表3　養育環境とADHD（ADHD関連症状）との関連

著者／年／国	研究デザイン	対象者	曝露評価	ADHDの評価	結果	考察
Tullyら／2004／UK [24]	後ろ向きコーホート	低体重出生の双子の5歳児2232組	母性的温かさ（ビデオ撮影された子どもの反応からコード化した感情表現）	教員と養育者によるADHD症状の調査票	ADHD得点において、児の体重と母性的温かさの間に有意な交互作用があった。IQとは関係しなかった	低体重で生まれた児のADHD症状は、母性的温かさにより緩和し、低体重出生児のADHD症状にある問題行動を防ぐ可能性がある
Jurvetzら／2007／Spain [25]	前向き出生コーホート	4歳児500名	授乳期間（聞き取り）	ADHD-DSM-Ⅳに基づく教員による調査票 MCSA（神経心理学検査） 教員によるCPSCS	授乳12週以上はADHD得点と社会適応の改善が認められた（ADHD） RR=0.56;95%CI.0.37-0.85; 社会適応 RR=0.57;95%CI.0.52-0.66） 授乳20週以上は実行機能と関連があった（β=4.9;95%CI.0.6-9.2） 交絡要因調整後も効果は維持した（12～20週:RR=0.56;95%CI.0.37-0.85; 21～28週::RR=0.47; 95%CI.0.27-0.80; 28週以上:RR=0.61; 95% CI.0.44-0.86）	12週以上の授乳はADHD症状改善に関連している
Swingら／2009／USA [26]	縦断研究	（2箇所のコーホート）	テレビやビデオゲームの視聴時間（13ヶ月間）	教員による注意機能の評価	テレビやビデオゲームの視聴は注意機能と非常に関連している(OR=1.81, 95%CI.1.56-2.11)	テレビだけでなくビデオゲームの視聴も注意機能に影響し、青年期との比較でも同様の結果であった
Pelsserら／2011／Netherlands [27]	クロスオーバーのRCT	6歳から12歳1323名	ダイエット(elimination diet;米,肉,野菜,梨、水など低刺激性の食品に,ジャガイモ,フルーツ,小麦などの特定の食品を補完するもの). 対照群は健康的な食事	小児科医によるADHD-RSとConners、SDQ,SPI	ダイエット群は対照群に比べ、ADHD症状評価指標のADHD-RS得点が23.7(95%CI 18.6-28.8; p<0.0001）差があり、両群を交差した結果、30人中16人（63%）に症状のぶり返しが認められた	ダイエット群は対照群よりADHD得点が低下した 食べ物由来のADHDにはダイエットプログラムの実施を考慮するとよい

表4　児の発育時期によるADHD発症にかかわる要因

曝露時期	原因
出生前	ニコチン，アルコール，鉛，PCB，毒物（マリファナ，コカイン），食品添加物，貧血，甲状腺機能低下症，ヨウ素欠乏，母の心理的ストレス，脳の発達異常，染色体異常，ウィルス性発疹症
出生時	早産，低出生体重，低酸素・虚血性脳症，髄膜炎，脳炎
出生後	受動喫煙，鉄欠乏，脂肪酸欠乏，強い心理社会的ストレス，ウィルス性髄膜炎，脳炎，脳外傷，甲状腺機能障害，中耳炎

※Linnetら（2003）[28]，Millichapら（2008）[29]，Banerjeeら（2007）[30]，Williamsら（2007）[31]を一部改編

としての可能性を報告していた．ADHD症状の緩和要因を明らかにすることは，ADHD症状の関連遺伝子を有しても，症状を和らげるあるいは発症を抑制するための環境情報を提供できる点で，社会に寄与できるだろう．

（3）児の生育時期によるADHD発症に関わる要因

児の生育時期，すなわちADHD発症リスクを網羅的にReviewした論文の結果を表4に整理した．

Linnetらは，ADHD評価を，DSM診断基準とほかの妥当な診断を用いているか，またはADHDのスクリーニングツールとADHD症状のテストを用い，1973年から2002年に発表された研究についてメタアナリシスを行った[29]．その結果，喫煙との関連が24本，アルコール摂取との関連が9本，心理社会的ストレスの関連が5本検出された．喫煙との関連は，妊娠中の母親の喫煙がADHD発症にもっとも強く関連していることが明らかとなった．妊娠中のストレスに関して一致した見解は認められなかったものの，出産後のADHD症状に若干影響することが示唆された．多くの研究で，思い出しバイアス，曝露評価，サンプル数の不足，交絡要因の補正が不十分であるなど方法論的な欠点が見出された．

Millichapは，ADHDの病因について，2007年までの論文を器質的，遺伝的，生物化学的要因から概観し，出

生前・出生時・出生後の環境リスク要因について整理した[30]．その結果，出生前・出生時のリスク要因には，妊娠中の喫煙とアルコール摂取，早産，ヨウ素欠乏が関連していた．

Banerjeeらは，疫学，神経心理学，神経イメージング，治療も含めADHDの関連要因について検討した結果，家族研究や双子研究などで遺伝的要因がADHD発症を規定している（79%）とはいえ，生物学的要因，環境要因もまたリスク要因であることを指摘し，食物添加物やダイエット，鉛汚染，喫煙とアルコール曝露，妊娠中の喫煙，低出生体重との関連を報告した[31]．

Williamsは胎児期の環境物質曝露がADHDや神経発達，精神健康などとの関連を前向きコーホート研究に絞って検討し[32]，鉛とPCBｓは脳の発達に，マリファナとアルコールは長期に（特に注意課題に対して）影響し，アルコールは妊娠年齢と飲酒頻度がより強く影響すると述べた．またコカインは年齢と共に影響は弱まるが，心理社会的要因がその影響を大きく緩和する一方で，喫煙との関連は妊娠中のニコチン曝露により遺伝子レベルの変化を介して影響すると報告した．

養育環境の中でも生活習慣である喫煙や飲酒といった発症リスクは，妊娠中および出産後の生活習慣改善により避けられるリスクである．

今回の先行研究の検索では，国内において出生前から出生後まで環境要因について評価した研究は未だ見られなかった．今後のADHDの発症環境要因の研究は，妊娠中から出生後の環境も考慮した長期の出生コーホート研究により，過去に関連が報告された要因を調整した解析，遺伝的要因分析も求められると考える．

なお，本研究では，農薬を始めとする環境化学物質曝露や遺伝的要因については，検討していない．近年蓄積されつつある遺伝的要因ならびに環境-遺伝交互作用については別の機会に整理したいと考える．

まとめ

ADHDの有病率は，日本では３～７％前後の報告が多いが，正確な疫学データは報告がなかった．発達支援やADHDの長期予後への対策を考える上でも，日本における疫学データが求められる。そのために統一された診断基準を用いた児童精神専門家による診断が望ましい．ADHDの環境リスク要因の解明には，喫煙曝露の影響について一致した見解が出ているが，十分なサンプルの出生コーホートにおいて，妊娠中から学童期までの曝露評価を客観的に行い，交絡要因を調整して再評価する必要がある．その上で，遺伝的要因がどの程度関連するか，環境-遺伝交互作用を検討するのが望ましい．環境リスク要因の解明は，学童期前からの養育と普通学級に通級している児童へのADHD症状に対する予防や症状緩和策にも繋がるものと考える．

【引用文献】

1）相原正男，畠山和男，青柳閣郎，他．山梨県立あけぼの医療センター発達障害外来患者の推移．平成20年度厚生労働省障害者保健福祉推進事業　障害者自立支援調査研究プロジェクト編：いま，発達障害が増えているか．社団法人日本発達障害福祉連盟，東京．2009；20-23.

2）渡部京太．第4章ADHDの中長期経過．齋藤万比古，渡部京太編．注意欠如・多動性障害-ADHD-の診断・治療ガイドライン．じほう,東京.2008；221-224.

3）吉益光一，山下洋，清原千賀子，他．注意欠陥多動性障害の疫学，治療と予防．日本公衛誌 2006；53(6):398-410.

4）渡部京太.ADHDの疫学と長期予後.精神科治療学 2010；25(6)：727-734.

5）Polanczyk G, Lima MS, Horta BL, et al. Worldwide Prevalence of ADHD: a systematic review and Metaregression Analysis. Am J Psychiatry 2007；164(6):942-948.

6）Postner K, Pressman AW and Greenhill LL. : ADHD in preschool children. In: (ed.), Brown TE. ADHD Comorbidities-Handbook for ADHD Complications in Children and Adults, American Psychiatric Publishing. Inc., Arlington, VA, 2009; 37-53.

7）Roy-Byrne P, Scheele L, Ward N, et al. Adult attention-deficit hyperactive disorder: Assessment guideline based on clinical presentation to a specialty clinic. Compr. Psychiatry 1997; 38: 133-140.

8）Dopheide JA and Pliszka SR. :Attention-deficit-hyperactivity disorder: un update. Pharmacotherapy 2009; 29:656-679.

9）岡田涼，大西将史，辻正次，他．日本の小中学生におけるADHD傾向　教師評定と保護者評定の違い．精神医学2011；53(3)：249-255.

10）小枝達也．5歳児健診の実際．小枝達也編．5歳児健診－発達障害の診療・指導エッセンス，診断と治療社，東京．2008；13－33.

11）大南秀明研究代表，通常の学級に在籍する特別な教育的支援を必要とする児童生徒に関する実態調査　調査結果 2002；http://www.mext.go.jp.

12）湯汲英史．発達障害の増加に関する医療面以外の調

査結果のまとめと今後の課題. 脳と発達 2010；42：S186

13) Barkley RA. Attention-deficit hyperactivity disorder. New York; The Guilford Press: 2006)；276-288.

14) Fergusson DM, Horwood LJ, Lynskey MT. Maternal smoking before and after pregnancy: effects on behavioral outcomes in middle childhood. Pediatrics 1993; 92: 815-822.

15) Bor W, Najman JM, Anderson MJ, et al. The relationship between low family income and psychological disturbance in young children: an Australian longitudinal study. Aust N Z J Psychiatry 1997; 31: 664-675.

16) Weissman MM, Warner V, Wickramaratne PJ, et al. Maternal smoking during pregnancy and psychopathology in offspring followed to adulthood. J Am Acad Child Adolesc Psychiatry 1999; 38: 892-899.

17) Breslau N, Chilcoat HD. Psychiatric sequelae of low birth weight at 11years ago. Bil Psychiatry 2000; 47: 1005-1011.

18) Hill SY, Lowers L, Locke-Wellman J, et al. Maternal smoking and drinking during pregnancy and the risk for child and adolescent psychiatric disorders. J Stud Alcohol 2000; 61: 661-668.

19) Kotimaa AJ, Moilanen I, Taanila A, et al. Maternal smoking and hyperactivity in 8-year-old children. J Am Acad Child Adolesc Psychiatry 2003; 42: 826-833.

20) Kahn RS, Khoury J, Nichols WC, et al. Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. J Pediatr 2003; 143: 104-110.

21) Batstra L, Hadders-Algra M, Neeleman J. Effect of antenatal exposure to maternal smoking on behavioral problems and academic achievement in childhood: prospective evidence from a Dutch birth cohort. Eary Hum Dev 2003; 75: 21-33.

22) Obel C, Linnet KM, Heriksen TB, et al. Smoking during pregnancy and hyperactivity-inattention in the offspring-comparing results from three Nordic cohorts. Int J Epidemiol. 2008；38(3):698-705.

23) Cho SC, Kin BN, Hong YC, et al. Effect of environmental exposure to lead and tobacco smoke on inattentive and hyperactive symptoms and neurocognitive performance in children. J of Child Psycho and Psych 2010；51(9)：1050-1057.

24) Hermann M, King K, Weitzman M. Prenatal tobacco smoke and postnatal secondhand smoke exposure and child neurodevelopment. Curr Opin Pediatr, 2008；20(2):184-190.

