Published in final edited form as:

Hum Genet. 2012 March ; 131(3): 341–351. doi:10.1007/s00439-011-1079-5.

Associations between gene polymorphisms in fatty acid metabolism pathway and preterm delivery in a US urban black population

Xin Liu,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Guoying Wang,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Xiumei Hong,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Hui-Ju Tsai,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan

Rong Liu,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Shanchun Zhang,

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Department of Epidemiology and Health Statistics, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310058, China

Hongjian Wang,

Correspondence to: Xin Liu, xnliu@childrensmemorial.org.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-011-1079-5) contains supplementary material, which is available to authorized users.

Conflict of interest None of the authors have a conflict of interest pertaining to this work.

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Colleen Pearson,

Department of Pediatrics, Boston Medical Center, Boston University School of Medicine, Boston, MA, USA

Katherin Ortiz,

Department of Pediatrics, Boston Medical Center, Boston University School of Medicine, Boston, MA, USA

Deli Wang,

Biostatistics Research Core, Children's Memorial Research Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Emmet Hirsch,

Department of Obstetrics and Gynecology, NorthShore University HealthSystem, Evanston, IL, USA

Department of Obstetrics and Gynecology, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA

Barry Zuckerman, and

Department of Pediatrics, Boston Medical Center, Boston University School of Medicine, Boston, MA, USA

Xiaobin Wang

Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Xin Liu: xnliu@childrensmemorial.org

Abstract

There is increasing evidence suggesting that higher intakes of fish or n-3 polyunsaturated fatty acids supplements may decrease the risk of preterm delivery (PTD). We hypothesized that genetic variants of the enzymes critical to fatty acids biosynthesis and metabolism may be associated with PTD. We genotyped 231 potentially functional single nucleotide polymorphisms (SNPs) and tagSNPs in 9 genes (*FADS1*, *FADS2*, *PTGS1*, *PTGS2*, *ALOX5*, *ALOX5AP*, *PTGES*, *PTGES2*, and *PTGES3*) among 1,110 black mothers, including 542 mothers who delivered preterm (<37 weeks gestation) and 568 mothers who delivered full-term babies (≥ 37 weeks gestation) at Boston Medical Center. After excluding SNPs that are in complete linkage disequilibrium or have lower minor allele frequency (<1%) or call rate (<90%), we examined the association of 206 SNPs with PTD using multiple logistic regression models. We also imputed 190 HapMap SNPs via program MACH and examined their associations with PTD. Finally, we explored gene-level and pathway-level associations with PTD using the adaptive rank truncated product (ARTP) methods. A total of 21 SNPs were associated with PTD (*p* value ranging from 0.003 to 0.05), including 3 imputed SNPs. Gene-level ARTP statistics indicated that the gene *PTGES2* was significantly associated

with PTD with a gene-based p value equal to 0.01. No pathway-based association was found. In this large and comprehensive candidate gene study, we found a modest association of genes in fatty acid metabolism pathway with PTD. Further investigation of these gene polymorphisms jointly with fatty acid measures and other genetic factors would help better understand the pathogenesis of PTD.

Introduction

Preterm delivery (PTD, <37 weeks of gestation) is a leading cause of infant mortality and morbidity. Despite considerable research and progress, the underlying causes and pathogenesis remain unclear, and the rate of PTD remains high in the US at about 12.3% in 2008 (Martin et al. 2010).

One interesting but unsettled area is the role of fatty acids in PTD. Linoleic acid (LA: 18:2n-6) and α -linolenic acid (ALA: 18:3n-3), the only two known essential fatty acids, are desaturated and elongated into longer chain n-6 (e.g., arachidonic acids (AA: 20:4n-6)), n-3 (e.g., eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA: 22:6n-3)) polyunsaturated fatty acids (PUFAs), respectively, and then into biologically functional eicosanoids: a series of prostaglandins (PGs) and leukotrienes (LTs). AA, EPA, and DHA are critical components of phospholipids and cell membranes, and thus are involved in the integrity and function of the membrane. Furthermore, bioactive products of these PUFAs can regulate blood pressure, blood viscosity, vasoconstriction, and immune and inflammatory responses (Yashodhara et al. 2009). However, the eicosanoids, derived from n-3 and n-6 PUFAs, exert different effects. Specifically, the metabolites of arachidonic acids (AA: 20:4n-6), including PGE₂, PGF_{2 α} , thromboxane A₂ (TXA₂), 5-hydroxyeicosatetraenoic acid (5-HETE), LTD₄, and LTC₄, have been demonstrated to be involved in connective tissue synthesis and breakdown as well as uterine contractions, and thus are important mediators of the parturition process (Allen and Harris 2001; Greenberg et al. 2008). Metabolic products from n-3 fatty acids (i.e., EPA and DHA), on the other hand, can down regulate the production of the above mediators and hence their downstream effects (Calder 2009). DHA also has been shown to give rise to resolvins and protectin D1, newly identified anti-inflammatory lipid mediators (Bazan 2007). As such, imbalanced PUFAs intake, particularly lower n-3 PUFAs intake, will lead to a high n-6/n-3 ratio and thus may increase the risk of PTD.

Previous studies, although not all, have demonstrated that higher fish or n-3 PUFAs supplement intake may decrease the risk of PTD or increase gestational length (Coletta et al. 2010; Decsi 2009; Guldner et al. 2007; Horvath et al. 2007; Makrides et al. 2006; Oken et al. 2004; Olsen and Secher 2002; Szajewska et al. 2006). However, these researches have mainly focused on the relationship between dietary/supplemental intake of fatty acids and PTD. The influence of genes involved in fatty acid metabolic pathways on PTD is largely unexplored. In this report, we conducted a comprehensive pathway-based association study to evaluate the association of fatty acid metabolism gene polymorphisms with PTD in a large US black population. Specifically, we examined SNPs in nine genes, which are well known to be involved in the polyunsaturated fatty acid metabolism pathway (details are

included under methods), including fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*), cyclooxygenase-1 and cyclooxygenase-2 (*PTGS1* and *PTGS2*), 5-lipoxygenase and its activating protein (*ALOX5* and *ALOX5AP*), and prostaglandin E synthases (*PTGES*, *PTGES2*, and *PTGES3*). To our knowledge, this is the first study of this kind in an inner city black population, which is at a high risk of PTD (Martin et al. 2010) and is known for a higher consumption of LA, AA, and EPA than are whites (Lovejoy et al. 2001).

