined the ethnic composition of their sample of women or adjusted for ethnicity. The potential for population stratification among a potentially multi-ethnic sample residing in Toronto⁴⁵ with the UK⁴⁸ and French Canadian⁵⁴ participants warrants further exploration. In addition, postmenopausal women who never used PMH or used PMH in the past^{45, 48} showed no appreciable difference in percent MD across genotypes of this polymorphism. Also, two additional studies, the Australian twin study of 307 postmenopausal women⁵⁰ and the Nurses' Health Study of 917 postmenopausal controls that participated in a case-control study of breast cancer³⁰ found no difference in MD across genotypes following adjustment for PMH use. In summary, the data suggest this polymorphism is not associated with MD regardless of menopausal status, although the C allele has been associated with lower circulating levels of *IGFBP-3*⁶⁸ and marginally significant decreased breast cancer risk⁶⁶.

***IGF-I* rs1520220 C>G and rs6220 A>G:** The rs1520220 C>G polymorphism is located in intron 3, while rs6220 A>G is located in the 3' UTR; neither polymorphism's function is known. Both were evaluated in two studies of premenopausal women comprising large samples of healthy women attending a mammography clinic (746 in Quebec, Canada⁵⁴ and 1,928 in the Netherlands³²). Only the Canadian study observed a significantly higher percent MD among women who carried two copies of the rs1520220 G allele⁵⁴ and both studies observed a marginally significantly higher percent MD with two copies of the rs6220 G allele^{32, 54}. Both studies^{32, 54} also showed higher serum IGF-I with each polymorphism, although significance was attained with two copies of the rs6220 only³². Rs6220 A>G has also been associated with increased breast cancer risk⁶⁹.

A third study among healthy (204 premenopausal and 917 postmenopausal) women in the Nurses' Health Study observed significantly lower percent MD among those with both copies

of the rs1520220 G allele, but no apparent difference was noted by menopausal status³⁰. The evidence thus far does not support a consistent relation of rs1520220 C>G with MD or with breast cancer risk^{70, 71}, although rs6220 A>G may be a potential candidate for premenopausal MD.

HSD3β1 Asn³⁶⁷Thr: One seemingly consistent finding among genes in the steroid hormone biosynthesis pathway (Figure 1) is the lower level of MD among Caucasian carriers of the Asn³⁶⁷Thr variant of hydroxysteroid dehydrogenase 3β1 (*HSD3β1*). This polymorphism has no known function. A cross-sectional study of 242 (~50% postmenopausal) Caucasian breast cancer patients with pre-diagnosis mammograms found a significantly lower percent MD among women with one or more copies of the Thr allele compared to no copies³⁹. This finding was confirmed in a study of 457 healthy Caucasian women (~67% postmenopausal) from 207 sisterhoods that also reported a significantly lower MD from one or more copies of the Thr allele⁵⁰. Analyses restricted to 176 premenopausal women with two copies of the Thr allele displayed a non-significantly higher MD in the former study³⁹, but a marginally significantly lower MD in the latter study of 150 premenopausal women⁵⁰. Potential reasons for the discrepancy could be variability from small sample sizes within subgroup analyses, the comparison of mammographic densities obtained retrospectively from women who were recruited as breast cancer cases with those of a selected group of twin-pairs, and the slightly lower age among the breast cancer cases compared to the twin-pairs, suggesting an enrichment of younger, higher-risk women in this³⁹ study. Although lower MD is seen for the Thr allele among Caucasian women after adjusting for menopausal status, the associations are inconsistent among premenopausal women, and not significant among postmenopausal women. The relevance of this polymorphism to MD, therefore, remains unclear. The association between the Asn³⁶⁷Thr variant and breast cancer risk has not been studied.

CYP19A1 (TTTA)n: The cytochrome P450 19A1 gene, also known as aromatase, contains a microsatellite repeat polymorphism that may alter the splice site during post-translational protein modification⁷²; however no functional effect of the polymorphism on estrogen biosynthesis is known⁷³ (Figure 1). Two studies of 486⁴¹ and 550³¹ Caucasian women found no statistical association with MD among carriers of the 10 repeat allele compared to carriers without this allele in models that adjusted for age, BMI and menopausal status^{31, 41}. In the same studies, carriers of the 12 repeat allele had marginally significantly lower MD levels than women without this allele in one³¹ but not in the other⁴¹ investigation. Both were case-control studies of breast cancer and used the computer-assisted thresholding method of MD assessment among controls. Differences in SNP associations with MD may be from chance or from subtle ancestral differences among the Caucasian samples. Additional analyses of haplotypes that captured variation across the entire *CYP19A1* gene with MD were not statistically significant³¹. Thus, the association of the *CYP19A1* 12 repeat allele with MD is inconclusive, and does not appear to be associated with breast cancer^{57, 74, 75}.