25) Tully LA, Arseneault L, Caspi A, et al. Does Maternal Warmth Moderate the Effects of Birth Weight on Twins' Attention-Deficit/Hyperactivity Disorder (ADHD) Symptoms and Low IQ? J of Consulting and Clinical Psychilogy 2004；72(2):218-226.

26) Julvetz J, Ribas-Fito N, Forms M, et al. Attention behavior and hyperactivity at age 4 and duration of breast-feeding. Acta Paediatica 2007；96(6); 842-847.

27) Swing EL, Gentile DA, Anderson CA, et al. Television and video game exposure and the development of attention problems. Pediatrics 2010；126(2): 214-221.

28) Pelsser LM, Franken K, Toorman J, et al. Effects a restricted elimination diet on the behavior of children with attention-deficit hyperactivity disorder (INCA study): a randomized controlled trial. Lancet 2011; 377:494-503.

29) Linnet KM, Dalsgaard S, Obel C, et al. Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. Am J Psychiatry 2003；160(6): 1028-1040.

30) Millichap JG. Etiologic classification of attention-deficit/hyperactivity disorder. Pediatrics 2008；121(2): e358-365.

31) Banerjee TD, Middleton F, Faraone SV. Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr 2007；96(9): 1269-1274.

32) Williams JH, Ross L. Consequences of prenatal toxin exposure for mental health in child and adolescents. Eur Child Adolesc Psychiatry 2007; 16: 243-253.

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Original Article

Effects of Maternal 5,10-Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms and Tobacco Smoking on Infant Birth Weight in a Japanese Population

Thamar Ayo Yila[1], Seiko Sasaki[1], Chihiro Miyashita[1], Titilola Serifat Braimoh[1], Ikuko Kashino[1], Sumitaka Kobayashi[1], Emiko Okada[1], Toshiaki Baba[1], Eiji Yoshioka[1], Hisanori Minakami[2], Toshiaki Endo[3], Kazuo Sengoku[4], and Reiko Kishi[5]

[1]Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[2]Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[3]Department of Obstetrics and Gynecology, School of Medicine, Sapporo Medical University, Sapporo, Japan
[4]Department of Obstetrics and Gynecology, School of Medicine, Asahikawa Medical College, Asahikawa, Japan
[5]Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan

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ABSTRACT

Background: Intracellular folate hemostasis depends on the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene. Because 5,10-*MTHFR* 677TT homozygosity and tobacco smoking are associated with low folate status, we tested the hypothesis that smoking in mothers with 5,10-*MTHFR* C677T or A1298C polymorphisms would be independently associated with lower birth weight among their offspring.

Methods: We assessed 1784 native Japanese mother-child pairs drawn from the ongoing birth cohort of The Hokkaido Study on Environment and Children's Health. Data (demographic information, hospital birth records, and biological specimens) were extracted from recruitments that took place during the period from February 2003 to March 2006. Maternal serum folate were assayed by chemiluminescent immunoassay, and genotyping of 5,10-*MTHFR* C677T/A1298C polymorphisms was done using a TaqMan allelic discrimination assay.

Results: The prevalence of folate deficiency (<6.8 nmol/L) was 0.3%. The 5,10-*MTHFR* 677CT genotype was independently associated with an increase of 36.40 g (95% CI: 2.60 to 70.30, $P = 0.035$) in mean infant birth weight and an increase of 90.70 g (95% CI: 6.00 to 175.50, $P = 0.036$) among male infants of nonsmokers. Female infants of 677TT homozygous passive smokers were 99.00 g (95% CI: −190.26 to −7.56, $P = 0.034$) lighter. The birth weight of the offspring of smokers with 5,10-*MTHFR* 1298AA homozygosity was lower by 107.00 g (95% CI: −180.00 to −33.90, $P = 0.004$).

Conclusions: The results suggest that, in this population, maternal 5,10-*MTHFR* C677T polymorphism, but not the 5,10-*MTHFR* A1298C variant, is independently associated with improvement in infant birth weight, especially among nonsmokers. However, 5,10-*MTHFR* 1298AA might be associated with folate impairment and could interact with tobacco smoke to further decrease birth weight.

Key words: birth weight; tobacco smoking; *MTHFR* SNPs; folate; Japan

INTRODUCTION

Intracellular folate hemostasis depends on the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene, which is located at position 36 on the short arm of chromosome 1. This gene codes for the enzyme *MTHFR*, which catalyses the irreversible conversion of 5,10-*MTHFR* to 5-metyltetrahydrofolate, a substrate for methylation of homocysteine to methionine. Thus far, 14 rare mutations in *MTHFR* have been described, but the 2 most common single nucleotide polymorphisms (SNPs) are 5,10-*MTHFR* C677T (dbSNP ID: rs1801133)—a missense mutation in exon 4, characterized by an alanine to valine substitution on codon 222—and 5,10-*MTHFR* A1298C (dbSNP ID: rs1801131)—a point mutation in exon 7 characterized by a glutamate to alanine substitution on codon 429.[1] 5,10-*MTHFR* C677T is located in the catalytic N-terminal domain of the enzyme, while 5,10-*MTHFR* A1298C is located in the regulatory domain of the enzyme.[2]

Address for correspondence. Dr. Reiko Kishi, Center for Environmental and Health Sciences, Hokkaido University. North 12, West 7, Kita-ku, Sapporo 060-0812, Japan (e-mail: rkishi@med.hokudai.ac.jp).

Biochemically, the 5,10-*MTHFR* C677T polymorphism is associated with thermolability and reduced enzyme activity. The metabolic consequences are folate deficiency and mild hyperhomocysteinemia, a risk factor for thrombotic vascular diseases. It has been suggested that oxidative stress, platelet aggregation, and endothelial cell dysfunction contribute to the vasculotoxicity of homocysteine, and 5,10-*MTHFR* polymorphisms have been widely investigated in relation to a spectrum of several disease outcomes. Specifically, several studies have identified maternal 5,10-*MTHFR* C677T polymorphisms as obstetric genetic risk factors for spina bifida, placenta-related vasculopathies, spontaneous fetal loss, preterm delivery (PTD), low birth weight (LBW), small for gestational age (SGA), neurodevelopmental delays, and other congenital anomalies.[3–15] However, several other investigators have found no such associations.[16–25] This confusion might be explained by the fact that phenotypic expression of this genetic trait depends on folate status and other environmental factors that vary by geographic region and race.

Although the functional consequences of the 5,10-*MTHFR* A1298C variant are not well known, it is a risk factor for neural tube defects,[26,27] and compound heterozygosity (5,10-*MTHFR* 677CT/1298AC) has been reported to have a biochemical profile similar to that of 677TT homozygosity.[1,28]

Maternal smoking during pregnancy is an established risk factor for intrauterine growth retardation (IUGR), SGA, PTD, and other adverse pregnancy outcomes.[29] More recently, smoking has been associated with nutritional deficiencies, including folate deficiency.[30–32] LBW secondary to IUGR or PTD remains a public health concern because it increases the risk of morbidity and mortality throughout life.

In Japan, there have been genetic association studies of the relation between folate and cardiovascular pathologies, cancers, *Helicobacter pylori* infection, and periodontal diseases. However, only a few such studies have investigated obstetric events, and none has considered infant birth size.[3,6,17,33–35] We therefore tested the hypothesis that maternal smoking in the presence of the 5,10-*MTHFR* C677T or A1298C polymorphisms independently reduces birth weight.

METHODS

Study design and participants

The study participants were native Japanese mother-child pairs drawn from an ongoing birth cohort: The Hokkaido Study on Environment and Children's Health. This ongoing cohort started in February 2003, and the details of the study have been previously described.[36] Briefly, all indigenous Japanese women who reserved antenatal care at any of 37 participating hospitals within Hokkaido during their first trimester of pregnancy were considered eligible. Health care personnel introduced the study, after which each potential participant was given an invitation that included a consent form, baseline questionnaire, and self-addressed envelopes for return of the signed consent forms and completed questionnaires. The participants were recruited between February 2003 and March 2006. Only participants with linked and integrated data (5772; 61.8%) were included in the baseline population of this study. The response rate for each variable was at least 70.0% from various sources. Based on the population allele frequencies of the 5,10-*MTHFR* C677T[37] and A1298C[38] polymorphic variants specific to Japanese and the prevalence of tobacco smoking during pregnancy,[39] minimum sample sizes were calculated by using genetic software.[40] We randomly selected 1805 extracted genomic DNAs, attempted to discriminate the alleles of 5,10-*MTHFR* polymorphisms, and successfully genotyped 1784, which were ultimately used in the data analysis (Figure 1). The Institutional Ethical Board for Human Gene and Genome Studies of Hokkaido University Graduate School of Medicine approved the study protocol.

Methods of data collection

Data were acquired from baseline self-administered questionnaires, hospital records of infant births, and postpartum self-administered questionnaires. Baseline information included biodata, lifestyle habits, drugs (including use of nutritional supplements), and gynecologic and obstetric histories. Infant birth records from hospitals had information about birth weight, gestational age at delivery, sex, obstetric events during index pregnancy, and congenital anomalies, among other information. Postpartum questionnaires collected information on infant anthropometric parameters, active or passive tobacco smoking during the index pregnancy, and whether the index pregnancy was eventful. Each variable in the dataset had a response rate of at least 70.0%, although the item on smoking status decreased from 99.0% at baseline to 70.0% after pregnancy. Whole-blood specimens were collected during the first trimester for serum folate assays; subsequent collections of whole blood specimens were stored at −80°C for genetic analyses.

Folate assay

Serum folate was assayed by a commercial laboratory (SRL, Inc. Tokyo, Japan) using an automated competitive protein binding (CPB) chemiluminescent enzyme immunoassay (CLEIA) technique according to the manufacturer's protocols. This type of assay has an intra- and inter-assay imprecision of 10.0% or less and has become common in in vitro studies, as it is less costly, faster, and convenient. In addition, the need for smaller samples is advantageous in large scale epidemiologic studies.[41] The specific assay method for this study was the ADVIA Centaur technique, which has a coefficient of variation between 4.0% to 4.3%.[42] Analyses were conducted in batches, which were scheduled with regard to recruitment period and laboratory procedure.

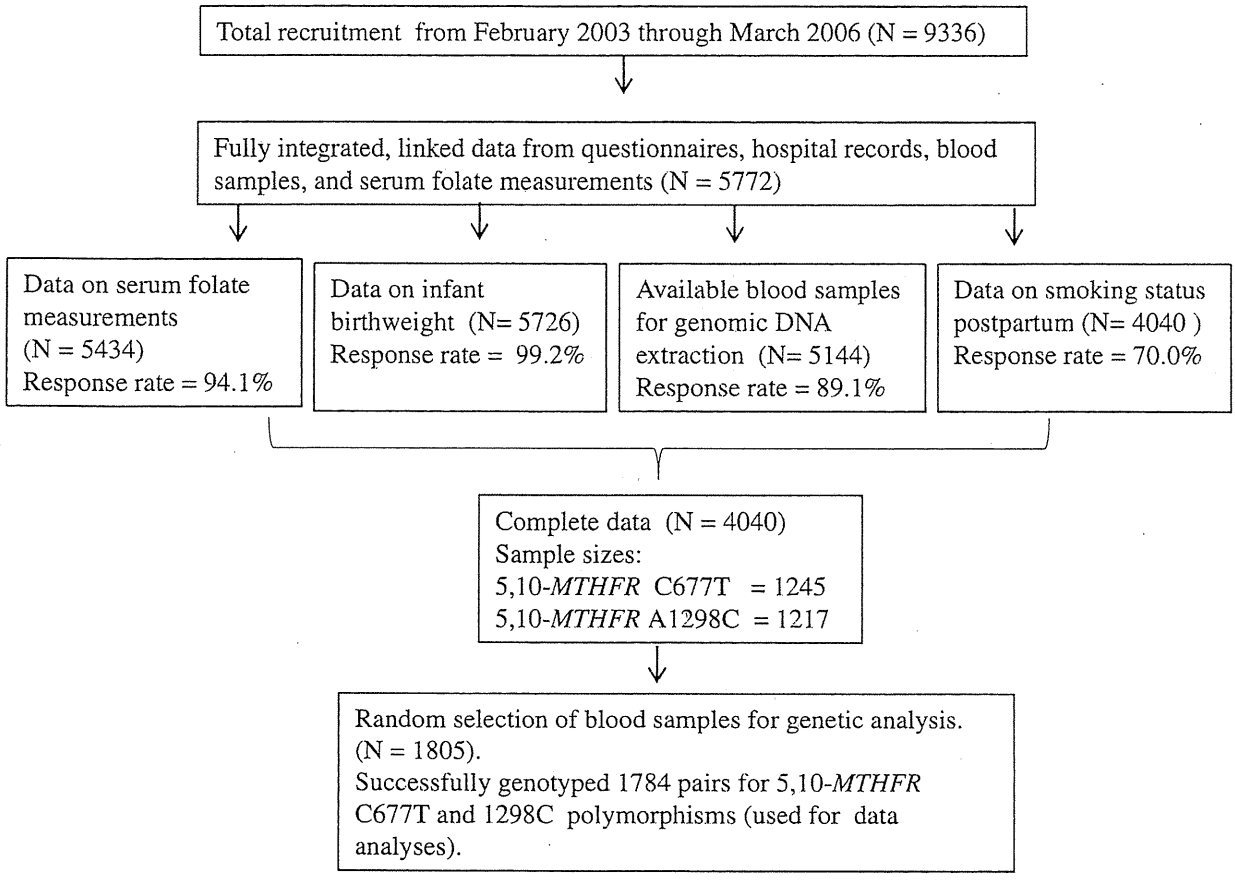

Figure 1. Study selection flow chart

Selection and genotyping of single nucleotide polymorphisms (SNPs)