Materials and methods

Study subjects

This study focused on 1,110 black mothers (African Americans and Haitians) who have been enrolled in the Boston Medical Center (BMC) since 1998. This included 542 mothers who delivered a singleton live baby at <37 gestational weeks (66% spontaneous PTD), and 568 mothers who delivered a term singleton baby (>37 gestational weeks) with birth weight greater than 2,500 g. Gestational age was assessed based on the first day of the last menstrual period as recorded in the maternal medical record and the early (<20 weeks) prenatal ultrasound as detailed in previous publications (Tsai et al. 2008; Wang et al. 2002). The Children's Memorial Hospital Institutional Review Board (IRB) in Chicago and the Boston University Medical Center IRB approved the study protocol. All participating mothers signed written informed consent.

We collected comprehensive epidemiological and clinical data, using standardized questionnaires and maternal and infant medical records, such as maternal age, pre-pregnancy body mass index (BMI), maternal smoking at 3 months pre-pregnancy and at each trimester, maternal alcohol drinking, and infant gender. In addition, maternal venous blood, cord blood samples and placental specimens were collected.

Candidate genes selection

Many genes are known to be involved in the PUFAs metabolism pathway and many other genes are yet to be discovered. We chose to examine nine genes that are critical to the synthesis of long-chain PUFAs and the derived eicosanoids (e.g., prostaglandins and leukotrienes) (Glaser et al. 2010). Specifically, we selected genes *FADS1* and *FADS2*, which encode the two known desaturases converting the essential fatty acids (LA and ALA) to their long-chain derivatives (AA, EPA, and DHA). Numerous studies have demonstrated that the genetic variants in these two genes contribute to the variation of PUFAs in plasma, RBCs, or breast milk (Glaser et al. 2010; Merino et al. 2010), and thereby could impact the productions of biologically functional eicosanoids and the development of PTD. We also selected the genes encoding the cyclooxygenases (*PTGS1* and *PTGS2*), and the lipoxygenase and its activating protein (*ALOX5* and *ALOX5AP*), which are involved in converting the above derived long-chain PUFAs to the series of PGs and LTs. Finally, we selected three known prostaglandin E synthases (*PTGES*, *PTGES2*, and *PTGES3*) needed in the synthesis of PGE₂, the most common prostanoid with the ability to stimulate uterine contractility (Allen and Harris 2001; Glaser et al. 2010; Hara et al. 2010; Merino et al. 2010).

SNP selection and genotyping

We selected the potentially functional SNPs and tagSNPs of nine fatty acids metabolism genes. Specifically, we focused on the validated common SNPs (minor allele frequency (MAF) >5%) that have been predicted to be functional variants based on the bioinformatics tool PupaSuite (<http://pupasuite.bioinfo.cipf.es/>). For example, SNPs result in nonsynonymous amino acid change, SNPs in putative transcription factor binding sites, exonic splicing enhancers, exonic splicing silencers, microRNAs and their targets, DNA triplex, etc. Genotype tagSNPs, which capture existing patterns of genotypes for all of the SNPs of a gene, were identified through the HapMap Project (<http://www.hapmap.org>). We chose a minimal set of tagSNPs such that each unselected SNP would be in high linkage disequilibrium (LD) ($r^2 \geq 0.8$) with the tagSNPs in a Yoruban population using a pair-wise tagging approach in the Tagger program (de Bakker et al. 2005). Functional SNPs were preferentially included as tagSNPs whenever possible. Of note, for each gene we selected SNPs from an extended region 5 kb upstream from the 5' end and 2 kb downstream from the 3' end of the gene region (HapMap Data Rel24/phaseII Nov08).

A total of 231 SNPs (Table S1) were genotyped using MassArray® technology exemplified by Sequenom (San Diego, CA) at the University of Chicago Core Facility. The blind reproducibility rate was >99.9% based on 56 duplicates and 4 triplicates.

Statistical methods

We tested Hardy–Weinberg equilibrium (HWE) and SNP–SNP correlations using the program HAPLOVIEW (Barrett et al. 2005) among 568 mothers who delivered a term baby. We excluded SNPs that are in complete linkage disequilibrium (LD, $n = 6$) or have lower MAF (<1%, $n = 6$) or call rate (<90%, $n = 13$). No SNP was dropped due to the deviation from HWE ($p < 0.001$). With a total of 206 SNPs, we conducted systematic association tests for the risk of PTD, including single-SNP association tests using typed and imputed SNPs, a gene-based pathway association test, and haplotype analysis.

We tested the association between each SNP and PTD using a logistic regression model after adjustment for maternal age (<20, 20–24, 25–30, 30–35, 35 years), education (<high school, high school, some college and above), parity (0, 1, 2), marital status, maternal active smoking, passive smoking, alcohol drinking and drug use during pregnancy, maternal pre-pregnancy BMI (<20, 20–24, 25–30, 30), and infant gender. Four genetic models (i.e., general, dominant, recessive, and multiplicative) were tested, and results from the general and best-fitted models were reported. We defined the best-fitted model as the one with the lowest χ^2 goodness-of-fit test statistics.

To further increase our SNP coverage of the candidate genes, we imputed untyped HapMap SNPs (i.e., Rel24/phaseII Nov08 and PhaseIII/Rel#2) using MACH (v1.) (Li et al. 2009, 2010) based on the available genotype data of 206 SNPs. We then applied MACH2DAT to test the association between imputed dosage data (i.e., not an integer copy of an allele with consideration for imputation uncertainty) and PTD. For those significant SNPs identified from MACH2DAT, we presented the associations of their ‘best-guess’ genotypes (i.e., 0, 1, 2 copies of allele) with PTD following the statistical tests above for the typed SNPs.