CYP19A1 +27(TCT)+/- and +268T>C: *CYP19A1* +27(TCT)+/- is an insertion/deletion polymorphism in intron 4 and is in linkage disequilibrium with *CYP19A1* (TTTA)n. *CYP19A1* +268T>C resides in exon 10 of the 3' untranslated region (UTR) of the gene, but has no known function (Figure 1). Neither polymorphism was significantly associated with percent MD among 550 Caucasian women participating as controls in a case-control study of breast cancer in the United States regardless of menopausal status or PMH use among postmenopausal women³¹ or among 1,286 postmenopausal women of unreported ethnicity participating in a prospective cohort study in the United Kingdom who were past or never users of PMH⁵³. A third study among 486 Caucasian controls in the United States also did not observe a significant association of the +268T>C polymorphism following adjustment for age and BMI and irrespective of menopausal status or use of PMH among postmenopausal women⁴¹. In the

absence of additional data, these polymorphisms do not appear to be associated with MD nor appear to be breast cancer risk factors^{74, 75}.

AR CAG repeat polymorphism: Two studies investigated this polymorphism in the androgen receptor gene (Figure 1). Longer transcripts may produce less active receptor^{76, 77}. MD did not vary by number of AR CAG repeats (≥ 22) among 538 (~73% postmenopausal) healthy Caucasian women participating as controls in a study of breast cancer⁴¹ or by ≥ 21 AR CAG repeats among 246 (~50% postmenopausal) Caucasian women with breast cancer whose screening mammograms were retrieved within the five years preceding their cancer diagnosis⁴⁰. The former study⁴¹ did not observe an effect on percent MD from PMH use, although women in the latter study⁴⁰ with the longer less active AR allele had higher MD if they also took a combination formulation of estrogen plus progesterone PMH. The associations with MD may reflect a large number of comparisons in the data of relatively small numbers of women⁴⁰. Moreover, the AR polymorphism is inconsistently associated with breast cancer risk^{78, 79}.

Tumor suppressor genes: Over 750 protein-truncating mutations exist in *BRCA1* and *BRCA2*^{80, 81}. Findings from early studies were mixed^{33–36} and relied on varied methods of qualitative assessments of MD in addition to very small sample sizes of 9 to 34 mutation carriers (Table 1). A recent well-designed study using the computer-assisted thresholding assessment found no difference in MD between 206 mutation carriers and 136 noncarriers after adjustment for age at mammography and BMI⁴⁹ (online Table). Although strong risk factors for heritable breast cancer, these mutations are not associated with MD.

CYP1A2 -154A>C (CYP1A1*1F): This polymorphism may induce enzyme activity⁸² to lower bioactive estrogen concentrations and, hypothetically, MD. A marginally significantly lower MD was observed overall among 268 pre- and 60 postmenopausal women screened in a mammography clinic, and particularly among premenopausal women⁴⁶, whereas a statistically significantly higher MD was reported among control subjects from a case control study of breast cancer in 97 pre- and 153 postmenopausal women³⁸. Interestingly, both studies sampled from among female residents in Hawaii. The former study⁴⁶ composed healthy women with an entry (baseline) mammogram, while the latter study³⁸ incorporated healthy women participating in two nested case-control studies nested within the Multiethnic Cohort Study who each had an average of 2.5 mammograms in order to reduce intra-individual variability in MD measurements. Given similarities in computerized mammographic assessments and adjustment with similar covariates, the findings from the latter study³⁸ may be more reliable due to increased precision of the measure. With only two studies, the influence of the polymorphism with MD is inconclusive, as is its association with breast cancer in the Shanghai Breast Cancer Study⁸³, although decreased risk has been observed among predominantly postmenopausal women in the Multiethnic Cohort Study⁵⁹.