We chose the 2 most common SNPs of this gene, namely C677T and A1298C, which have minor allele frequencies (MAF) of 35.2%[37] and 19.0%,[38] respectively, among Japanese. Genomic DNAs were extracted using a Maxwell 16 Instrument (Promega Corporation, WI, USA). DNA amplifications were performed in batches on 96-well micro-amp reaction plates using validated TaqMan probes for *MTHFR* C677T and A1298C (assay IDs: C_1202883_20 and C_850486_20), respectively, on a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) with an end-point allelic discrimination (AD) assay on a 7300/ 7500 Real-time PCR System[43] (Applied Biosystems, Foster City, CA, USA). We randomly selected 95 samples (5.0% of the successfully genotyped samples) and repeated genotyping to check for genotyping quality. The results were 100% concordant.

Definition of variables

Environmental exposures

Overall smoking status was classified into 3 categories using both self-reported active smoking and passive exposure to environmental tobacco smoke (ETS) at home. Nonsmokers had no history of active smoking or exposure to ETS at home. Nonsmokers and quitters with ETS exposure were classified as the passive smoking group, while smokers consisted of active smokers irrespective of ETS exposure status. Quitters with no ETS exposure during the first trimester had mean infant birth weights similar to those of nonsmokers; hence, they were added to the nonsmoking group. Mothers who quit during the second or third trimesters were added to the active smoking group.

Genetic exposures

The 5,10-*MTHFR* C677T and A1298C genotypes were categorized as dominant homozygous, heterozygous, and recessive homozygous genotypes (677CC, 677CT, and 677TT; and 1298AA, 1298AC, and 1298CC, respectively).

Statistical analyses

Univariate ANOVA with multiple comparison tests was performed to assess the main effects of maternal 5,10-*MTHFR* C677T and A1298C polymorphisms and smoking on serum folate levels, while ANCOVA was used to investigate the interactive association between smoking and 5,10-*MTHFR* C677T and A1298C polymorphisms in relation to folate status and infant birth weight. Serum folate was log-transformed before the analyses and back-transformed after the analyses. Known major predictors of infant birth weight (infant sex, gestational age at delivery, maternal age, maternal

prepregnancy weight, maternal height, parity, and alcohol intake during pregnancy) were adjusted for in the multivariate regression analyses. Use of a folic acid supplement, which was highly correlated with serum folate levels, was also included as a covariate. Smoking status was adjusted for when we assessed the predictive power of each SNP on birth weight. Categorical covariates were dichotomized to fit the regression equation. Subgroups with few participants (ie, subgroups for the 1298CC genotype) were excluded from the regression analyses. We used the codominant genetic model and per-allele approach. Our preliminary analyses revealed that mean serum folate was highest for the *MTHFR* 1298AC genotype. Because adequate folate status is an integral part of our hypothesis, we decided that it was biologically plausible to set 1298AC as the reference category in the regression analyses. Predictors were entered simultaneously into the equation. Assessment of the 5,10-*MTHFR* C677T and A1298C genotypes for deviation from the Hardy-Weinberg equilibrium, and other evaluations of data quality, were conducted using Haploview version 4.2 software.[44] All other analyses were performed using SPSS version 16.00 for Windows (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at less than 0.05.

RESULTS

The maternal mean serum folate (SD) level was 16.4 (1.5) nmol/L. The prevalence of folate deficiency (<6.8 nmol/L) was 0.3%; most (73.0%) mothers had adequate folate status (≥13.6 nmol/L). The prevalence of folic acid supplementation was 10.0%. Mean infant birth weight (SD) was 3040 (374) g. The prevalence of active smoking during pregnancy was 15.9%, while that of passive smoking was 53.0%. The distributions of the 5,10-*MTHFR* C677T and A1298C genotypes did not deviate from the Hardy-Weinberg equilibrium ($P = 0.546$ and 0.909, respectively). The frequencies of *MTHFR* 677CC, 677CT, and 677TT were 37.3%, 46.7%, and 16.0%, respectively, while those of *MTHFR* 1298AA, 1298AC, and 1298CC were 62.7%, 33.1%, and 4.2%, respectively. A strong LD ($D' = 0.943$) between *MTHFR* C677T and A1298C was also observed, and the minor allele frequencies were 0.392 and 0.205, respectively (Table 1). These findings were similar to those of previous studies of Japanese populations.[6,37,38,45–47] We used 2-way analysis of variance to assess the main effects of 5,10-*MTHFR* on maternal mean serum folate concentration. Carrying the T allele was associated with a decrease in mean serum folate level, and the lowest level (14.1 nmol/L) was observed in the 677TT homozygous group. Tukey's honestly significant differences (HSD) of 1.0 nmol/L ($P = 0.008$) and 3.8 nmol/L ($P < 0.001$) were observed between 677CC versus 677CT and between 677CC versus 677TT, respectively. In contrast, carrying the 1298C allele was associated with higher mean serum folate levels. A Tukey's HSD of 1.5 nmol/L ($P < 0.001$) was observed between 1298AA versus 1298AC (Figure 2). Mean serum folate levels in the analysis of covariance were generally lower among smokers for all 5,10-*MTHFR* C677T genotypes, and the lowest level was found among 677TT homozygotes (11.8 nmol/L, $P_{interaction} < 0.001$). With regard to 5,10-*MTHFR* A1298C genotypes, the lowest mean folate level was observed among smokers with the 1298AA homozygous genotype, ($P_{interaction} < 0.001$; Figure 3).

We initially explored the role of maternal serum folate during the first trimester as a predictor of infant birth weight and found no significant linear association. After stratification by folate status, low folate status (<13.6 nmol/L) was associated with a nonsignificant reduction in birth weight. After stratification by birth weight status, low folate status was associated with a 34.00 g ($P = 0.045$) reduction in mean birth weight of infants in the normal birth weight group (data not shown).

To investigate whether the maternal 5,10-*MTHFR* C677T and A1298C polymorphisms were independently associated with birth weight, we conducted a multiple regression analysis with adjustments for known major predictors of birth weight.

Table 1. Characteristics of 1784 mother-child pairs

Characteristic	*n* (%)
Maternal age (years)	30.0 (4.3)[e]
Maternal height (cm)	158.0 (5.1)[e]
Prepregnancy weight (kg)	53.0 (9.3)[e]
Maternal serum folate (nmol/L)	16.4 (1.5)[e]
Gestational age at delivery (weeks)	38.9 (1.3)[e]
Infant birth weight (g)	3040 (374)[e]
Infant sex	
Male	873 (48.9)
Female	911 (51.1)
Parity	
Nulliparous	391 (21.9)
Parous	1393 (78.1)
Alcohol intake during pregnancy	
No	1499 (84.0)
Yes	285 (16.0)
Tobacco smoking during pregnancy	
Nonsmoker	555 (31.1)
Passive smoker	946 (53.0)
Smoker	283 (15.9)
Folic acid supplementation	
No	1601 (89.7)
Yes	183 (10.3)
Maternal *MTHFR* C677T genotype[a,b]	
CC	666 (37.3)
CT	833 (46.7)
TT	285 (16.0)
CT/TT	1118 (62.7)
Maternal *MTHFR* A1298C genotype[c,d]	
AA	1118 (62.7)
AC	591 (33.1)
CC	75 (4.2)
AC/CC	666 (37.3)

[a]HWE = Hardy-Weinberg equilibrium $P = 0.5463$
[b]MAF = Minor allele frequency = 0.392
[c]HWE = Hardy-Weinberg equilibrium $P = 0.9091$
[d]MAF = Minor allele frequency = 0.205
[e]Mean (SD) *MTHFR* = Methylenetetrahydrofolate reductase gene

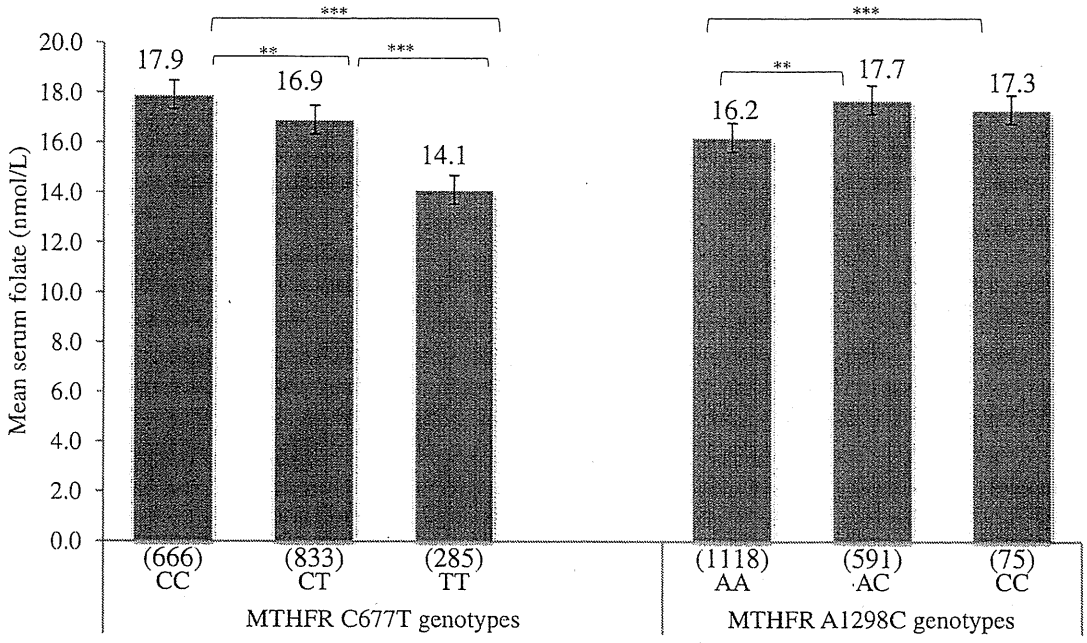

Figure 2. Maternal mean serum folate levels across *MTHFR* C677T and A1298C genotypes. $^{**}P < 0.01$, $^{***}P < 0.001$ Univariate analysis with Tukey's honestly significant differences test. Values in parentheses are counts in each group.