Finally, we summarized the associations between each gene and then the pathway with the risk of PTD using the adaptive rank truncated product (ARTP) methods (Yu et al. 2009a). Specifically, we permuted the preterm status 10,000 times and performed association tests for the 206 typed SNPs for each permuted data set. This leads to the empirical distribution of p values under the situation of no association, and as such the gene-level and pathway-level ARTP statistics are robust to the multiple comparisons. For the genes with significant gene-based p values, we further conducted two-locus and three-locus haplotype analyses via sliding window using an R package *haplo.stats* (Schaid et al. 2002).

Results

Characteristics of study subjects

This study consisted of 1,110 black mothers who delivered babies at BMC, including 542 PTD cases and 568 term controls. Compared to mothers with term babies, those with premature babies were more likely to be less educated, unmarried, active and passive smokers during pregnancy, and illicit drug users ($p < 0.05$) (Table 1). No statistically significant difference across term status was noted with regards to parity, infant gender, maternal age, pre-pregnant BMI, or alcohol drinking. All of these variables were considered to be potential confounders/risk factors and were included in the following regression analyses.

Genetic associations of typed and imputed SNPs with PTD

Table 2 summarizes the information on nine tested genes, including the regions of focus in the present study, and numbers of typed and imputed HapMap SNPs (detailed in Supplementary Table 1). The mean densities for typed and typed plus imputed SNPs were in the range of 650–4,000 bp and 350–2,400 bp, respectively. Relative position and LD information among typed SNPs for each of the nine genes are shown in Supplementary Figures S1a–i.

Table 3 lists twenty-one SNPs that were significantly associated with PTD at the nominal level of 0.05 based on the best-fitted models. The most statistically significant associations were observed in gene *ALOX5*, including four typed SNP (rs745986, rs7089406, rs4540799, and rs10751382) and one imputed SNP (rs3780900). Specifically, mothers carrying the T allele of rs4540799 had a higher risk of PTD than those carrying the CC genotypes (OR = 1.57, 95%CI: 1.17–2.10, $p = 0.003$). A similar association was found for three SNPs. None of these typed SNPs had any predicted function based on two bioinformatics tools: PupaSuite and FuncPred (Table 3 footnote). For gene *PTGES2*, three out of seven typed SNPs showed significant associations with PTD. Mothers with the CC genotype of the potentially functional SNPs (rs4837243 and rs7047254) were more likely to develop PTD than non-CC carriers with ORs of 3.53 (95%CI: 1.35–9.27) and 1.37 (95%CI: 1.01–1.97), respectively. A marginally significant association was observed for a tagSNP (rs7862523) and PTD (OR = 0.72, 95%CI: 0.52–0.98). It should be noted that none of the associations were significant after correcting for multiple testing based on Bonferroni correction.

These significant findings were not materially changed when ancestral proportion estimates were included in the regression model (Table S2a). Of note, only 923 of the studied subjects had such information available from the Illumina panel of 1,509 African ancestry informative markers (unpublished data). The results also were not substantially changed when analyzing PTD subgroups: 391 PTD subjects who delivered between 32 and 37 weeks of gestation [PTD (32–37 weeks)] or 361 spontaneous PTD mothers who delivered as a result of spontaneous cervical effacement and dilation or premature rupture of membranes without uterine contractions (Table S2a). We also explored the associations among PTD with placental histologic evidence of intrauterine inflammation ($n = 126$, defined in a previous study (Gupta et al. 2007)), PTD subjects who delivered <32 weeks of gestation [PTD (<32 weeks)] ($n = 151$), and medically indicated PTD ($n = 181$) (Table S2b); however, some estimates might not be reliable due to the small sample size.

Gene-based association and haplotype test of association

Figure 1 shows the associations between the typed SNPs and PTD. The gene-based p value derived from ARTP methods was only significant for gene *PTGES2* ($p = 0.01$), and pathway-level test statistic was not statistically significant ($p = 0.06$). Two-locus and three-locus haplotype association tests for gene *PTGES2* did not provide additional or more significant associations compared to the single-SNP test (Fig. 2).

Discussion

Imbalanced PUFAs intake has been demonstrated to be associated with PTD, but not in all of the studies to date. Lower fish intake in early pregnancy was found to increase the risk of PTD in a prospective cohort of Danish women (Olsen and Secher 2002), but not in other studies in the Danish population (Kesmodel et al. 1997; Olsen et al. 1990, 1995). Although randomized controlled trials (RCT) and studies using biomarkers for PUFAs also showed conflicting results (Bonham et al. 2008; Horvath et al. 2007; Makrides et al. 2006; Olsen et al. 1991; Pontes et al. 2006; Reece et al. 1997; Szajewska et al. 2006), meta-analyses of RCTs have demonstrated that n-3 long-chain PUFAs supplementation during pregnancy was associated with prolonged gestation (Makrides et al. 2006; Szajewska et al. 2006).

Individual variations in genetic susceptibility may be one of the explanations for these inconsistent findings, given the mounting evidence for the effect of gene–environment ($G \times E$) interactions on the risk of PTD (Crider et al. 2005; Macones et al. 2004; Tsai et al. 2008; Wang et al. 2002; Yu et al. 2009b). If the gene–fatty acid interactions do exist, then genetic polymorphisms themselves could have moderate marginal effects on PTD and thereby be detected by an association study alone. In this large US black population, we found modest associations between gene polymorphisms in enzymes critical to fatty acids metabolism and the risk of PTD. In addition, the significant SNP-based and gene-based associations for gene *PTGES2* and PTD suggested that membrane-associated PGE synthase-2 (PTGES-2) might play important roles in total PGE₂ production and hence the risk of PTD.