CYP1A1 Ile⁴⁶²Val and CYP1B1 Val⁴³²Leu: Higher CYP1A1 enzyme expression⁸² or activity⁸⁴ and CYP1B1 enzyme activity^{85, 86} may lower bioactive estrogen concentrations (Figure 1). Neither polymorphism is associated with MD among predominantly premenopausal⁴⁶ or predominantly postmenopausal⁴¹ healthy women. Also, *CYP1B1* Val⁴³²Leu was not associated with MD among 1,260 cohort members who were past or never users of PMH⁵³. MD among 140 current users of PMH with both copies of the *CYP1B1* Leu allele, however, was lower than Val/Val carriers, although of borderline significance only⁴¹. This relation among current users was not evaluated in the other studies and requires confirmation. Neither polymorphism is strongly associated with breast cancer^{57, 59, 84, 87}.

Summary: The hypothesis-dependent approach focusing on candidate genes that may influence breast cancer has produced mostly conflicting results regardless of whether the polymorphisms have known functional effects (Table 2). Indeed, the accumulated evidence indicates that the *COMT* Val¹⁵⁸Met and *IGF-I* rs6220 3' UTR polymorphisms (or variants in strong linkage disequilibrium with these polymorphisms) influence percent MD in premenopausal women, and the *ESR1* intronic *Xba I* and *Pvu II* variants may lead to higher percent MD among women taking PMH. The remaining studies are inconclusive, mostly from too few independent investigations or from between-study heterogeneity in sample characteristics.

Linkage Analysis

A major limitation of candidate gene studies is the low prior probability that an association is true⁸⁸. Selecting candidate genes in a region with prior evidence of genetic linkage directly addresses this weakness. Because the regions identified by linkage analysis are hypothesis-independent, they can identify a gene or genes that are not anticipated. In multifactorial complex traits like MD this is particularly important.

Two linkage analyses of MD have been conducted. Both used a large breast cancer family study as the sampling frame. The first was a small sibpair study of 71 sisterhoods in 22 families and used 147 microsatellite markers placed across the autosomes at a spacing of 30 centiMorgans (cM). The results indicated a possible linkage to chromosome 689. The second larger study included 889 members of 89 multigenerational families and 403 markers spaced an average of 9 cM apart⁹⁰. The strongest evidence for linkage was identified on chromosome 5p with a maximum log odds for linkage (or LOD score) of 2.9. Finer mapping of this region with a dense set of markers strengthened the evidence for linkage (LOD=4.2). The 1-LOD region which contains 45 genes has not been previously implicated in breast cancer and is currently being pursued. Further, although this locus was not linked to BMI, it was only apparent after adjustment for BMI, and could explain up to 22% of the total variability in MD among women. Two other suggestive regions for linkage were also identified on chromosome 12, one containing the *IGF-I* gene. Linkage analysis, then, can result in identification of novel genes that may influence both MD and breast cancer risk.

Genome Wide Association Studies

Genome wide association studies (GWAS) utilize a large number of polymorphisms positioned across the genome. Polymorphisms are not confined to coding or regulatory regions and the function of the majority of these polymorphisms is unknown. No GWAS has been reported for MD, but six polymorphisms identified from three GWASs of breast cancer^{91–93} have been evaluated by one investigative team in relation to MD (online Table). In this study of 516 Caucasian breast cancer cases, Lee et al.⁵¹ observed no association of these variants with MD using the mammogram of the contralateral (non-cancerous) breast, although a positive association with MD of rs3817198 T>C within the lymphocyte-specific protein 1 (*LSP1*) gene on chromosome 11p was seen specifically in women with ER+/PR+ tumors. One of the LSP1 protein functions is to regulate adhesion to fibrinogen matrix proteins, which may have implications for MD.

Summary and Future Directions

In summary, there is strong evidence for a genetic component to MD. Both hypothesis-dependent and hypothesis-free approaches have provided clues to relevant genes and/or loci. Candidate gene approaches yielded replicated associations between *COMT* Val¹⁵⁸Met and *IGF-I* rs6220 A>G and premenopausal MD and *ESR1* (*Xba I* and *Pvu II*) polymorphisms and postmenopausal MD, which possibly translate to breast cancer risk. However, many

polymorphisms have only been examined in a few studies, limiting the conclusions that can be drawn (Table 2). Technological advances make genome-wide scans feasible and may reveal novel candidate genes, while haplotype block or tagSNP approaches are advantageous to study all common variation within a gene. Linkage analyses identified a candidate region on chromosome 5p encompassing 45 genes that are also under consideration for their influence on MD.