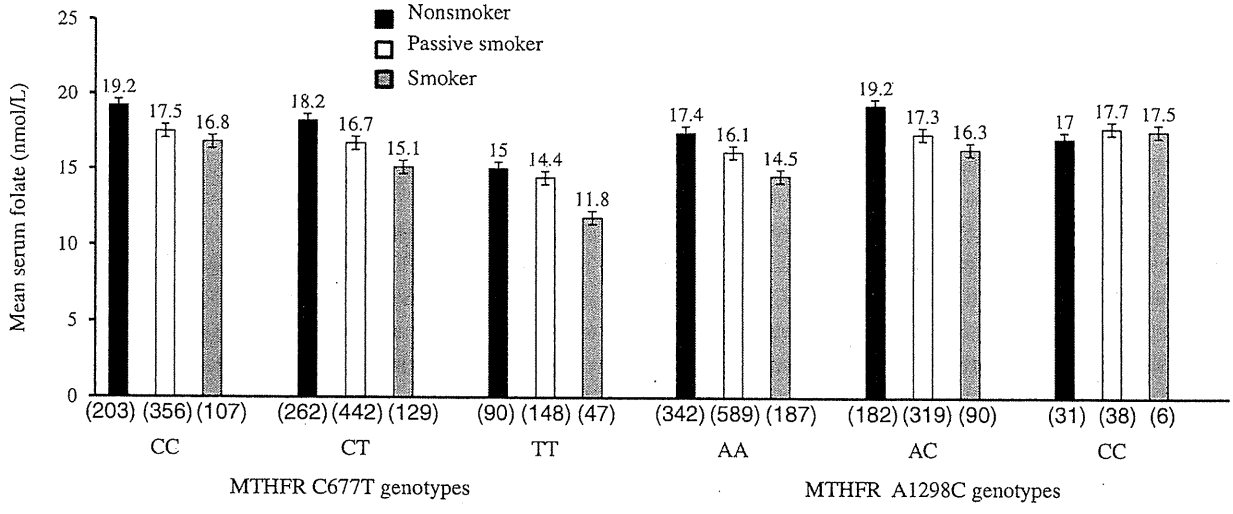

Figure 3. Maternal mean serum folate levels across *MTHFR* C677T and A1298C genotypes according to smoking status. ANCOVA $P_{interaction} < 0.001$. For MTHFR C677T and A1298C. Values in parentheses are counts in each group.

Among infants of 677CT heterozygous mothers, adjusted mean birth weight was highest (3061 g) for 5,10-*MTHFR* C677T. The 677CT genotype was associated with a 36.40 g increase in mean infant birth weight (95% CI: 2.60 to 70.30, $P = 0.035$). Carrying the 677T allele was associated with a marginally significant 27.00 g increase in infant birth weight (95% CI: −3.76 to −59.47, $P = 0.084$).

Polymorphism in 5,10-*MTHFR* A1298C or carrying the 1298C allele was not significantly independently associated with birth weight, although the adjusted mean infant birth weight was highest (3048 g) in the 1298AC heterozygous group. The adjusted mean birth weight of infants of active tobacco smokers was lowest (2978 g) and was 85.00 g (95% CI: 133.30 to −36.80, $P = 0.001$) less than that of children born to nonsmokers (Table 2).

We stratified mothers by tobacco smoking status. Among nonsmokers, male infants of 677CT genotype mothers were 90.00 g (95% CI: −2.11 to 182.50, $P = 0.05$) heavier than the infants in the reference category, which was marginally statistically significant, while among passive smokers, female

Table 2. Association of maternal 5,10-*MTHFR* C677T, A1298C genotypes and tobacco smoking with infant birth weight (*N* = 1784)

Maternal 5,10-*MTHFR* polymorphisms/smoking status	*n*	Adjusted mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	P_{trend}[c]
[a]*MTHFR* C677T genotype				
CC	666	3024.70 (14.51)	Reference	
CT	833	3061.13 (12.98)	36.40 (17.30) [2.60, 70.30]*	
TT	285	3015.15 (22.75)	4.00 (23.50) [−42.20, 50.10]	0.07
[a]*MTHFR* C677T allele				
C	2165	3044.97 (9.68)	Reference	
T	1403	3049.52 (11.29)	27.86 (16.12) [−3.76, 59.47]†	
[a]*MTHFR* A1298C genotype				
AA	1118	3036.58 (11.17)	Reference	
AC	591	3048.71 (15.67)	14.47 (16.78) [−18.45, 47.38]	
CC	75	3028.59 (44.69)	−27.65 (39.36) [−104.85, 49.55]	0.49
[a]*MTHFR* A1298C allele				
A	2827	3040.77 (9.1)	Reference	
C	741	3046.43 (14.78)	9.74 (16.16) [−21.95, 41.43]	
[b]Smoking status				
Nonsmoker	555	3062.81 (14.11)	Reference	
Passive smoker	946	3046.08 (10.70)	−14.40 (17.90) [−49.50, 20.60]	
Smoker	283	2978.29 (19.70)	−85.01 (24.60) [−133.30, −36.80]**	0.001

**$P < 0.01$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight) B (Unstandardized coefficients) SE (standard error). (CI) confidence interval. (*MTHFR*) Methylenetetrahydrofolate reductase. Grams (g). [a]Multiple linear regression adjusted for gestational age, infant sex, maternal age, prepregnancy weight, height, parity, smoking during pregnancy, alcohol intake during pregnancy, and folic acid supplement use. [b]Multiple linear regression adjusted for gestational age, infant sex, maternal age, prepregnancy weight, height, parity, and folic acid supplement intake. [c]Polynomial univariate analysis.

infants of 677TT homozygous mothers were 99.00 g (95% CI: −190.26 to −7.56, $P = 0.03$) lighter than reference. None of the minor-allele genotypes among smokers showed any significant effect on infant birth weight as compared with those in the major-allele genotypes (data not shown). Per-allele analyses revealed that carrying the 677T allele was associated with a 68.00 g (95% CI: −121.74 to −15.27, $P = 0.012$, $P_{trend} = 0.003$) reduction in mean birth weight among infants of smokers and an 89.00 g (95% CI: −168.89 to −9.56, $P = 0.028$, $P_{trend} = 0.018$) reduction among female infants (Table 3a). Furthermore, smoking in mothers carrying the 1298A allele was associated with a 92.00 g (95% CI: −144.46 to −40.96, $P < 0.001$, $P_{trend} = 0.091$) reduction in mean birth weight. Males were lighter by 79.00 g (95% CI −150.73 to −8.58, $P = 0.028$, $P_{trend} = 0.228$), while females were lighter by 107.00 g (95% CI: −182.78 to −31.54, $P = 0.006$, $P_{trend} = 0.112$; Table 3b).

In cross-classification interactive analyses, infants born to nonsmokers with 5,10-*MTHFR* 677CT genotypes had the highest mean birth weight (3092 g); male newborns were 90.70 g (95% CI: 6.00 to 175.50, $P = 0.036$) heavier than the male infants of nonsmoking 677CC mothers, ($P_{interaction} = 0.020$; Table 3a). The 5,10-*MTHFR* 1298AA genotype was associated with a 107.00-g (95% CI: −165.67 to −47.52, $P < 0.001$) decrease in mean infant birth weight among smokers. Stratification by infant sex did not yield obvious differences in birth weight, $P_{interaction} = 0.040$; Table 3b). When 1298AC was set as the reference category, the 5,10-*MTHFR* 1298AA genotype was associated with a 107.00-g (95% CI, −180.00 to −33.90, $P = 0.004$) decrease in mean infant birth weight in smokers; the effect was more obvious in male infants (117.00 g; 95% CI: −218.60 to −14.70, $P = 0.025$; data not shown).

DISCUSSION

Among nonsmokers, we found an association of maternal 5,10-*MTHFR* 677CT heterozygosity with higher infant birth weight, while 5,10-*MTHFR* 677TT homozygosity was associated with lower birth weight among female infants of passive tobacco smokers. In addition, among smokers, 5,10-*MTHFR* 1298AA homozygosity was associated with low folate status and lower birth weight. To our knowledge, this is the first study to report such findings for a Japanese population.

Maternal 5,10-*MTHFR* C677T, *MTHFR* A1298C and serum folate status

Our results showed an association between the 5,10-*MTHFR* 677T allele and low folate status, which agrees with the findings of earlier reports.[6,46] 677TT homozygosity was associated with low folate status, and values were much lower among active and passive smokers, which suggests independent and combined effects of tobacco smoke and 5,10-*MTHFR* C677T polymorphism on folate status.

In contrast, the 5,10-*MTHFR* 1298C allele was associated with higher serum folate levels, while the 1298AA genotype was associated with lower folate levels. Although the metabolic and clinical functions of this SNP have not been fully characterized, it is currently being studied by a number of investigators. In a recent study of Koreans, mean plasma

Table 3a. Association of maternal 5,10-*MTHFR* C677T polymorphism and tobacco smoking with infant birth weight (*N* = 1784)

5,10-*MTHFR*	Smoking status	Overall (*N* = 1784)			Males (*N* = 873)			Females (*N* = 911)		
		n	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g
C677T genotype										
CC	Nonsmoker	203	3008.84 (25.84)	Reference	93	3080.01 (37.75)	Reference	110	2949.95 (34.58)	Reference
	Passive smoker	356	3035.93 (19.85)	9.5 (29.3) [−47.9, 66.9]	187	3037.8 (27.16)	−39.6 (40.5) [−119.2, 39.9]	169	3033.88 (29.16)	59.3 (41.6) [−22.3, 140.8]
	Smoker	107	3017.29 (37.52)	−38.7 (39.8) [−116.8, 39.4]	52	3109.23 (52.51)	−37.7 (55.8) [−147.3, 71.8]	55	2928.76 (51.18)	55.3 (56.5) [−166.2, 55.6]
CT	Nonsmoker	262	3092.43 (24.44)	60.0 (31.0) [−0.9, 120.9]†	133	3162.77 (35.43)	90.7 (43.20) [6.0, 175.50]*	129	3020.99 (32.59)	34.1 (44.1) [−52.4, 120.6]
	Passive smoker	442	3068.18 (17.15)	47.5 (28.2) [−7.9, 102.9]	198	3114.72 (24.29)	21.2 (40.3) [−57.8, 100.3]	244	3030.45 (23.78)	62.1 (39.1) [−14.6, 138.8]
	Smoker	129	2973.99 (32.43)	−53.1 (37.8) [−127.2, 21.0]	70	3041.21 (35.16)	−39.8 (51.1) [−140.0, 60.4]	59	2894.24 (55.93)	−63.5 (55.2) [−171.9, 44.9]
TT	Nonsmoker	90	3087.77 (38.76)	59.1 (42.0) [−23.3, 141.4]	43	3123.57 (54.59)	15.1 (58.8) [−100.6, 130.5]	47	3055.09 (55.04)	91.7 (59.0) [−24.0, 207.4]
	Passive smoker	148	2984.38 (31.57)	−14.4 (36.1) [−85.2, 56.3]	74	3045.41 (45.87)	11.1 (50.1) [−87.2, 109.4]	74	2922.5 (42.44)	−39.7 (51.1) [−140.0, 60.6]
	Smoker	47	2974.11 (58.61)	−49.1 (53.9) [−154.8, 56.7]	23	3067.13 (73.23)	−53.7 (74.9) [−200.7, 93.3]	24	2884.96 (88.5)	−48.3 (76.7) [−198.8, 102.2]
	$P_{interaction}$			0.03			0.02			0.14
C677T allele[d]										
C	Nonsmoker	465	3059.64 (15.39)	Reference	226	3137.62 (21.58)	Reference	239	2987.47 (21.95)	Reference
	Passive smoker	798	3053.06 (11.64)	−13.37 (19.69) [−51.98, 25.25]	385	3075.26 (56.28)	−60.94 (26.60) [−113.15, −8.73]*	413	3030.17 (16.61)	35.80 (29.20) [−21.51, 93.11]
	Smoker	236	2979.64 (21.57)	−63.45 (33.22) [−128.61, 1.71]†	122	3046.22 (29.14)	−57.52 (46.52) [−148.82, 33.78]	114	2909.50 (32.18)	−78.85 (47.97) [−173.00, 15.30]
	$P_{interaction}$			0.45			0.75			0.06
T	Nonsmoker	352	3084.10 (17.66)	Reference	176	3159.40 (24.30)	Reference	176	3015.04 (25.68)	Reference
	Passive smoker	590	3055.28 (13.57)	−9.93 (17.12) [−23.65, 43.52]	272	3103.07 (19.34)	12.38 (24.16) [−35.03, 59.79]	318	3009.48 (19.08)	7.75 (24.40) [−40.15, 55.64]
	Smoker	176	2975.05 (24.89)	−68.50 (27.14) [−121.74, −15.27]*	93	3042.31 (33.20)	−48.48 (36.51) [−120.13, 23.18]	83	2909.60 (37.38)	−89.22 (40.59) [−168.89, −9.56]*
	$P_{interaction}$			0.34			0.48			0.46