Three forms of PGE synthases have been previously known and well-studied (Hara et al. 2010; Murakami and Kudo 2004). The observations of constitutive expression PTGES2 in contrast to stimulus-inducible PTGES expression suggested that the former may have more

important effects on PGE₂ production than the latter. However, PGE₂ levels in several tissues were not altered in PTGES2-deficient mice (Jania et al. 2009), which indicated that PTGES2 may not be an essential enzyme in PGE₂ synthesis. A non-synonymous SNP (rs13283456: Arg298His) in gene *PTGES2* was reported to be associated with the parameters of the metabolic syndrome (Lindner et al. 2007), type II diabetes (Fisher et al. 2007; Nitz et al. 2007) and BMI (Fischer et al. 2009), and thus support the biological relevance of PTGES2 to inflammation and insulin sensitivity, which also are part of the biological mechanisms underlying the pregnancy complications of PTD. The effect of rs13283456 on PTD is unknown; it was not included in our original SNP selection due to low MAF in Africans (<5%) and was dropped from the imputed SNPs list due to low imputation quality (rsq < 0.3). In turn, our study demonstrated that two other SNPs in *PTGES2* (rs4837243 and rs7047254) not only were significantly associated with PTD, but also appeared to be pronounced among spontaneous PTD, PTD (<32 weeks), and PTD with placental histologic evidence of intrauterine inflammation (Table S2a and S2b). PTD accompanied by histologic chorioamnionitis was shown to be significantly correlated with spontaneous and PTD (<32 weeks) in this cohort (Tsai et al. 2008). As such, our findings suggest that *PTGES2* gene polymorphisms may influence the risk of PTD through an inflammatory pathway.

Among the three significantly associated SNPs in gene *PTGES2* with PTD, rs4837243 and rs7047254 are located at the predicted transcription binding sites according to the bioinformatics tool FuncPred, indicating that they may have an impact on gene transcription, particularly for rs7047254. This SNP is located ~2 kb upstream of the transcriptional start site for gene *PTGES2*, and also at a region with high regulatory potential based on sequence alignments of seven species (King et al. 2005), and thus is likely to exhibit regulatory activity. rs4837243 is located within an intronic region ~50 nt from its 5' splice site, and is exactly within two functional RNA elements (each with 34 nt) bound by a splicing regulator, FOX2 (Yeo et al. 2009). Interestingly, gene *PTGES2* has at least three known alternatively spliced transcripts. Observed together, rs4837243 may play a regulatory role in alternative splicing of gene *PTGES2*. As such, functional studies of these significantly associated SNPs will be needed once our findings are confirmed by replication studies in different populations.

Our study has several merits. This is the first genetic association study on the fatty acid metabolism pathway and PTD, and is currently the largest candidate gene study of PTD in a black urban population in the US. We comprehensively selected functional and genotype tagSNPs for each gene, and we also imputed untyped SNPs based on both HapMap II and III data. None of the previous genetic studies of PTD have undertaken such dense SNP selection with consideration for coverage (i.e., tagSNP selection) and potential function (i.e., bioinformatics prediction). We found that 11 out of 21 significantly associated SNPs (Table 3) are not included by Illumina's Omni2.5 Bead-Chip, including two from the gene *PTGES2* (rs7862523 and rs7047254). This may partially explain the 'missing heritability' from the genome-wide association study (GWAS), given that current commercial arrays provide a low coverage for the common variations (MAF > 5%) in candidate genes (Bhangale et al. 2008; Li et al. 2008), particularly in the black population. It has been demonstrated that

Human1M only covers 55% of common SNPs ($r^2 = 0.8$) for 76 genes resequenced in HapMap African samples (Bhangale et al. 2008). Besides the comprehensive SNP selection, the genotype imputation of the present study limits the possibility of missing a causal SNP. Finally, the permutation-based ARTP method reduces the false positive rate from multiple tests. Therefore, although the observed associations were moderate in the present study, the chance of having false positive or false negative findings was relatively low.

This current study also has limitations. A major limitation is the lack of replication; this is particularly important in light of the moderate associations that we found. Thus, our findings need to be confirmed in independent studies, and furthermore, the underlying biological mechanisms need to be explored. Our study did not cover all of the important candidate genes relevant to the fatty acids metabolism pathway, for example, *ELOVLs* (elongase for long-chain PUFAs synthesis) and *PTGERs* (PGE2 receptors); these gene families may be considered in the future. Also, the joint effects of maternal and fetal genotypes should be examined in future studies. In the past decade, our team and others have demonstrated the important role of genetic susceptibility and $G \times E$ interactions in PTD (Gomez et al. 2010; Hao et al. 2004; Jones et al. 2010; Nukui et al. 2004; Plunkett and Muglia 2008; Romero et al. 2010; Suh et al. 2008; Tsai et al. 2008; Wang et al. 2002; Yu et al. 2009b). The majority of these previous studies were focused on inflammatory genes, interleukins and their receptors, pathogen recognition genes, remodeling of connective tissues and response to environmental toxins, etc. Whether the SNPs evaluated in this study can interact with PUFAs measures to affect the risk of PTD (i.e., gene–fatty acid interactions) and whether imbalanced PUFAs, SNPs and epigenetic marks of the fatty acids metabolic pathway genes interact to influence the risk of PTD are important questions for future study.

In summary, we conducted a comprehensive candidate gene study of PTD in a large U.S. black population. We found a modest genetic association of fatty acid metabolism pathway gene SNPs with PTD. Our findings underscore the value in further exploring the role of gene variants involved in fatty acid metabolic pathways in PTD. Our findings also stress the need to simultaneously explore fatty acid measures, genetic variants and epigenetic marks in order to better understand the complex interplay of genetic susceptibility, dietary intake, and gene regulation on the risk of PTD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all of the participating mothers and their families enrolled in the Boston Medical Center Cohort study; their efforts have helped further our understanding of the causes of preterm delivery. We thank the nursing staff of Labor and Delivery at Boston Medical Center for their continuous support and assistance to the study, as well as Lingling Fu for data management and Ann Ramsay for administrative support. We thank Dr. Kai Yu from the NCI for his helpful instruction on ARTP methods, and also Dr. Fuhong He from the Beijing Institute of Genomics, Chinese Academy of Sciences for her help on bioinformatics analyses. We thank Tami R. Bartell for English editing. The study was supported in part by grants from the National Institutes of Health (R01 HD041702, R01ES11682, R21ES11666, and R21HD066471), and March of Dimes Birth Defects Foundation (20-FY98-0701, 20-FY02-56).