Future investigations should incorporate well-characterized study populations including specification of age, race and ethnicity, quantify MD and its changes in the most objective manner possible, stratify subjects by design (rather than post-hoc) by menopause and specific type of hormone regimen with mammographic densities reflecting the timing of PMH exposure, and consider assessment of earlier periods in life when MD changes are most dramatic and the inter-individual variation is greatest. Several protocols reviewed in this Perspective drive these recommendations and are strong studies for future comparisons and synthesis^{32, 48, 52}. Also, the reviewed studies underscore the importance of measuring and accounting for non-heritable factors to allow better detection of genetic influences on MD. Several of the polymorphisms examined thus far have inconclusive functional significance, requiring research to understand tissue response to genetic polymorphisms that alter local biosynthesis, metabolism or catabolism of hormones or growth factors. Ultimately, identification of genetic risk factors for early markers of disease may permit better risk prediction and targeted early preventive or therapeutic strategies for breast cancer.

Textbox 2 Breast density classification methods

Qualitative		Quantitative		
Wolfe ⁴	BI-RADS ^{5, 8}	Planimeter (outlining tool) ⁶	Boyd 6-category scale ^{7, 94}	Computer-assisted threshold method ⁹⁵
Visual assessment of the relative percentage of fat (appears dark) and epithelial/stromal (appear white) tissue obtained from mammographic x-rays. Four categorical patterns of breast density are described: N1 – “normal”, almost completely fatty breast; P1 – mainly fatty breast with prominent ducts, up to 25% density; P2 – prominent ducts, more than 25% density; and DY – no visible ducts, diffuse and extensive nodular density.	Standardized reporting of visual assessment of mammographic findings by the American College of Radiology Breast Imaging Reporting and Data Systems (BI-RADS). Four categorical patterns of breast density are described: Category 1 – entirely fatty breast; Category 2 – breast containing scattered stromal tissue; Category 3 – heterogeneously dense breast; Category 4 – extremely dense breast.	An acetate overlay is placed over radiographic image of the breast to outline the breast and to trace dysplastic elements. The total breast and dense areas are measured with an outlining tool. Agreement (kappa) between Wolfe score and planimeter = 0.91. Results can be reported as percent density distribution by quartiles or quintiles.	Radiologist assessment of the relative percentage of fat and epithelial/stromal tissue is obtained from mammographic x-rays. Individual scores for a given density estimate are assigned the midpoint of six potential categories of breast density: 0%; 5% for 0–10%; 17.5% for 10–25%; 37.5% for 25–50%; 62.5% for 50–75%; and 87.5% for 75–100%.	Mammographic films are digitized (Figure) and 2 different threshold values selected: one separates the image of the breast from background (red line) and the other identifies the edges of the regions representing radiographically dense tissue (green line). Percent density is the proportion of total breast area calculated from the histogram of pixel values in the 2 different threshold areas. Results can be reported as mean percent density by genotype.

Textbox 3 Polymorphisms in the IGF-1 axis

Components of the IGF axis may influence the tissue composition of the breast (Figure 2). High circulating levels of IGF-1, or higher IGF-1 in relation to IGFBP-3, correlate with higher premenopausal mammographic density^{47,88–91} similar to their association with breast cancer risk⁹⁶. Six studies reported 29 polymorphisms in *IGF-1* and 16 polymorphisms in *IGFBP-3* or *IGFBP-1* with mammographic density. Most polymorphisms were examined in only one study and are described in the online Table. In addition to the three SNPs discussed in the text, five polymorphisms have each been evaluated by two investigative teams:

***IGF-1* polymorphisms.** Four polymorphisms (rs2946834 G>A, rs7965399 T>C, rs1019731 C>A, and rs1549593 G>T) have either been evaluated among ~700 premenopausal women^{32, 54} or among 204 premenopausal and 917 postmenopausal women combined³⁰. Of these, rs2946834 G>A in the 3' UTR, was inversely related to percent density among pre- and postmenopausal women combined who carry both copies of the A allele³⁰, but not among premenopausal women only³². Different sample compositions make interpretation difficult. Polymorphisms rs7965399 T>C, rs1019731 C>A, and rs1549593 G>T were each evaluated in premenopausal women and in pre- and postmenopausal women combined with no significant differences observed in density across genotypes^{30, 32, 54}.

***IGFBP-3* rs3110697C>T.** This polymorphism was investigated in two studies, one among 741 premenopausal women⁵⁴ and another among 204 premenopausal and 917 postmenopausal women evaluated separately and combined³⁰. Neither showed appreciable differences in density across genotypes.

***IGF-1* and *IGFBP-3* haplotypes.** The Nurses' Health Study³⁰ and EPIC-Prospect³² explored haplotypes with mammographic density. None were significantly associated with percent MD^{29,31}.