***$P < 0.001$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight), B (Unstandardized coefficient) SE (Standard error). CI (Confidence interval), g (Grams). [a]Multiple linear regression adjusted for infant sex, gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [b]Multiple linear regression adjusted for gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [c]Excluded from the regression analyses. [d]P_{trend} by smoking status (C allele = 0.021, Males = 0.045, Females = 0.040 and T allele = 0.003, males = 0.073, Females = 0.018).

homocysteine was higher among 1298AA homozygotes as compared with those carrying the 1298C allele.[48] Because serum folate is inversely correlated with plasma homocysteine, we inferred that our study population might have a similar plasma homocysteine distribution across genotypes. A report from Portugal noted that 1298AC heterozygosity was associated with a high plasma folate level and that the level was lowest among 1298CC homozygotes,[49] which is similar to the findings of the present study. Our findings contradict those of a study of a Dutch population, in which *MTHFR* A1298C alone was not associated with any biochemical abnormalities except when in combination with *MTHFR* C677T, specifically compound heterozygosity 5,10-*MTHFR* 677CT/1298AC.[28] The fact that our findings were similar to those from a report on a Korean population is genetically plausible because racial, geographic, and nutritional disparities might account for differences in the functional characteristics of 5,10-*MTHFR* SNPs.[50,51]

Effects of maternal 5,10-*MTHFR* A1298C polymorphism and tobacco smoke on infant birth weight

Smokers carrying 1298A alleles delivered infants with lower mean birth weights, especially female infants; however, these results must be interpreted with caution because alleles do not act in isolation. The effect of the maternal 5,10-*MTHFR* 1298AA genotype in reducing the birth weight of infants delivered by tobacco smokers might be due to low folate status associated with the 1298AA genotype. Perhaps some essential folate-dependent cellular processes were compromised. Cells that lack folate have been observed to accumulate in the S-phase of the cell cycle. Such cells have higher uracil misincorporation and DNA damage,[52] which might have a role in the impairment of fetal growth. Moreover, chronic deficits in extracellular and intracellular folate due to the effects of tobacco smoke might have been severe enough to inflict nutritional stress. In our study, we could not examine the role of the 1298CC genotype among

Table 3b. Association of maternal 5,10-*MTHFR* A1298C polymorphism and tobacco smoking with infant birth weight (*N* = 1784)

5,10-*MTHFR*	Smoking status	Overall (*N* = 1784)			Males (*N* = 873)			Females (*N* = 911)		
		n	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g
A1298C genotype										
AA	Nonsmoker	342	3071.9 (20.47)	Reference	169	3145.63 (28.99)	Reference	173	3000.74 (27.94)	Reference
	Passive smoker	589	3036.45 (15.32)	−29.04 (22.36) [−72.91, 14.82]	266	3069.39 (23.03)	−59.35 (31.34) [−120.86, 2.16]	323	3009.36 (20.43)	−3.07 (32.08) [−66.03, 59.90]
	Smoker	187	2973.15 (26.53)	−106.59 (30.12) [−165.67, −47.52]***	94	3045.5 (31.69)	−104.19 (41.36) [−185.37, −23.03]*	93	2900.02 (41.47)	−113.45 (44.05) [−199.91, −26.99]*
AC	Nonsmoker	182	3058.19 (29.74)	−1.66 (30.08) [−60.66, 57.34]	83	3110.86 (45.78)	13.56 (42.70) [−70.26, 97.37]	99	3015.1 (38.68)	−13.88 (42.68) [−97.64, 69.89]
	Passive smoker	319	3049.36 (20.36)	−15.20 (25.59) [−65.39, 34.98]	173	3066.73 (25.49)	−51.98 (34.39) [−119.47, 15.51]	146	3028.88 (32.66)	21.20 (38.41) [−54.19, 96.59]
	Smoker	90	3027.25 (42.41)	−57.04 (39.11) [−133.74, 19.66]	46	3120.85 (51.65)	−59.18 (53.47) [−164.14, 45.78]	44	2927.12 (65.41)	−64.21 (58.00) [−178.05, 49.62]
CC	Nonsmoker	31	2959.81 (55.2)	−120.92 (61.81) [−242.16, 0.32]†	17	3037.65 (79.96)	−107.17 (81.49) [−267.12, 52.77]	14	2865.29 (69.02)	−142.72 (94.43) [−328.05, 42.61]
	Passive smoker	38	3092.97 (65.72)	13.12 (61.81) [−97.08, 123.31]	20	3158.9 (98.97)	14.55 (75.56) [−133.75, 162.84]	18	3019.72 (84.17)	6.64 (84.21) [−158.64, 171.91]
	Smoker	6[c]	2976.17 (247.92)	—	5[c]	3054.6 (288.05)	—	1[c]	2584	—
	$P_{interaction}$			0.04			0.03			0.22
A1298C allele[d]										
A	Nonsmoker	524	3069.05 (14.52)	Reference	252	3140.25 (20.45)	Reference	272	3004.92 (20.68)	Reference
	Passive smoker	908	3045.91 (10.93)	−15.55 (17.17) [−49.23, 18.12]	439	3075.06 (15.24)	−35.44 (24.30) [−83.13, 12.26]	469	3016.12 (15.67)	−6.65 (24.40) [−45.24, 50.54]
	Smoker	277	2978.20 (19.91)	−92.71 (26.38) [−144.46, −40.96]***	140	3043.18 (27.23)	−79.65 (36.21) [−150.73, −8.58]*	137	2912.57 (29.35)	−107.16 (38.53) [−182.78, −31.54]**
	$P_{interaction}$			0.15			0.22			0.47
C	Nonsmoker	213	3049.58 (22.66)	Reference	100	3124.26 (32.36)	Reference	113	2980.20 (31.83)	Reference
	Passive smoker	357	3056.17 (17.48)	−18.49 (19.73) [−20.21, 57.20]	193	3084.82 (23.06)	−4.28 (26.42) [−56.13, 47.58]	164	3031.86 (26.57)	41.38 (29.65) [−16.81, 99.57]
	Smoker	97	3009.71 (33.78)	−29.12 (34.99) [−97.75, 39.50]	51	3070.73 (44.90)	−19.37 (47.02) [−111.66, 72.92]	45	2942.56 (51.53)	−46.16 (52.88) [−149.95, 57.64]
	$P_{interaction}$			0.37			0.72			0.51

***$P < 0.001$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight). B (Unstandardized coefficient) SE (Standard error). CI (Confidence interval), g (Grams). [a]Multiple linear regression adjusted for infant sex, gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [b]Multiple linear regression adjusted for gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [c]Excluded from the regression analyses. [d]P_{trend} by smoking status (A allele = 0.091, Males = 0.228, Females = 0.112 and C allele = 0.752, males = 0.828, Females = 0.271).

smokers because of its low frequency. However, previous reports observed that the maternal 1298CC genotype was associated with a greater reduction in the risk of low birth weight as compared with the 1298AA genotype.[21] The 1298CC homozygous genotype was also reported to be protective against IUGR in Canadians.[53] Hyperhomocysteinemia might have increased the risk of placental vasculopathy via oxidative stress, endothelial cell dysfunction, and/or coagulopathies leading to feto-placental hypoperfusion.[5]

Adequate serum vitamin B_{12} status has been shown to decrease total plasma homocysteine levels in Japanese.[54] However, among smokers, the possible coexistence of nutritional deficiencies, including vitamin B_{12} deficiency, might have compromised the methylation of homocysteine to methionine, resulting in impaired fetal growth. The folate level was probably not adequate to silence the phenotypic expression of 1298AA among smokers. Higher exogenous folate may be needed to correct deficits and maintain ideal levels for optimal fetal growth.

With regard to the 5,10-*MTHFR* gene structure, the A1298C variant is located on the regulatory C-terminal domain, which contains protein retention signals that prevent delivery of proteins to the secretory pathway. It is possible that allosteric inhibitory interplay in the s-adenosyl methionine (SAM) and s-adenosyl homocysteine (SAH) cycle is involved in the functional behavior of this SNP in relation to folate status and mediation of fetal growth. Recently, the 5,10-*MTHFR* A1298C polymorphism was found to be associated with increased folate levels in red blood cells, in an inverse relationship with 5,10-*MTHFR* C677T polymorphism,[55] which suggests that both SNPs have different functional characteristics with regard to phenotypic expression.

Due to limited evidence on the functional role of the 5,10-*MTHFR* A1298C polymorphism, especially among Japanese,

further research is needed to verify this observation and elucidate the biological mechanisms associated with this SNP.

Effects of the maternal 5,10-*MTHFR* C677T polymorphism and tobacco smoke on infant birth weight

The 5,10-*MTHFR* 677T allele is associated with low folate and high homocysteine levels. In this study, the 677T allele was associated with lower birth weight in offspring of active smokers. The 677CT genotype was protective against low birth weight, especially among male offspring, but only in the absence of active or passive tobacco smoke. This might be due to the presence of higher mean serum folate levels among nonsmokers. In a Korean population, the 677T allele had a weak protective association with lung carcinoma.[56] The protective effect of adequate folate status might be mediated by the stabilization of flavin-adenine-dinucleotide (FAD) binding at the catalytic domain.[2] The birth weight of female infants of 677TT homozygous passive smokers was significantly lower than that of female infants born to passive smokers of similar genotypes. Male fetuses were favored, probably because pregnant mothers carrying male fetuses have a higher nutritional intake than those with female fetuses.[57] Poorly understood phenomena on fetal sex-specific signals have been implicated in fetal growth, especially in response to glucocorticoid activity that might modify the fetal response to stress.[58]

Study strengths and limitations

The participants were indigenous Japanese; hence, we overcame issues of population stratification in genetic association studies. Overall, this study might be limited by selection bias, as we utilized integrated data from only 61% of the total recruitment during the study period. However, because we randomly selected the final study population based on known pooled population frequencies of the genetic factors and tobacco smoking among pregnant women, we believe that this study does not substantially differ from one with a higher participation rate (ie, ≥70%). The sample size of the study was adequate to detect gene-environment interactions; however, multiple comparisons and small subgroup sample sizes might have affected the study power. There could be misclassification bias from self-reported tobacco smoking; however, a previous study reported very low misclassification bias among Japanese women.[59] Therefore, the findings of the present study are likely to be reliable. Nevertheless, this does not diminish the importance of using biomarkers like cotinine. The findings might have been confounded by other B vitamins, which were not studied, or by other unidentified sources common to cohort study designs. Finally, this study was hospital-based; therefore, our findings should not be generalized. Further studies of other factors in the folate-homocysteine pathway should prove interesting.

Public health implications

Analysis of the 5,10-*MTHFR* A1298C polymorphism shows that the frequency of the 1298AA genotype is greater than 60.0% in the Japanese population.[38] Its association with low folate status is thus a considerable public health concern. With the recent increasing prevalence of smoking among young Japanese women, particularly in Hokkaido,[60] maternofetal morbidity and mortality might also increase. Smoking cessation and targeted use of folic acid supplements could therefore prove to be very important public health tools in this population.

Conclusions

Our findings suggest that the maternal 5,10-*MTHFR* C677T polymorphism is independently associated with higher infant birth weight, especially among nonsmokers, while the 5,10-*MTHFR* A1298C variant is not independently associated with birth weight. In addition, the 5,10-*MTHFR* 1298AA polymorphism might be associated with folate impairment and could interact with tobacco smoke to further decrease birth weight.