References

- Allen KG, Harris MA. The role of n-3 fatty acids in gestation and parturition. *Exp Biol Med* (Maywood). 2001; 226:498–506. [PubMed: 11395920]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265.10.1093/bioinformatics/bth457 [PubMed: 15297300]
- Bazan NG. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr Opin Clin Nutr Metab Care*. 2007; 10:136–141. [PubMed: 17285000]
- Bhangale TR, Rieder MJ, Nickerson DA. Estimating coverage and power for genetic association studies using near-complete variation data. *Nat Genet*. 2008; 40:841–843. [PubMed: 18568023]
- Bonham MP, Duffy EM, Wallace JM, Robson PJ, Myers GJ, Davidson PW, Clarkson TW, Shamlaye CF, Strain JJ. Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). *Prostaglandins Leukot Essent Fatty Acids*. 2008; 78:343–350. [PubMed: 18585023]
- Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie*. 2009;10.1016/j.biochi.2009.01.008
- Coletta JM, Bell SJ, Roman AS. Omega-3 Fatty acids and pregnancy. *Rev Obstet Gynecol*. 2010; 3:163–171. [PubMed: 21364848]
- Crider KS, Whitehead N, Buus RM. Genetic variation associated with preterm birth: a HuGE review. *Genet Med*. 2005; 7:593–604. [PubMed: 16301860]
- de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet*. 2005; 37:1217–1223. [PubMed: 16244653]
- Decsi T. Effects of supplementing LCPUFA to the diet of pregnant women: data from RCT. *Adv Exp Med Biol*. 2009; 646:65–69.10.1007/978-1-4020-9173-5_7 [PubMed: 19536664]
- Fischer A, Grallert H, Bohme M, Gieger C, Boomgaarden I, Heid I, Wichmann HE, Doring F, Illig T. Association analysis between the prostaglandin E synthase 2 R298H polymorphism and body mass index in 8079 participants of the KORA study cohort. *Genet Test Mol Biomarkers*. 2009; 13:223–226.10.1089/gtmb.2008.0111 [PubMed: 19371221]
- Fisher E, Nitz I, Lindner I, Rubin D, Boeing H, Mohlig M, Hampe J, Schreiber S, Schrezenmeir J, Doring F. Candidate gene association study of type 2 diabetes in a nested case–control study of the EPIC-Potsdam cohort—role of fat assimilation. *Mol Nutr Food Res*. 2007; 51:185–191.10.1002/mnfr.200600162 [PubMed: 17266179]
- Glaser C, Heinrich J, Koletzko B. Role of FADS1 and FADS2 polymorphisms in polyunsaturated fatty acid metabolism. *Metabolism*. 2010; 59:993–999.10.1016/j.metabol.2009.10.022 [PubMed: 20045144]
- Gomez LM, Sammel MD, Appleby DH, Elovitz MA, Baldwin DA, Jeffcoat MK, Macones GA, Parry S. Evidence of a gene–environment interaction that predisposes to spontaneous preterm birth: a role for asymptomatic bacterial vaginosis and DNA variants in genes that control the inflammatory response. *Am J Obstet Gynecol*. 2010; 202:386e1–6.10.1016/j.ajog.2010.01.042 [PubMed: 20350647]
- Greenberg JA, Bell SJ, Ausdal WV. Omega-3 fatty acid supplementation during pregnancy. *Rev Obstet Gynecol*. 2008; 1:162–169. [PubMed: 19173020]
- Guldner L, Monfort C, Rouget F, Garlantezec R, Cordier S. Maternal fish and shellfish intake and pregnancy outcomes: a prospective cohort study in Brittany, France. *Environ Health*. 2007; 6:33.10.1186/1476-069X-6-33 [PubMed: 17958907]
- Gupta M, Mestan KK, Martin CR, Pearson C, Ortiz K, Fu L, Stubblefield P, Cerda S, Kasznica JM, Wang X. Impact of clinical and histologic correlates of maternal and fetal inflammatory response on gestational age in preterm births. *J Matern Fetal Neonatal Med*. 2007; 20:39–46. [PubMed: 17437198]
- Hao K, Wang X, Niu T, Xu X, Li A, Chang W, Wang L, Li G, Laird N, Xu X. A candidate gene association study on preterm delivery: application of high-throughput genotyping technology and advanced statistical methods. *Hum Mol Genet*. 2004; 13:683–691. [PubMed: 14976157]