The associations of the aforementioned individual polymorphisms and haplotypes with density are too few to interpret definitively. Furthermore, associations with breast cancer risk have not been found for selected polymorphisms and haplotypes^{70, 71, 97}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Textbox 1 Glossary terms

Stroma	The supportive framework of a biologic tissue with an extensive extracellular matrix that serves to support cells, separate tissues and regulate intercellular communication.
Familial aggregation	A tendency for the trait under study to occur or cluster in multiple family members more often than would be expected by chance.
Parent-offspring/twin studies	Investigates the proportion of variation in the trait under study that is explained by unmeasured additive genetic factors.
Segregation analysis	Tests hypotheses about whether the existence of major genes account for the observed pattern of familial aggregation of the trait and provides evidence for the mode of inheritance of the genes using pedigree data. Statistical models compare Mendelian inheritance patterns of a trait to a model in which there are no restrictions on mode of inheritance or other model parameters.
Linkage analysis	The process of determining the approximate chromosomal location of a gene associated with the trait being studied by looking for evidence of cosegregation with other marker genes whose locations are already known.
Genetic association studies	Tests whether a polymorphism in a specific candidate gene explains the inter-individual variation in the trait being studied.
Linkage disequilibrium (LD)	The nonrandom association of alleles at two or more loci on chromosomes in a population more than would be expected by chance.

Haplotype blocks	Configurations of alleles within regions on the chromosome which tend to be inherited together (that is, in high LD). Little genetic variability is observed within this region among individuals in a population.
tagSNPs	A reduced set of single nucleotide polymorphisms that identify or “tag” other SNPs with which it is in high LD.

Figure 1. Pathways of Steroid Hormone Synthesis, Metabolism and Sensitivity of Tissues

Steroid hormone biosynthesis. The cytochrome p450 (CYP) 17A1 enzyme catalyzes the conversion of pregnenolone and progesterone to the hormones, dehydroepiandrosterone (DHEA) and androstenedione, respectively, which are further metabolized to estrone (E1) and 17β-estradiol (E2) by the CYP19A1 (aromatase) enzyme. Androgen conversion to estrogen in adipose tissue by CYP19A1 is an important source of bioactive endogenous estrogens among postmenopausal women. The hydroxysteroid dehydrogenase (HSD) enzyme HSD3β1 catalyzes the interconversion of pregnenolone and progesterone and of DHEA and androstenedione, while HSD17β1 catalyzes the interconversions of androstenedione and testosterone and of E1 and E2. Testosterone and E2 bind to the serum sex hormone binding globulin (SHBG), which regulates the bioavailability of these hormones in target cells. **A. Estrogen catabolism.** The CYP1 family of enzymes are phase I enzymes that function in the oxidative metabolic activation and deactivation of compounds including several steroid hormones. E1 and E2 preferentially undergo 2-hydroxylation (2-OH-E1 or -E2) by the CYP1A1 and 1A2 enzymes, whereas E1 and E2 preferentially undergo 4-hydroxylation (4-OH-E1 or -E2) by CYP1B1. Catecholestrogens are deactivated by catechol-O-methyltransferase (COMT). UDP-glucuronosyltransferase (UGT) 1A1 is a detoxifying enzyme, which converts endogenous substrates including E1 or E2 and their metabolites to inactive hydrophilic glucuronides. **B. Progesterone catabolism.** The aldoketo reductase family 1, member C4 (AKR1C4) enzyme catalyzes the conversion of progesterone and androstenedione to their corresponding alcohols. **C. Gene transcription.** Progesterone, testosterone and E2 bind to their respective nuclear receptor proteins, progesterone receptor (PR), androgen receptor (AR) and estrogen receptor alpha (ERα), and activate genes with

corresponding responsive elements. The steroid receptor coactivator protein, amplified in breast-1 (AIB1) interacts with ER α in a ligand-dependent manner to increase estrogen-dependent transcription.