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Conflicts of interest: None declared.

REFERENCES

1. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) associated with decreased enzyme activity. Mol Genet Metab. 1998;64:169–72.
2. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA. 2002;99:5606–11.
3. Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T. Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet. 1997;34:525–6.
4. Isotalo PA, Wells GA, Donnelly JG. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet. 2000;67:986–90.
5. van der Molen EF, Arends GE, Nelen WL, van der Put NJ, Heil SG, Eskes TK, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene as a new risk factor for placental vasculopathy. Am J Obstet Gynecol. 2000;182:1258–63.
6. Murakami S, Matsubara N, Saitoh M, Miyakaw S, Shoji M, Kubo T. The relation between plasma homocysteine concentration and methylenetetrahydrofolate reductase gene polymorphism in pregnant women. J Obstet Gynaecol Res. 2001;27:349–52.
7. Hefler L, Jirecek S, Heim K, Grimm C, Antensteiner G, Zeillinger R, et al. Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. J Soc Gynecol Investig. 2004;11:42–4.
8. Valdez LL, Quintero A, Garcia E, Olivares N, Celis A, Rivas F Jr, et al. Thrombophilic polymorphisms in preterm delivery. Blood Cells Mol Dis. 2004;33:51–6.
9. Johnson WG, Scholl TO, Spychala JR, Buyske S, Stenroos ES, Chen X. Common dihydrofolate reductase 19-base pair deletion allele: a novel risk factor for preterm delivery. Am J Clin Nutr. 2005;81:664–8.
10. Relton CL, Pearce MS, Burn J, Parker L. An investigation of folate-related genetic factors in the determination of birthweight. Paediatr Perinat Epidemiol. 2005;19:360–7.
11. Mtiraoui N, Zammiti W, Ghazouani L, Braham NJ, Saidi S, Finan RR, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses. Reproduction. 2006;131:395–401.
12. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83:993–1016.
13. Stonek F, Hafner E, Philipp K, Hefler LA, Bentz EK, Tempfer CB. Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. Obstet Gynecol. 2007;110:363–8.
14. Ozbek N, Ataç FB, Verdi H, Cetintaş S, Gürakan B, Haberal A. Relationship between small-for-gestational age births and maternal thrombophilic mutations. Thromb Res. 2008;122:175–8.
15. Pilsner JR, Hu H, Wright RO, Kordas K, Ettinger AS, Sánchez BN, et al. Maternal *MTHFR* genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. Am J Clin Nutr. 2010;92:226–34.
16. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod. 2000;15:458–62.
17. Kobashi G, Yamada H, Asano T, Nagano S, Hata A, Kishi R, et al. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and preeclampsia in Japanese women. Am J Med Genet. 2000;93:122–5.
18. Gebhardt GS, Scholtz CL, Hillermann R, Odendaal HJ. Combined heterozygosity for methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C is associated with abruptio placentae but not with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol. 2001;97:174–7.
19. McCowan LM, Craigie S, Taylor RS, Ward C, McLintock C, North RA. Inherited thrombophilias are not increased in "idiopathic" small-for-gestational-age pregnancies. Am J Obstet Gynecol. 2003;188:981–5.
20. Chen DF, Hu YH, Yang F, Wu BY, Chen L, Fang ZA, et al. Mother's and child's methylenetetrahydrofolate reductase C677T polymorphism is associated with preterm delivery and low birth weight. Beijing Da Xue Xue Bao. 2004;36(3):248–53.
21. Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. Am J Med. 2004;117:26–31.
22. Resch B, Gallistl S, Kutschera J, Mannhalter C, Muntean W, Mueller WD. Thrombophilic polymorphisms—factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations—and preterm birth. Wien Klin Wochenschr. 2004;116:622–6.
23. Also-Rallo E, Lopez-Quesada E, Urreizti R, Vilaseca MA, Lailla JM, Balcells S, et al. Polymorphisms of genes involved in homocysteine metabolism in preeclampsia and in uncomplicated pregnancies. Eur J Obstet Gynecol Reprod Biol. 2005;120:45–52.
24. Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ. Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. Am J Obstet Gynecol 2006;195:1231.e1–11.
25. Ananth CV, Peltier MR, De Marco C, Elsasser DA, Getahun D, Rozen R, et al; New Jersey-Placental Abruption Study Investigators. Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption. Am J Obstet Gynecol 2007;197:385.e1–7.
26. Richter B, Stegmann K, Röper B, Böddeker I, Ngo ET, Koch MC. Interaction of folate and homocysteine pathway genotypes evaluated in susceptibility to neural tube defects (NTD) in a German population. J Hum Genet. 2001;46(3):105–9.
27. Chango A, Fillon-Emery N, Mircher C, Bléhaut H, Lambert D, Herbeth B, et al. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr.

2005;94:166–9.
28. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998; 62:1044–51.
29. Hayashi K, Matsuda Y, Kawamichi Y, Shiozaki A, Saito S. Smoking during pregnancy increases risks of various obstetric complications: a case-cohort study of the Japan Perinatal Registry Network database. J Epidemiol. 2011;21(1):61–6. Epub 2010 Nov 13.
30. Brown KS, Kluijtmans LA, Young IS, Murray L, McMaster D, Woodside JV, et al. The 5,10-methylenetetrahydrofolate reductase C677T polymorphism interacts with smoking to increase homocysteine. Atherosclerosis. 2004;174:315–22.
31. Ozerol E, Ozerol I, Gökdeniz R, Temel I, Akyol O. Effects of smoking on serum concentrations of total homocysteine, folate, Vitamain B12, and Nitric Oxide in Pregnancy: A preliminary study. Fetal Diagn Ther. 2004;19:145–8.
32. Jauniaux E, Johns J, Gulbis B, Spasic-Boskovic O, Burton GJ. Transfer of folic acid inside the first-trimester gestational sac and the effect of maternal smoking. Am J Obstet Gynecol 2007:197:58.e1–6.
33. Takamura N, Kondoh T, Ohgi S, Arisawa K, Mine M, Yamashita S, et al. Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan. Eur J Nutr. 2004;43:285–7.
34. Kobashi G, Kato EH, Morikawa M, Shimada S, Ohta K, Fujimoto S, et al. *MTHFR* C677T Polymorphism and factor V Leiden mutation are not associated with recurrent spontaneous abortion of unexplained etiology in Japanese women. Semin Thromb Hemost. 2005;31:266–71.
35. Kobashi G. Genetic and environmental factors associated with the development of hypertension in pregnancy. J Epidemiol. 2006;16:1–8.
36. Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int J Epidemiol. 2011;40:611–8. Epub 2010 May 26.
37. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151:862–77.
38. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol. 2003;157:571–82.
39. Sasaki S, Kondo T, Sata F, Saijo Y, Katoh S, Nakajima S, et al. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod. 2006;12(2):77–83.
40. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, 2006. Available from: http://hydra.usc.edu/gxe.
41. Bock JL. The New Era of Automated Immunoassay. Am J Clin Pathol. 2000;113:628–46.
42. Sujaku K, Ogiwara T, Kawasaki Y. Determination of vitamin B12 and folate by ADVIA Centaur. J Anal Bio-Sci. 2006; 29:235–42 (in Japanese).
43. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res. 2001;11:1262–8.
44. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet. 2005; 76:887–93.
45. Matsuo K, Suzuki R, Hamajima N, Ogura M, Kagami Y, Taji H, et al. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. Blood. 2001;97(10):3205–9.
46. Lwin H, Yokoyama T, Date C, Yoshiike N, Kokubo Y, Tanaka H. Are the associations between life-style related factors and plasma total homocysteine concentration different according to polymorphism of 5,10-methylenetetrahydrofolate reductase gene (C677T *MTHFR*)? A cross-sectional study in a Japanese rural population. J Epidemiol. 2002;12:126–35.
47. Yin G, Kono S, Toyomura K, Hagiwara T, Nagano J, Mizoue T, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci. 2004;95:908–13.
48. Kim NK, Choi YK, Kang MS, Choi DH, Cha SH, An MO, et al. Influence of combined methylenetetrahydrofolate reductase (*MTHFR*) and thymidylate synthase enhancer region (TSER) polymorphisms to plasma homocysteine levels in Korean patients with recurrent spontaneous abortion. Thromb Res. 2006;117:653–8.
49. Castro R, Rivera I, Ravasco P, Jakobs C, Blom HJ, Camilo ME, et al. 5,10-Methylenetetrahydrofolate reductase 677C→T and 1298A→C mutations are genetic determinants of elevated homocysteine. QJM. 2003;96:297–303.
50. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr. 2000;72:324–32.
51. Jhaveri MS, Wagner C, Trepel JB. Impact of extracellular folate levels on global gene expression. Mol Pharmacol. 2001;60:1288–95.
52. Courtemanche C, Elson-Schwab I, Mashiyama ST, Kerry N, Ames BN. Folate deficiency inhibits the proliferation of primary human $CD8^+$ T lymphocytes in vitro. J Immunol. 2004;173: 3186–92.
53. Infante-Rivard C, Rivard GE, Yotov WV, Génin E, Guiguet M, Weinberg C, et al. Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. N Engl J Med. 2002;347:19–25.
54. Moriyama Y, Okamura T, Kajinami K, Iso H, Inazu A, Kawashiri M, et al. Effects of serum B vitamins on elevated plasma homocysteine levels associated with the mutation of methylenetetrahydrofolate reductase gene in Japanese. Atherosclerosis. 2002;164:321–8.
55. Parle-McDermott A, Mills JL, Molloy AM, Carroll N, Kirke PN, Cox C, et al. The *MTHFR* 1298CC and 677TT genotypes have opposite associations with red cell folate levels. Mol Genet Metab. 2006;88:290–4.
56. Cui LH, Shin MH, Kim HN, Song HR, Piao JM, Kweon SS, et al. Methylenetetrahydrofolate reductase C677T polymorphism in patients with lung cancer in a Korean population. BMC Med Genet. 2011 Feb 22;12:28.
57. Tamimi RM, Lagiou P, Mucci LA, Hsieh CC, Adami HO,

Trichopoulos D. Average energy intake among pregnant women carrying a boy compared with a girl. BMJ. 2003;326:1245–6.
58. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta and fetus. Endocr Rev. 2006;27:141–69.
59. Tsutsumi A, Kagawa J, Yamano Y, Nakadate T, Shimizu S. Relation between Cotinine in the Urine and Indices Based on Self-Declared Smoking Habits. Environ Health Prev Med. 2002;6:240–7.
60. Smoking Rate Highest in Hokkaido: A survey by the Japan Tobacco Inc. 2009. Available from: http://e.nikkei.com/e/fr/tnks/Nni20100331D30HH717.htm.