- Hara S, Kamei D, Sasaki Y, Tanemoto A, Nakatani Y, Murakami M. Prostaglandin E synthases: understanding their pathophysiological roles through mouse genetic models. *Biochimie*. 2010; 92:651–659.10.1016/j.biochi.2010.02.007 [PubMed: 20159030]
- Horvath A, Koletzko B, Szajewska H. Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Br J Nutr*. 2007; 98:253–259. [PubMed: 17419889]
- Jania LA, Chandrasekharan S, Backlund MG, Foley NA, Snouwaert J, Wang IM, Clark P, Audoly LP, Koller BH. Microsomal prostaglandin E synthase-2 is not essential for in vivo prostaglandin E2 biosynthesis. *Prostaglandins Other Lipid Mediat*. 2009; 88:73–81.10.1016/j.prostaglandins.2008.10.003 [PubMed: 19010439]
- Jones NM, Holzman C, Friderici KH, Jernigan K, Chung H, Wirth J, Fisher R. Interplay of cytokine polymorphisms and bacterial vaginosis in the etiology of preterm delivery. *J Reprod Immunol*. 2010; 87:82–89.10.1016/j.jri.2010.06.158 [PubMed: 20965572]
- Kesmodel U, Olsen SF, Salvig JD. Marine n-3 fatty acid and calcium intake in relation to pregnancy induced hypertension, intrauterine growth retardation, and preterm delivery. A case–control study. *Acta Obstet Gynecol Scand*. 1997; 76:38–44. [PubMed: 9033242]
- King DC, Taylor J, Elnitski L, Chiaromonte F, Miller W, Hardison RC. Evaluation of regulatory potential and conservation scores for detecting cis-regulatory modules in aligned mammalian genome sequences. *Genome Res*. 2005; 15:1051–1060.10.1101/gr.3642605 [PubMed: 16024817]
- Li M, Li C, Guan W. Evaluation of coverage variation of SNP chips for genome-wide association studies. *Eur J Hum Genet*. 2008; 16:635–643. [PubMed: 18253166]
- Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet*. 2009; 10:387–406.10.1146/annurev.genom.9.081307.164242 [PubMed: 19715440]
- Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol*. 2010; 34:816–834.10.1002/gepi.20533 [PubMed: 21058334]
- Lindner I, Helwig U, Rubin D, Fischer A, Marten B, Schreiber S, Doring F, Schrezenmeir J. Prostaglandin E synthase 2 (PTGES2) Arg298His polymorphism and parameters of the metabolic syndrome. *Mol Nutr Food Res*. 2007; 51:1447–1451.10.1002/mnfr.200700144 [PubMed: 17979097]
- Lovejoy JC, Champagne CM, Smith SR, de Jonge L, Xie H. Ethnic differences in dietary intakes, physical activity, and energy expenditure in middle-aged, premenopausal women: the Healthy Transitions Study. *Am J Clin Nutr*. 2001; 74:90–95. [PubMed: 11451722]
- Macones GA, Parry S, Elkousy M, Clothier B, Ural SH, Strauss JF 3rd. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene–environment interaction in the etiology of spontaneous preterm birth. *Am J Obstet Gynecol*. 2004; 190:1504–1508. (discussion 3A). [PubMed: 15284722]
- Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *Cochrane Database Syst Rev*. 2006; 3:CD003402. [PubMed: 16856006]
- Martin JA, Osterman MJ, Sutton PD. Are preterm births on the decline in the United States? Recent data from the National Vital Statistics System. *NCHS Data Brief*. 2010; 39:1–8. [PubMed: 20604990]
- Merino DM, Ma DW, Mutch DM. Genetic variation in lipid desaturases and its impact on the development of human disease. *Lipids Health Dis*. 2010; 9:63.10.1186/1476-511X-9-63 [PubMed: 20565855]
- Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. *Prog Lipid Res*. 2004; 43:3–35. pii: S0163782703000377. [PubMed: 14636669]
- Nitz I, Fisher E, Grallert H, Li Y, Gieger C, Rubin D, Boeing H, Spranger J, Lindner I, Schreiber S, Rathmann W, Gohlke H, Doring A, Wichmann HE, Schrezenmeir J, Doring F, Illig T. Association of prostaglandin E synthase 2 (PTGES2) Arg298His polymorphism with type 2 diabetes in two

- German study populations. *J Clin Endocrinol Metab.* 2007; 92:3183–3188.10.1210/jc.2006-2550 [PubMed: 17566096]
- Nukui T, Day RD, Sims CS, Ness RB, Romkes M. Maternal/newborn GSTT1 null genotype contributes to risk of preterm, low birthweight infants. *Pharmacogenetics.* 2004; 14:569–576. [PubMed: 15475730]
- Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. *Am J Epidemiol.* 2004; 160:774–783.10.1093/aje/kwh282 [PubMed: 15466500]
- Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *BMJ.* 2002; 324:447. [PubMed: 11859044]
- Olsen SF, Olsen J, Frische G. Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. *Int J Epidemiol.* 1990; 19:971–977. [PubMed: 2084030]
- Olsen SF, Hansen HS, Sommer S, Jensen B, Sorensen TI, Secher NJ, Zachariassen P. Gestational age in relation to marine n-3 fatty acids in maternal erythrocytes: a study of women in the Faroe Islands and Denmark. *Am J Obstet Gynecol.* 1991; 164:1203–1209. [PubMed: 1827949]
- Olsen SF, Hansen HS, Secher NJ, Jensen B, Sandstrom B. Gestation length and birth weight in relation to intake of marine n-3 fatty acids. *Br J Nutr.* 1995; 73:397–404. [PubMed: 7766563]
- Plunkett J, Muglia LJ. Genetic contributions to preterm birth: implications from epidemiological and genetic association studies. *Ann Med.* 2008; 40:167–195. [PubMed: 18382883]
- Pontes PV, Torres AG, Trugo NM, Fonseca VM, Sichieri R. n-6 and n-3 Long-chain polyunsaturated fatty acids in the erythrocyte membrane of Brazilian preterm and term neonates and their mothers at delivery. *Prostaglandins Leukot Essent Fatty Acids.* 2006; 74:117–123. [PubMed: 16364619]
- Reece MS, McGregor JA, Allen KG, Harris MA. Maternal and perinatal long-chain fatty acids: possible roles in preterm birth. *Am J Obstet Gynecol.* 1997; 176:907–914. [PubMed: 9125620]
- Romero R, Velez Edwards DR, Kusanovic JP, Hassan SS, Mazaki-Tovi S, Vaisbuch E, Kim CJ, Chaiworapongsa T, Pearce BD, Friel LA, Bartlett J, Anant MK, Salisbury BA, Vovis GF, Lee MS, Gomez R, Behnke E, Oyarzun E, Tromp G, Williams SM, Menon R. Identification of fetal and maternal single nucleotide polymorphisms in candidate genes that predispose to spontaneous preterm labor with intact membranes. *Am J Obstet Gynecol.* 2010; 202:431e1–34.10.1016/j.ajog.2010.03.026 [PubMed: 20452482]
- Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet.* 2002; 70:425–434. [PubMed: 11791212]
- Suh YJ, Ha EH, Park H, Kim YJ, Kim H, Hong YC. GSTM1 polymorphism along with PM10 exposure contributes to the risk of preterm delivery. *Mutat Res.* 2008; 656:62–67.10.1016/j.mrgentox.2008.07.006 [PubMed: 18706519]
- Szajewska H, Horvath A, Koletzko B. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2006; 83:1337–1344. [PubMed: 16762945]
- Tsai HJ, Liu X, Mestan K, Yu Y, Zhang S, Fang Y, Pearson C, Ortiz K, Zuckerman B, Bauchner H, Cerda S, Stubblefield PG, Xu X, Wang X. Maternal cigarette smoking, metabolic gene polymorphisms, and preterm delivery: new insights on G × E interactions and pathogenic pathways. *Hum Genet.* 2008; 123:359–369. [PubMed: 18320229]
- Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, Niu T, Wise PH, Bauchner H, Xu X. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA.* 2002; 287:195–202. [PubMed: 11779261]
- Yashodhara BM, Umakanth S, Pappachan JM, Bhat SK, Kamath R, Choo BH. Omega-3 fatty acids: a comprehensive review of their role in health and disease. *Postgrad Med J.* 2009; 85:84–90. [PubMed: 19329703]
- Yeo GW, Coufal NG, Liang TY, Peng GE, Fu XD, Gage FH. An RNA code for the FOX2 splicing regulator revealed by mapping RNA–protein interactions in stem cells. *Nat Struct Mol Biol.* 2009; 16:130–137.10.1038/nsmb.1545 [PubMed: 19136955]