Figure 2. The IGF Axis

The insulin-like growth factor (IGF)-1 plays a role in regulating cellular growth, differentiation and apoptosis in normal breast and in mammary carcinogenesis and works in conjunction with growth hormone (GH), sex steroids and other hormones. Growth hormone-releasing hormone (GHRH) and its receptor (GHRHR) are both involved in the regulation of pituitary synthesis and release of GH. Although most IGF-1 is synthesized in the liver in response to pituitary GH secretion, local production occurs in tissues including the breast. This autocrine and paracrine production of IGF-1 within IGF-1-responsive tissues is of major physiological importance and supports a role for IGF-1 as a potent mitogen in the development of some cancers including breast cancer¹¹⁸. IGF-1 production is also influenced by estradiol, which directly stimulates IGF-1 production in estrogen-responsive tissues through the association of estrogen receptor α (ER α) with IGF1R¹¹⁹ and indirectly stimulates IGF-1 production in the liver through pituitary release of GH. In circulation, IGF-1 binds to one of six different IGFBP, the majority of which is bound to IGFBP-3. IGFBP-3 is largely dependent on hepatic production and can prolong the half-life of IGF-1 and prevent it from interacting with cell surface receptors (IGF1R), where it is capable of modulating IGF-1's biological response¹¹⁸ by activating downstream signaling molecules including insulin receptor substrate 1 (IRS1) and PI3K¹¹⁸. IGFBP-3 production is negatively regulated by GH to limit apoptosis and antiproliferation.

Qualitative		Quantitative		
Wolfe 4	BI-RADS 5, 8	Planimeter (outlining tool) 6	Boyd 6-category scale 7, 94	Computer-assisted threshold method 95
Visual assessment of the relative percentage of fat (appears dark) and epithelial/stromal (appear white) tissue obtained from mammographic x-rays. Four categorical patterns of breast density are described: N1 – “normal”, almost completely fatty breast; P1 – mainly fatty breast with prominent ducts, up to 25% density; P2 – prominent ducts, more than 25% density; and DY – no visible ducts, diffuse and extensive nodular density.	Standardized reporting of visual assessment of mammographic findings by the American College of Radiology Breast Imaging Reporting and Data Systems (BI-RADS). Four categorical patterns of breast density are described: Category 1 – entirely fatty breast; Category 2 – breast containing scattered stromal tissue; Category 3 – heterogeneously dense breast; Category 4 – extremely dense breast.	An acetate overlay is placed over radiographic image of the breast to outline the breast and to trace dysplastic elements. The total breast and dense areas are measured with an outlining tool. Agreement (kappa) between Wolfe score and planimeter = 0.91. Results can be reported as percent density distribution by quartiles or quintiles.	Radiologist assessment of the relative percentage of fat and epithelial/stromal tissue is obtained from mammographic x-rays. Individual scores for a given density estimate are assigned the midpoint of six potential categories of breast density: 0%; 5% for 0–10%; 17.5% for 10–25%; 37.5% for 25–50%; 62.5% for 50–75%; and 87.5% for 75–100%.	Mammographic films are digitized (Figure) and 2 different threshold values selected: one separates the image of the breast from background (red line) and the other identifies the edges of the regions representing radiographically dense tissue (green line). Percent density is the proportion of total breast area calculated from the histogram of pixel values in the 2 different threshold areas. Results can be reported as mean percent density by genotype.

Table 1

Characteristics of genetic association studies of mammographic density . *

Author, year	Study/ Setting	Design	Subject selection	Participants	Density Assessment	Genes studied	Covariates [†]
Helvie, 1997 ³³	Michigan, U.S.	Case-series	Clinic-based	9 mutation + cases	BI-RADS	BRCA1/2	None; visual assessment only
Chang, 1999 ³⁴	Singapore/ Hong Kong	Case-series	Clinic-based	9 mutation + cases; 19 mutation – cases	Visual assessment, 5-point scale	BRCA1/2	A
Huo, 2000 ³⁵	Chicago, U.S.	Retrospective	Screening mammogram clinic & cancer risk clinic	15 mutation + cases; 30 “low risk” women	Computer based, 7-point scale	BRCA1/2	A
Tilanus-Linthorst, 2002 ³⁶	Netherlands	Cross-sectional	Clinic breast cancer cases, mammogram at time of diagnosis	34 mutation + cases; 34 sporadic cancers	BI-RADS	BRCA1/2	A, U
Haiman, 2002 ³⁹	Los Angeles, U.S.	Cross-sectional	Cancer registry cases with pre-diagnosis mammogram	176 Premenopausal 171 Postmenopausal	Computer based, USC	CYP17A1, COMT, HSD17β1, HSD3β1	A–H
Lillie, 2004 ⁴⁰				191 Premenopausal 195 Post menopausal	Computer based, USC	AR	A–C
Haiman, 2003 ⁴¹	U.S.	Cross-sectional	Control subjects in a case-control study of breast cancer	94 Premenopausal 392 Postmenopausal	Computer based, UT	CYP17A1, CYP19A1, COMT, CYP1A1, CYP1B1, UGT1A1, AR, AIB1	A, C–F, I
Tamimi, 2007 ³⁰				204 Premenopausal 917 Postmenopausal	Computer based, UT	IGF-1, IGFBP-1, IGFBP-3	A, C–F, H, I, X
Hong, 2003 ⁴²	Toronto, Canada	Cross-sectional	Screening mammogram clinic	181 Premenopausal 171 Postmenopausal	Computer based, UT	COMT	A, B
Hong, 2004 ⁴³				181 Premenopausal 173 Postmenopausal	Computer based, UT	CYP17A1	A–C, E, G, H, J
Mulhail, 2005 ⁴⁴				177 Premenopausal 171 Postmenopausal	Computer based, UT	GHI, GHRHR	A–D
Lai, 2004 ⁴⁵				206 Premenopausal 206 Postmenopausal	Computer based, UT	IGF-1, IGFBP-3	A, C, E, I, S
Maskarinec, 2004 ⁴⁶	Hawaii, U.S.	Cross-sectional and 2 dietary intervention trials	Screening mammogram clinic	268 Premenopausal 60 Postmenopausal	Computer based, UT	CYP17A1, COMT, CYP1A1, CYP1A2, CYP1B1	A–F
Lord, 2005 ³⁷	Los Angeles, U.S.	MD changes over time	Subjects in a randomized control trial of PMH & atherosclerosis	232 Postmenopausal	Computer based, USC	COMT, CYP1B1, UGT1A1, AKR1C4	A–C, L–P
Van Duijnhoven, 2005 ⁴⁷	Netherlands	Cross-sectional	Large prospective cohort participants	181 Premenopausal 432 Postmenopausal	Computer based, UT	ESR1	A, C, D–H, K, I, Q