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胎生期低栄養と小児の健康

那須 民江*1, 岸 玲子*2

*1名古屋大学大学院医学系研究科環境労働衛生学 *2北海道大学環境健康科学教育センター

Malnutrition of Fetal Age and the Infant Health

Tamie NAKAJIMA*1 and Reiko KISHI*2

*1*Nagoya University Graduate School of Medicine, Department of Occupational and Environmental Health*
*2*Hokkaido University, Center for Environmental and Health Sciences*

国内外問わず子供の健康に対する環境リスクの増大が懸念され，化学物質を中心とした有害物質に対する子供の脆弱性について関心が高まっている。いままで行われてきた多くの化学物質研究は親世代への影響を追及するものであり，次世代の子供の成長まで観察する研究は少ない。我が国では環境省がいちはやくこの問題に着手し，全国調査「エコチル調査」が開始し，胎児期から13歳に達するまで定期的に子供たちの健康状態を確認する出生コーホート調査を開始した。この研究会では，「胎児期の栄養と子供の健康」ということに焦点をあて，議論をしてみたい。

「胎生期の低栄養」で注目されるのは，まず，妊娠期の母親のたんぱく質や脂肪，あるいはビタミン等の摂取不足に起因する栄養不足によるものである。福岡は，妊娠期母親の低栄養は次世代の児の生活習慣病リスク（生活習慣病胎児期発症説）に影響を与えるという新しい概念を紹介する。つまり，成人病のリスクは子宮内環境から始まっているということで，成人期の健康管理のみでは生活習慣病の予防は不十分であることを示唆するものである。これらの考え方は，大人の心疾患による死亡率が乳幼児の死亡率や乳児の出生時体重に関連する等，子宮内環境は成人の心疾患による死亡率に影響を与えるというBarker (1) の仮説に端を発し，「健康および成人病の素因は，胎芽，胎児，乳児期の環境に影響を受けて形成され，この変化は出生後変わることなく，その素因と環境との相互作用で健康および疾病が形成される」というDOHaD（Developmental Origins of Health and Disease）説が生まれた。

一方，妊婦のペルフルオロオクタン酸（PFOA）やペルフルオロオクタンスルフォン酸濃度と児の出生時体重は逆相関することがいくつかの疫学研究によって明らかにされている (2–4)。動物実験では，妊娠期のPFOA曝露が生存仔数を減らすという報告がされている (5, 6)。しかし，これらのメカニズムや「低体重」が児の発達や成人期の病気にどのような影響を与えるかまでは明らかにされていない。当然，化学物質曝露によってもたらされた「低体重児」が小児の健康にどのような影響をもたらすか，興味が持たれる。林らは母親が十分な栄養摂取状況下であっても，プラスチック可塑剤として汎用されているフタル酸ジ（2-エチルヘキシル）（DEHP）曝露が妊娠期母親の血漿中トリグリセライドや必須脂肪酸濃度を低下させ，「低栄養」を招来することに着目し，新しい視点から，胎仔期化学物質曝露の次世代健康影響の一端を報告する。

文　献

(1) Barker DJ. The origins of the developmental origins theory. J Internal Med. 2007;Suppl 1:45–49.
(2) Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. Correlation between prenatal exposure to perfluorinated chamicals and reduced fetal growth. Environ Health Perspect. 2009;117:660–667.
(3) Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007;115:1670–1676.
(4) Fei C, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect. 2007;115:1677–1682.
(5) Hayashi Y, Ito Y, Yamagishi N, Yanagiba Y, Tamada H, Wang D, Ramdhan DH, Naito H, Harada Y, Kamijima M, Gonzales FJ, Nakajima T. Hepatic peroxisome proliferator-activated receptor alpha may have an important role in the toxic effects of di(2-ethylhexyl)phthalate on offspring of mice. Toxicology. 2011;289:1–10.
(6) Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, Nakayama S, Lindstrom AB, Strynar MJ, Lau C. Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha.Toxicol Sci. 2007;98:571–581.

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Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants ☆

Emiko Okada [a], Seiko Sasaki [a], Yasuaki Saijo [b], Noriaki Washino [a], Chihiro Miyashita [a], Sumitaka Kobayashi [a], Kanae Konishi [a], Yoichi M. Ito [c], Rie Ito [d], Ayako Nakata [d], Yusuke Iwasaki [d], Koichi Saito [d], Hiroyuki Nakazawa [d], Reiko Kishi [e,*]

[a] Department of Public Health, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Department of Health Science, Asahikawa Medical University, Asahikawa, Japan
[c] Department of Biostatistics, Division of Advanced Medical Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[d] Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan
[e] Center for Environmental and Health Sciences, Hokkaido University, North 12 West 7 Kita-ku, Sapporo 060-0812, Japan

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ABSTRACT

Background: Recent studies have shown effects of prenatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on infants in the general environmental levels. Laboratory animal studies have shown that exposure to PFOS and PFOA is associated with immunotoxic effects.
Objectives: To investigate the relationship between maternal PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. Cord blood immunoglobulin (Ig) E levels were also evaluated.
Methods: We conducted a prospective cohort study of pregnant women from 2002 to 2005 in Sapporo, Japan. Maternal PFOS and PFOA levels were measured in relation to cord blood IgE concentrations (n=231) and infant allergies and infectious diseases (n=343). Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at 18 months of age. Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured.
Results: Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis media in the 18 month-old infants (adjusted for confounders).
Conclusions: Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases at age in 18 months.

1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are end metabolites of perfluorinated chemicals (PFCs). PFOS and PFOA are persistent organic pollutants and are widely used in consumer products (e.g., surface-active agents, flame retardants, adhesives, and pesticides). PFOS and PFOA have recently been found to be widespread contaminants in the environment, wildlife, and humans (Lau et al., 2007). The half-life of PFOS in humans is 3.8 years, and that of PFOA is 5.4 years (Olsen et al., 2007). Possible health effects of PFOS and PFOA exposure in humans are a concern because of bioaccumulation and persistence in the environment, in animals, and in humans. It has been reported that PFC concentrations in human blood

Abbreviations: ATS—DLD, American Thoracic Society—Division of Lung Diseases; BMI, body mass index; CI, confidence interval; IgA, immunoglobulin A; ISAAC, International Study of Asthma and Allergies in Childhood; ND, non-detectable; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; PFC, perfluorinated chemical; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate

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Ethics approval: This study was conducted with written informed consent from all patients and was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

* Corresponding author. Fax: +81 11 706 4725.
E-mail address: rkishi@med.hokudai.ac.jp (R. Kishi).

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increase with age (Harada et al., 2004, 2007). The main sources of human exposure are drinking water (Hölzer et al., 2008), foods such as red meat and animal fat (Halldorsson et al., 2008), contamination from food packaging (Begley et al., 2005; Tittlemier et al., 2007), and indoor dust (Björklund et al., 2009; Shoeib et al., 2005).

PFOS and PFOA pass the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Some studies have reported a negative association between prenatal PFOS and PFOA exposure and birth weight (Apelberg et al., 2007; Fei et al., 2007). We previously observed a strong positive correlation between PFOS concentrations in maternal blood and cord blood (Inoue et al., 2004a), and a negative correlation between relatively low levels of maternal blood PFOS concentration and birth weight among female infants (Washino et al., 2009).

The prevalence of allergies has recently increased in children (Zöllner et al., 2005). The main environmental risk factors associated with asthma and other wheezy disorders are genetics, viruses, bacteria, tobacco exposure, and allergic sensitization, as determined by long-term studies (Bisgaard and Bønnelykke, 2010). Certain oxidants, airborne particulate matter, diesel exhaust particles, and polycyclic aromatic hydrocarbons have also been suggested as risk factors for asthma (Ho, 2010). It has been suggested that susceptibility to the effects of environmental chemicals may be higher during prenatal development when many physiological systems are developing, including the immune system (Luster et al., 2003). In an epidemiological study, the Danish National Birth Cohort showed that prenatal exposure to PFOA or PFOS was not associated with risk of hospitalization for infectious diseases in early childhood (Fei et al., 2010). In the C8 Health Project that investigated residents in the vicinity of a PFOA plant, it was shown that immunoglobulin (Ig) A and C reactive protein levels had a significant decreasing trend with increasing PFOA levels in blood samples; this pattern was also indicated for IgE, but only in females (Fletcher et al., 2009). Effects of PFOS and PFOA exposure on immunity and allergy in laboratory animals include immunosuppression, suppression of IgM antibody production (Dewitt et al., 2008; Keil et al., 2008; Peden-Adams et al., 2007), and increased total IgE response in ovalbumin-sensitized mice (Fairley et al., 2007). However, reports that have examined effects of PFC on immunity and allergy in a prospective study are few. It is therefore necessary to epidemiologically evaluate the effects of PFC exposure on immunity and allergy in humans, and to determine relationships between PFC exposure and immunity and allergy in humans.

The aim of this study was to ascertain possible relationships between maternal PFOS and PFOA levels and allergies and infectious diseases in their infants during the first 18 months of life using a prospective cohort study. IgE concentrations in cord blood were also evaluated.

2. Materials and methods

2.1. Study population

This prospective birth cohort study was based on infants delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan, which is an obstetrics and gynecology hospital (Hokkaido Study on Environment and Children's Health). Details regarding the study population, data collection, and the content of the questionnaires have been previously described (Kishi et al., 2011). In brief, between July 2002 and October 2005, participants were native Japanese women who enrolled at 23–35 weeks of gestation and were residents of Sapporo City or surrounding areas. Of 1796 women asked to participate, 514 agreed (participation rate of 28.6%). Among the 514 women, 10 were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before follow-up. Thirteen women were excluded due to death of the infant, relocation, or voluntary withdrawal for the follow-up period from delivery to 18 months.

2.2. Data collection

Participants completed a self-administered questionnaire during the second trimester of pregnancy. The questionnaire included information related to previous medical history, educational level, household income, smoking status, alcohol intake, caffeine intake, and food intake frequency during pregnancy. Medical information including maternal age, maternal height, maternal pre-pregnancy weight, pregnancy complications, parity, gestational age, infant gender, birth weight, and birth length were obtained from medical records. At 18 months post-delivery, participants completed another self-administered questionnaire. Of the 491 women to whom the questionnaire was mailed, 390 responded (recovery rate of 79.4%). The questionnaire included information related to breast-feeding, infant weight and length at 18 months, smoking status of parents, environmental tobacco smoke exposure at 18 months, pets in the home, living environment, day care attendance at 18 months, infant vaccination, and previous or current medical history of infant allergies and infectious diseases (food allergy, eczema, asthma, febrile convulsion, respiratory syncytial virus (RSV) disease, otitis media, and other diseases). For this study, all participating women provided written informed consent, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.3. Assessment of infant allergies and infections

Infant allergies and infectious diseases that developed during the first 18 months of life were assessed based on mothers' self-administered questionnaire at 18 months post-delivery. Food allergy was defined as a positive response to the following question: "Has your child ever had symptoms such as hives, swelling of the lip, emesis, diarrhea, or respiratory distress when they ate food allergens including milk, egg rice gruel, egg-drop, shrimp, or other foods?" Eczema was defined using a modified part of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (ISAAC Steering Committee, 1998). The part contained six questions: "(1) Has your child ever had an eczema in the past? If yes; (2) Has your child ever had an itchy rash, which was coming and going for at least 6 months? If yes; (3) Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?; (4) Has your child ever had dry skin in the past?; (5) Has your child ever had a doctor's diagnosis or diagnostic possibility for an eczema in the past?; (6) Does an itchy rash at any time affect any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes at present?" Wheezing was defined using a modified part of the Japanese version of the American Thoracic Society—Division of Lung Diseases (ATS—DLD) questionnaire (Nishima et al., 2009). The part contained five questions: "(1) Has your child ever had an attack of wheezing and/or shortness of breath in the past?; (2) Has your child ever had twice or more attacks in the past?; (3) Has your child ever had a doctor's diagnosis possibility for a bronchial asthma, asthmatic, or pediatric asthma in the past?; (4) Could wheezing be heard during an attack?; (5) Has your child ever had shortness of breath and wheezing during an attack?" To estimate the proportion of allergies or infectious diseases, we defined an outcome based on the following criteria: if infants had a positive response to the following medical question: "Has your child ever had a doctor's diagnosis, hospitalization, or medical treatment for the following diseases: asthma, eczema, other allergic diseases, otitis media, febrile convulsion, RSV diseases or other diseases, including chicken pox, bronchitis, rhinitis, pneumonia, skin infection and other viral infections?"

2.4. Measurement of PFOS and PFOA concentrations in maternal serum

Detailed sampling and laboratory methods for measuring PFOS and PFOA have been previously described (Washino et al., 2009). In brief, a 40 mL blood sample was taken from the maternal peripheral vein after the second trimester of pregnancy. When this was not possible because of anemia of the mother, a blood sample was taken after delivery. All samples were stored at −80 °C until analysis. The analytical detection method used was a variation of a method published previously (Nakata et al., 2005a, 2005b), and the methods for developing this variation of the analytical approach have also been described elsewhere (Inoue et al., 2004a, 2004b). Human serum samples (0.1 mL) were mixed with 0.2 mL internal standard solution containing acetonitrile, centrifuged at 1450g for 10 min, and the supernatant was transferred to a polypropylene tube. An aliquot of the filtered sample solution was subjected to column-switching liquid chromatography–tandem mass spectrometry. As a result of loss to follow-up, lack of serum specimen, and laboratory capacity, the concentrations of PFOS and PFOA were measured in 447 maternal serum samples. For participants with a concentration below the detection limit, a value equal to half of the detection limit was used for calculation purposes.