- Yu K, Li Q, Bergen AW, Pfeiffer RM, Rosenberg PS, Caporaso N, Kraft P, Chatterjee N. Pathway analysis by adaptive combination of P-values. *Genet Epidemiol.* 2009a; 33:700–709.10.1002/gepi.20422 [PubMed: 19333968]
- Yu Y, Tsai HJ, Liu X, Mestan K, Zhang S, Pearson C, Ortiz K, Xu X, Zuckerman B, Wang X. The joint association between F5 gene polymorphisms and maternal smoking during pregnancy on preterm delivery. *Hum Genet.* 2009b; 124:659–668. [PubMed: 19020903]

Fig. 1. Summary of associations between 206 SNPs and preterm delivery in the BMC birth cohort: single-SNP association (*in dot*) and gene-based association (*p* value under the gene symbol)

Fig. 2.
Summary of associations between gene *PTGES2* and preterm delivery in the BMC birth cohort

Table 1

Epidemiological characteristics of study subjects from BMC birth cohort stratified by preterm status

Epidemiological characteristics	Preterm delivery (<i>N</i> = 542) <i>N</i> (%)	Term delivery (<i>N</i> = 568) <i>N</i> (%)	<i>p</i> value
Age (years)			
<20	58 (11)	67 (12)	
20–24	129 (24)	127 (22)	
25–29	120 (22)	128 (23)	
30–34	117 (22)	125 (22)	
35	118 (22)	121 (21)	0.96
Education			
Middle school	128 (24)	174 (31)	
≤High school	231 (43)	166 (29)	
>High school	175 (33)	224 (40)	<0.0001
Parity			
0	229 (42)	230 (40)	
1	142 (26)	165 (29)	
1	171 (32)	173 (30)	0.57
Marital status			
Married	150 (28)	211 (37)	
Other	391 (72)	356 (63)	0.0008
Smoking during pregnancy			
Never	399 (74)	471 (83)	
Quitter	41 (8)	48 (9)	
Persistent	95 (18)	46 (8)	<0.0001
Passive smoking during pregnancy			
No	393 (74)	442 (80)	
Yes	138 (26)	114 (21)	0.03
Illicit drug use			
No	442 (82)	509 (90)	
Yes	98 (18)	57 (10)	0.0001
Alcohol use			
No	512 (95)	543 (96)	
Yes	29 (5)	24 (4)	0.38
Maternal pre-pregnant BMI (kg/m) ²			
<20	51 (9)	44 (8)	
20–24	188 (35)	219 (39)	
25–29	168 (31)	192 (34)	
30	135 (25)	113 (20)	0.12
Infant sex			
Male	277 (51)	298 (52)	
Female	265 (49)	270 (48)	0.65
Gestational age (weeks)	33.2 ± 3.6 ^a	39.5 ± 1.3 ^a	<0.0001

Epidemiological characteristics	Preterm delivery (<i>N</i> = 542) <i>N</i> (%)	Term delivery (<i>N</i> = 568) <i>N</i> (%)	<i>p</i> value
Birth weight (grams)	2055 ± 776 ^a	3310 ± 483 ^a	<0.0001

Numbers of missing information for education, marital status, smoking during pregnancy, passive smoking during pregnancy, illicit drug use, and alcohol use are, 12, 2, 10, 23, 4, and 2 respectively *p* values are based on *t* test statistics for continuous variables and Chi-square test statistics for categorical variables

^aMean ± SD

Table 2

Summary of typed and imputed SNPs in nine candidate genes involved in fatty acids metabolism pathway

Gene	Gene description	Chromosome: position ^a	Number of typed SNPs	Number of imputed SNPs ^b
FADS1	Fatty acid desaturase 1	Chr11: 61321679–61345886	6	7
FADS2	Fatty acid desaturase 2	Chr11: 61347289–61393401	22	21
PTGS1	Cyclooxygenase-1 (COX-1)	Chr9: 124168050–124199802	48	43
PTGS2	Cyclooxygenase-2 (COX-2)	Chr1: 184905593–184921179	24	9
ALOX5	5-lipoxygenase (5-LOX)	Chr10: 45184635–45263567	51	41
ALOX5AP	5-LOX-activating protein	Chr13: 30202669–30238556	44	50
PTGES	Prostaglandin E synthase	Chr9: 131538433–131560165	20	12
PTGES2	Prostaglandin E synthase 2	Chr9: 129920794–129935533	7	3
PTGES3	Prostaglandin E synthase 3	Chr12: 55341649–55373156	9	4
Total			231	190

^aPosition (genome build 36) focused in this study^bSNPs with high imputation quality (rsq > 0.3) and were listed in Supplemental table S1

Table 3

Significant associations between typed and imputed SNPs of genes involved in fatty acids metabolism pathway with preterm delivery in the BMC birth cohort