Author, year	Study/ Setting	Design	Subject selection	Participants	Density Assessment	Genes studied	Covariates [†]
Van Duijnhoven, 2006 ⁵²	Netherlands and U.K.	MD changes over time		781 non PMH users 795 PMH users	Computer based, UT	ESR1, PGR	A, C-E, G-I, K, N, P-R
Verheus, 2007 ³²	Netherlands	Cross-sectional		1,928 Premenopausal	Computer based, UT	IGF-1	C (no observed effect from A, C-G, K, Q, R)
		MD changes over time		656 Premenopausal women who became Postmenopausal during an average 4.4 years	Computer based, UT	IGF-1	None (no observed effect from A, D-G, K, M, N, Q, R)
Warren, 2006 ⁵³	U.K.	Cross-sectional	Large prospective cohort participants	1,260 Postmenopausal non PMH users	6-category Boyd scale	CYP17A1, CYP19A1, COMT, CYP11B1, ESR1, PGR, SHBG	B, E, G, K, L
dos Santos Silva, 2006 ⁴⁸	U.K.	Cross-sectional	Large prospective cohort participants	215 Premenopausal 238 Postmenopausal	Computer based, UK	IGFBP-3	A, C, F, J, K, L, Q, T
Mitchell, 2006 ⁴⁹	U.K.	Cross-sectional	Large prospective cohort participants	206 mutation + carriers; 136 mutation – relatives	Computer based, UT	BRCA1/2	A, C-E
Takata, 2007 ³⁸	Hawaii and Los Angeles, U.S.	Average of repeated mammogram assessments at time of breast cancer diagnosis	Nested case-control study, pre-treatment mammograms for cases	Cases: 92 Premenopausal 233 Postmenopausal Controls: 97 Premenopausal 153 Postmenopausal	Computer based, UT	COMT, CYP1A2	B-H, V, W
Olson, 2007 ³¹	Upper mid-west, U.S.	Cross-sectional	Control subjects in a case-control study of breast cancer	550 women	Computer based, UT	CYP19A1	A, C-F,Y
Olson, 2008 ⁵⁵				402 Postmenopausal 136 Premenopausal	Computer based, UT	COMT	A, C-F,Y
Stone, 2007 ⁵⁰	Australia	Cross-sectional and within-sister pair analysis	Twin and sister-pairs (104 MZ, 182 DZ and 171 singletons)	150 Premenopausal	Computer based, UT	COMT, HSD3β1, IGFBP-3, HER2, XPD, XRCC3	A, C-E, K
Lee, 2008 ⁵¹	Los Angeles, U.S.	Cross-sectional	Cancer registry cases with mammogram	516 women	Computer based, USC	rs889312 – chr5q C>A rs3803662 – chr16q T>C rs3817198 – chr11p LSP1 T>C rs13281615 – chr8q A>G rs13387042 – chr2q A>G rs2981582 – chr10q FGFR2 T>C	A-D
Diorio, 2008 ⁵⁴	Quebec, Canada	Cross-sectional	Screening mammogram clinic	741 Premenopausal	Computer based, UT	IGF-1, IGFBP-3, IGF1R, IRS1, PIK3CB	C, E, I, K, V, Z (no observed effect from F–