To examine possible effects of other environmental chemicals as confounding factors, we measured the concentrations of 7 polychlorinated dibenzo-*p*-dioxins

(PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-*ortho*-polychlorinated biphenyl (PCB) congeners, 8 mono-*ortho*-PCB congeners, and 58 non-dioxin-like PCB congeners in maternal blood (n=426) using high-resolution gas chromatography/high resolution mass spectrometry at the Fukuoka Institute of Health and Environmental Sciences (Todaka et al., 2007, 2008).

2.5. Measurement of IgE in cord serum

At the time of delivery, a blood sample (10–30 mL) was collected from the umbilical cord. All samples were stored at −80 °C until analysis. Concentrations of total IgE in cord serum were measured using an enzyme-linked immunosorbent assay (IMx® analyzer, ABBOTT JAPAN CO., LTD., Tokyo, Japan). IgE concentrations were measured in 268 cord serum samples at SRL, Inc. (Tokyo, Japan).

2.6. Statistical analysis

First, we analyzed a possible association between maternal PFOS and PFOA levels and cord blood IgE levels. For analysis of correlations between cord blood IgE levels and characteristics of mothers and infants, we used the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test. Because data did not fall into a normal distribution, PFOS, PFOA, and IgE concentrations were converted to a $\log_{10}$ scale. We calculated the residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels. Polynomial regression analysis was performed as the residual of potential confounding variables for dependent variables and $\log_{10}$-transformed maternal PFOS or PFOA levels for the independent variable, because the cubic polynomial regression model was better fitted than the linear regression model. Confounding variables were selected based on covariates that influenced cord blood IgE levels in univariate analyses and possible risk factors reported in previous studies, and also based on the change in estimate criterion. Variables considered in the analysis are: maternal age, maternal allergic history, infant gender, birth season, distance from home to highway, and sampling period. Parity was introduced into the final models because maternal PFOS and PFOA concentrations varied significantly primiparous and multiparous. Deep sea fish intake during pregnancy was also considered because IgE levels varied significantly, but since the results did not change, the variable was not introduced into the final models. For the fully adjusted model, polynomial regression analysis was applied adjusted for maternal age, maternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, birth season, distance from home to highway (<100 m or ≥ 100 m), and blood sampling period (during pregnancy/after delivery). We further performed stratified analysis by infant gender.

Second, we analyzed possible associations between maternal PFOS and PFOA concentrations and the development of infant allergies and infectious diseases during the first 18 months of life. For analysis of correlations between maternal PFOS and PFOA concentrations and characteristics of parents and infants, the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test were used. For analysis of correlations between allergies and infectious diseases during the first 18 months of life and characteristics of parents and infants, we used the Student's *t*-test and the Chi-square test. To assess risk factors or protective factors for infant illnesses, the characteristics of parents and infants were introduced as explanatory variables in binominal logistic regression analyses. Crude and adjusted logistic regression analyses were performed to evaluate associations between PFOS and PFOA concentrations and the risk of allergies and infections among all infants, male infants, and female infants. In logistic models, we evaluated odds ratios (ORs) for the risk of allergies and infection with $\log_{10}$-transformed maternal PFOS and PFOA levels. Multivariate analyses were adjusted for confounding variables that influenced allergies or infections in univariate analyses, possible risk factors reported in previous studies, and the sampling period. The fully adjusted model used logistic regression analysis of allergic disease adjusted for maternal age, maternal educational level (≤ 9 years, 10–12 years, 13–16 years, and ≥ 17 years), pre-pregnancy body mass index (BMI), maternal allergic history (yes/no), paternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, breast-feeding period (<4 months or ≥ 4 months), environmental tobacco smoke exposure at 18 months (yes/no), day care attendance at 18 months (yes/no), and blood sampling period (during pregnancy/after delivery) and logistic regression analysis of infectious disease adjusted for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco smoke exposure at 18 months, day care attendance at 18 months, and blood sampling period. Results were considered statistically significant when $p<0.05$.

3. Results

Concentrations of PFOS and PFOA (ng/mL) in maternal serum (n=343) and total IgE (IU/mL) in cord serum (n=231) were measured (Table 1). Detection limits for both PFOS and PFOA concentrations were 0.5 ng/mL. PFOS was detected in all samples, and PFOA was below the detection limit in 22 samples (6.4%); PFOS concentrations ranged from 1.3 to 16.2 ng/mL and the median value was 5.2 ng/mL. PFOA concentrations ranged from below the detection limit to 5.3 ng/mL and the median value was 1.3 ng/mL. The detection limit of IgE concentration was 0.05 IU/mL, and 39 samples (16.9%) were below the detection limit. Concentrations ranged from below the detection limit to 10.9 IU/mL and the median value was 0.21 IU/mL.

Possible associations between maternal serum PFOS and PFOA concentrations and various characteristics of parents and infants (n=343) were measured (Table 2). Univariate analyses indicated that increasing age of the mother was significantly associated with lower PFOS and PFOA concentrations. Concentrations in serum from multiparous women were significantly lower than from primipara women and concentrations in samples taken after delivery were significantly lower than in those taken during pregnancy.

Cord blood IgE concentrations were also measured in relation to several characteristics of the parents and infants (n=231). Statistically significant differences in IgE levels by maternal allergic history, infant gender, and deep sea fish intake during pregnancy ($p<0.05$) were observed (data not shown).

Infant allergies and infectious diseases during the first 18 months of life (n=343) were also determined (Table 3). The numbers of infants who developed allergies or infections up to age 18 months were as follows: food allergy, 57 (16.6%); eczema, 37 (10.8%); wheezing, 33 (9.6%); otitis media, 61 (17.8%); chicken pox, 16 (4.7%); bronchitis, 9 (2.6%); RSV diseases, 7 (2.0%); rhinitis, 6 (1.7%); pneumonia, 6 (1.7%); skin infection, 5 (1.5%); other virus infections (rotavirus, adenovirus, and cytomegalovirus), 15 (4.4%). Thus, we did not include chicken pox, bronchitis, RSV diseases, rhinitis, pneumonia, skin infection, and other viral infections in subsequent analyses because the numbers of cases of infection were very low except for otitis media, and sufficient statistical power could not be ensured in the multivariate analysis. Possible associations between characteristics of participants and infant allergies and infectious diseases were analyzed. There was no significant difference between allergies and infectious diseases with regard to gender.

We observed statistically significant differences ($p<0.05$) for eczema by paternal allergic history, for wheezing by paternal

Table 1
Concentrations of PFOS and PFOA in maternal serum (n=343) and concentrations of IgE in cord serum (n=231).

	Detection limit	ND[a], no. (%)	Mean	Minimum	25th	Median	75th	Maximum	Geometric mean
Maternal serum PFOS (ng/mL)[b]	0.5	0 (0)	5.6	1.3	3.4	5.2	7.2	16.2	5.0
Maternal serum PFOA (ng/mL)[c]	0.5	22 (6.4)	1.4	ND	0.8	1.3	1.7	5.3	1.2
Cord serum IgE (IU/mL)[d]	0.05	39 (16.9)	0.62	ND	0.08	0.21	0.58	10.9	0.22

[a] ND: not detected.
[b] PFOS: perfluorooctane sulfonate.
[c] PFOA: perfluorooctanoate.
[d] IgE: immunoglobulin E.

Table 2
Maternal PFOS and PFOA concentrations in relation to characteristics of parents and infants (n=343).

	No.	(%)	PFOS (ng/ml)[a]		PFOA (ng/ml)[b]	
			Median	(25th–75th)	Median	(25th–75th)
Parental characteristics						
Maternal age (years)	31.3 ± 4.4[c]		r=−0.149[e]		r=−0.114[e]	
Maternal pre-pregnancy BMI (kg/m^2)[d]	21.2 ± 3.5[c]		r=−0.077[e]		r=−0.053[e]	
Annual household income (million yen)[f]						
<5	224	(65.3)	5.2	(3.4–7.1)	1.3	(0.8–1.7)
≥5	118	(34.4)	5.5	(3.3–7.2)	1.3	(0.9–1.8)
Maternal educational level (years)						
≤9	4	(1.1)	6.7	(3.4–8.2)	0.75	(0.3–1.1)
10–12	139	(40.5)	4.8	(3.3–6.4)	1.3	(0.8–1.8)
13–16	195	(56.9)	5.6	(3.5–7.6)	1.3	(0.8–1.7)
≥17	5	(1.5)	3.0	(2.8–6.4)	0.8	(0.4–1.5)
Maternal smoking status during pregnancy						
Nonsmoker	292	(85.1)	5.3	(3.5–7.4)	1.3	(0.8–1.8)
Smoker	51	(14.9)	4.6	(2.8–6.6)	1.2	(0.9–1.6)
Parity[f]						
Primiparous	163	(47.5)	5.7	(3.9–8.0)	1.5	(1.2–2.2)
Multiparous	179	(52.2)	4.8	(3.0–6.7)	0.9	(0.6–1.4)
Blood sampling period						
During pregnancy	246	(71.7)	5.6	(4.1–7.6)	1.4	(0.9–1.8)
After delivery	97	(28.3)	3.6	(2.5–6.1)	1.1	(0.7–1.6)
Maternal allergic history						
No	251	(73.2)	5.3	(3.3–7.4)	1.3	(0.8–1.7)
Yes	92	(26.8)	4.6	(3.4–6.6)	1.3	(0.8–1.8)
Paternal allergic history						
No	280	(81.6)	–	–	–	–
Yes	63	(18.4)	–	–	–	–
Infant characteristics						
Gender						
Male	169	(49.3)	–	–	–	–
Female	174	(50.7)	–	–	–	–
Birth season						
Spring (March–May)	103	(30.0)	–	–	–	–
Summer (June–August)	70	(20.4)	–	–	–	–
Autumn (September–November)	70	(20.4)	–	–	–	–
Winter (December–February)	100	(29.2)	–	–	–	–
Breast-feeding period (months)						
<4	70	(16.5)	–	–	–	–
≥4	273	(83.5)	–	–	–	–
Environmental tobacco smoke exposure at 18 months						
No	210	(61.0)	–	–	–	–
Yes	133	(39.0)	–	–	–	–
Day care attendance at 18 months[f]						
No	269	(78.4)	–	–	–	–
Yes	72	(21.0)	–	–	–	–
Distance from home to highway[f]						
<100 m	183	(53.3)	–	–	–	–
≥100 m	159	(46.4)	–	–	–	–

[a] PFOS: perfluorooctane sulfonate.
[b] PFOA: perfluorooctanoate.
[c] Mean ± SD.
[d] BMI: body mass index.
[e] r: Spearman's correlation coefficient.
[f] Missing data; annual household income (1), parity (1), day care attendance at 18 months (2), distance from home to highway (1).

allergic history, pre-pregnancy BMI, and day care attendance, and for otitis media by parity and by day care attendance (data not shown).

Next, possible associations between maternal serum PFOS and PFOA concentrations and immune system parameters were examined. Two samples with IgA levels >10 mg/dL were considered to be contaminated by maternal blood and were excluded. Ultimately, 231 mother-infant pairs were included in the analysis, for whom PFOS, PFOA, and IgE had been measured. Table 4 shows the results of cubic polynomial regression analysis between $\log_{10}$-transformed maternal serum PFOS or PFOA level and residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels (n=231). In analyses stratified by infant gender, cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, no significant associations were observed between maternal PFOS or PFOA level and cord blood IgE levels among male infants.

Fig. 1 shows scatterplots and the linear regression model, and the cubic polynomial regression model for the $\log_{10}$-transformed