Genes	Function ^a	Cases/controls ^b	General model		Best-fitted models	
			OR (95%CI) ^c	p value	Model	OR (95%CI) ^c
FADS2						
rs2072114	tagSNP					
AA		406/420	Ref			
AG		111/133	0.87 (0.65–1.18)	0.37		
GG		16/8	2.55 (1.01–6.42)	0.05	Rec	2.63 (1.04–6.60) 0.04
rs7119667	ESE; miRNA					
CC		467/462	Ref			
CG		67/98	0.68 (0.48–0.98)	0.04		
GG		3/5	0.62 (0.13–2.85)	0.54	Dom	0.68 (0.48–0.97) 0.03
PTGS1						
rs10306227 ^d	–					
GG		499/503	Ref			
AG		40/62	0.59 (0.37–0.93)	0.02		
AA		3/3	0.74 (0.14–3.83)	0.72	Dom	0.60 (0.38–0.93) 0.02
rs10306120 ^d	–					
GG		502/541	Ref			
GA		37/27	1.64 (0.96–2.79)	0.07		
AA		3/0	NA		Dom	1.79 (1.06–3.02) 0.03
rs10306121	Triplex					
AA		303/351	Ref			
AT		201/182	1.34 (1.02–1.75)	0.03		
TT		33/30	1.28 (0.75–2.20)	0.37	Dom	1.33 (1.03–1.71) 0.03
rs3842788	ESE/ESS					
GG		310/297	Ref			
AG		186/215	0.79 (0.60–1.03)	0.08		
AA		36/46	0.72 (0.44–1.16)	0.18	Dom	0.77 (0.60–1.00) 0.05

Genes	Function ^a	Cases/controls ^b	General model		Best-fitted models		
			OR (95%CI) ^c	p value	Model	OR (95%CI) ^c	p value
rs10306150	tagSNP						
GG		153/182	Ref				
CG		264/287	1.06 (0.80–1.42)	0.69			
CC		121/97	1.50 (1.04–2.15)	0.03	Rec	1.44 (1.05–1.97)	0.02
PTGS2							
rs5276	ESE; miRNA						
GG		378/390	Ref				
AG		148/151	0.98 (0.74–1.30)	0.91			
AA		11/25	0.40 (0.18–0.86)	0.02	Rec	0.40 (0.18–0.86)	0.02
ALOX5							
rs745986	tagSNP						
AA		378/418	Ref				
AG		142/131	1.32 (0.99–1.76)	0.06			
GG		18/16	1.71 (0.84–3.51)	0.14	Multi	1.31 (1.04–1.67)	0.02
rs3780900 ^d	–						
GG		521/517	Ref				
GA		21/51	0.43 (0.25–0.76)	0.004			
–		0/0	NA		Dom	0.43 (0.25–0.76)	0.004
rs7089406	tagSNP						
GG		505/504	Ref				
CG		32/54	0.50 (0.30–0.81)	0.005			
CC		2/3	0.59 (0.09–3.91)	0.58	Dom	0.50 (0.31–0.81)	0.005
rs4540799	tagSNP						
CC		382/441	Ref				
CT		141/108	1.63 (1.21–2.20)	0.002			
TT		9/12	1.02 (0.41–2.51)	0.97	Dom	1.57 (1.17–2.10)	0.003
rs10751382	tagSNP						
AA		343/394	Ref				
AG		173/148	1.39 (1.05–1.83)	0.02			
GG		15/19	1.03 (0.50–2.10)	0.94	Dom	1.35 (1.03–1.76)	0.03

Genes	Function ^a	Cases/controls ^b	General model		Best-fitted models			
			OR (95%CI) ^c	p value	Model	OR (95%CI) ^c	p value	
ALOX5AP								
rs4769055	TFBS	338/331	Ref					
		170/192	0.89 (0.68–1.16)	0.38				
		26/43	0.52 (0.30–0.90)	0.02	Rec	0.54 (0.31–0.93)	0.03	
rs10507391	tagSNP	355/379	Ref					
		162/155	1.10 (0.84–1.46)	0.49				
		15/29	0.50 (0.25–0.99)	0.05	Rec	0.49 (0.25–0.96)	0.04	
rs3885907	tagSNP	224/208	Ref					
		231/261	0.77 (0.58–1.01)	0.06				
		81/94	0.77 (0.53–1.13)	0.18	Dom	0.77 (0.59–0.99)	0.04	
PTGES								
rs2302821	ESE; miRNA	223/217	Ref					
		251/255	0.93 (0.71–1.22)	0.62				
		66/92	0.66 (0.45–0.96)	0.03	Rec	0.68 (0.48–0.97)	0.03	
rs10739757	tagSNP	279/294	Ref					
		226/218	1.07 (0.82–1.39)	0.63				
		32/51	0.59 (0.36–0.97)	0.04	Rec	0.57 (0.35–0.93)	0.02	
PTGES2								
rs7862523	tagSNP	459/461	Ref					
		73/96	0.77 (0.54–1.09)	0.14				
		3/8	0.30 (0.07–1.18)	0.08	Multi	0.72 (0.52–0.98)	0.03	
rs4837243	TFBS	382/401	Ref					
		137/153	0.96 (0.72–1.27)	0.76				

Genes Variants	Function ^a	Cases/controls ^b	General model		Best-fitted models		
			OR (95%CI) ^c	p value	Model	OR (95%CI) ^c	p value
rs7047254	TFBS	19/6	3.49 (1.33–9.19)	0.01	Rec	3.53 (1.35–9.27)	0.01
		154/171	Ref				
		254/285	1.01 (0.76–1.36)	0.92			
		127/110	1.38 (0.97–1.97)	0.07	Rec	1.37 (1.01–1.86)	0.04

Genetic mode of inheritance: Dom (dominant), Rec (recessive), Multi (multiplicative)

^aPredicted by PupaSuite (<http://pupasuite.bioinfo.cipf.es/>) and FuncPred (<http://manicore.niehs.nih.gov/snpfunc.htm>): TFBS (transcription factor binding sites); ESE (exonic splicing enhancers); ESS (exonic splicing silencer); miRNA (microRNA)

^bTotal number of cases and controls are not equal to 1,110 and not the same with each other due to the different call rates of the SNPs

^cAdjusted for maternal age (<20, 20–24, 25–30, 30–35, 35 years), education (<high school, high school, and some college and above), parity (0, 1, 2), marital status, maternal active smoking, passive smoking, alcohol drinking and drug use during pregnancy, maternal pre-pregnancy BMI (<20, 20–24, 25–30, 30), and infant gender. *p* values were based on Wald test without adjustment of multiple comparisons

^dImputed HapMap SNPs