Author, year	Study/ Setting	Design	Subject selection	Participants	Density Assessment	Genes studied	Covariates [‡]
							I, O, Q, R, AA–EE

* Abbreviations: BI-RADS, Breast Imaging Reporting and Data Systems; MD, Mammographic Density; USC, University of Southern California; UT, University of Toronto.

[‡] Covariates: A_{age}, B_{race} or ethnicity, CBMI, D_{menopausal status/PMH} use, E_{parity}, F_{age at first birth}, G_{age at menarche}, H_{family history of breast cancer}, I_{alcohol intake}, J_{waist circumference or waist to hip ratio}, K_{smoking}, L_{years since menopause}, M_{change in BMI during study period}, N_{density at baseline}, O_{past use of PMH}, P_{study arm/population}, Q_{oral contraceptive use}, R_{physical activity}, S_{coffee intake}, T_{time since blood draw}, U_{year of breast cancer diagnosis}, V_{age at mammogram}, W_{case-control status}, X_{history of benign breast disease}, Y_{geographic region of residence}, Z_{previous breast biopsies}, AA height, BB total energy intake, CC lactation, DD education, EE season of recruitment.

Table 2

Summary of association studies between polymorphisms and mammographic density.

No significant effect in >1 study	Mixed results*	Significantly lower density observed in the only study to examine it	Significantly lower density in >1 study	Significantly higher density in the only study to examine it	Significantly higher density in >1 study
<i>CYP17A1</i> +27T>C ^{39, 41, 43, 46, 53}	<i>CYP19A1</i> +268T>C ^{41, 53}	<i>COMT</i> +701A>G ⁵⁵	<i>HSD3β1</i> Asn ³⁶⁷ Thr among Caucasians adjusted for menopausal status ^{39, 50}	<i>HSD3β1</i> Asn ³⁶⁷ Thr among African Americans ³⁹	<i>ESR1</i> [†] Xba I ^{47, 52} Pvu II ^{47, 52} among postmenopausal women taking PMH
<i>CYP1A1</i> M2A>G ^{41, 46}	<i>CYP1A2</i> * 1FA>C ^{38, 46}	<i>IGF-1</i> -26989G>A ³⁰	<i>COMT</i> Val ¹⁵⁸ Met among premenopausal women adjusted for ethnicity ^{42, 46}	<i>AKR1C4</i> [†] Leu ³¹¹ Val ³⁷	<i>IGF-1</i> +1830T>C ^{31, 53} among premenopausal women
<i>PROGINS</i> complex [†] 51, 52	<i>CYP1B1</i> [†] Val ⁴³² Leu ^{37, 41, 46, 53}	<i>IGF-1</i> T>C rs7136446 (change in density from pre to post menopause) ³²		<i>PGR</i> [†] +331G>A ⁵²	
<i>IGF-1</i> -17526T>C ^{30, 32, +4996C>A^{29, 31, C>A} rs1549593^{30, 54}}	<i>COMT</i> Val ¹⁵⁸ Met ^{37-39, 41, 50, 53} among postmenopausal women	<i>PIK3CB</i> T>C rs361072 ⁵⁴		<i>AIB1</i> (CAG)n repeat ⁴¹	
<i>BRCA1/BRCA2</i> ^{32, 35, 48}	<i>UGT1A1</i> [†] (TA)n TAA repeat ^{37, 41}			<i>IGF-1</i> (CA)n repeat ⁴⁵	
	<i>AR</i> (CAG)n repeat ^{40, 41}			<i>IGF-1</i> A>G rs7956547 ³²	
	<i>IGF-1</i> 3' UTR G>A ^{30, 32, -178C>G^{30, 32, 54}}			<i>IGFBP-1</i> -574G>A ³⁰	
	<i>IGFBP-3</i> -202C>A ^{45, 48, 50, 54}			<i>GHI</i> Val ²⁶ Val ⁴⁴ , Gly ³⁰ Gly ⁴⁴	

* No consistent association in >1 study (e.g., studies that vary in significant findings or significant studies that vary in direction of effect).

[†] Examined the change in density phenotype due to PMH.