Table3. Cord serum IgE concentration in relation to characteristics (n=231).

		IgE (IU/mL)	
	No. (%)	Median (25^{th}-75^{th})	p-value
Maternal age (years)[a]	30.2±4.8[d]	r= -0.099	0.13
Maternal pre-pregnancy BMI (kg/m^2)[a]	20.9±3.1[d]	r= 0.008	0.91
Maternal educational level[b]			
≤ 9	8 (3.5)	0.26 (0.095-0.73)	0.78
10-12	97 (42.0)	0.19 (0.06-0.57)	
13-16	122 (52.8)	0.245 (0.09-0.643)	
≥ 17	4 (1.7)	0.28 (0.183-0.43)	
Maternal smoking status during pregnancy[c]			
Nonsmoker	181 (78.4)	0.23 (0.09-0.59)	0.62
Smoker	50 (21.6)	0.195 (0.06-0.485)	
Parity[c]			
0	120 (51.9)	0.225 (0.08-0.65)	0.62
≥ 1	111 (48.1)	0.20 (0.08-0.57)	
Maternal allergic history[c]			
No	168 (72.7)	0.19 (0.06-0.488)	0.002 **
Yes	63 (27.3)	0.30 (0.17-0.86)	
Paternal allergic history[c]			
No	191 (82.7)	0.21 (0.08-0.58)	0.80
Yes	40 (17.3)	0.21 (0.105-0.618)	
Inshore fish intake during pregnancy[c]			
< 1time/week	122 (52.8)	0.195 (0.09-0.43)	0.23
≥ 1time/week	109 (47.2)	0.26 (0.065-0.865)	
Deep sea fish intake during pregnancy[c]			
< 1time/week	102 (44.2)	0.185 (0.068-0.39)	0.026 *
≥ 1time/week	129 (55.8)	0.26 (0.095-0.845)	
Distance of highway to home[c]			
< 100 m	107 (46.5)	0.25 (0.10-0.86)	0.065
≥ 100 m	123 (53.5)	0.19 (0.07-0.43)	
Gestational age (weeks)[a]	39.3±1.1[d]	r=0.048	0.47
Infant sex[c]			
Male	103 (44.6)	0.30 (0.11-0.92)	0.007 **
Female	128 (55.4)	0.19 (0.063-0.42)	
Birth season[b]			
Spring	80 (34.6)	0.195 (0.09-0.553)	0,22
Summer	68 (29.4)	0.175 (0.034-0.368)	
Autumn	36 (15.6)	0.325 (0.12-0.828)	
Winter	47 (20.3)	0.26 (0.07-0.60)	

[a] Spearman's correlation test, [b] Kruskal-Wallis test, and [c] Mann-Whitney *U*-test. [d] Mean±SD.
Statistically significant, * p<0.05, ** p<0.01.

Table4. Infant allergic and infection disease during the first 18 months of life (n=343).

	No. (%)
Allergic Symptoms	
Food allergy	57 (16.6)
Eczema	37 (10.8)
Asthma	33 (9.6)
Infection	
Otitis media	61 (17.8)
Chicken pox	16 (4.7)
Bronchitis	9 (2.6)
RSV disease[a]	7 (2.0)
Rhinitis	6 (1.7)
Pneumonia	6 (1.7)
Skin infection	5 (1.5)
Other viral infections[b]	15 (4.4)

[a] RSV ; respiratory syncytial virus disease.

[b] rotavirus, rotavirus, adenovirus and cytomegalovirus.

Table5. Regression coefficients between maternal blood PFOS and PFOA levels (ng/mL) and cord blood IgE levels (IU/mL).

	Overall (n=231)			Male (n=103)			Female (n=128)		
	Beta[b]	(95%CI)	p-value	Beta[b]	(95%CI)	p-value	Beta[b]	(95%CI)	p-value
log_{10}PFOS[a]	-0.085	(-0.644, 0.158)	0.233	-0.076	(-0.923, 0.466)	0.516	-0.098	(-0.783, 0.254)	0.315
log_{10}PFOA[a]	0.045	(-0.225, 0.432)	0.535	0.026	(-0.562, 0.694)	0.836	0.074	(-0.25, 0.558)	0.452

[a] Multiple regression model adjusted for maternal age, maternal allergic history, intake of deep sea fish during pregnancy, distance of highway to home, infant sex, parity, birth season and blood sampling period.

[b] Beta coefficients represent the change in IgE levels for a 10-fold increase PFOS or PFOA levels.

Table6. Adjusted odds ratio (95% CI) for infant allergic and infection disease during the first 18 months of life according to maternal PFOS levels in quartiles.

PFOS (ng/mL)	1.3-3.4		3.5-5.2			5.3-7.2			7.2-16.2			p-for trend
	No. (%)		No. (%)	OR	(95%CI)	No. (%)	OR	(95%CI)	No. (%)	OR	(95%CI)	
Overall (n=343)												
Food allergy[a]	12 (14.3)	Reference	8 (9.8)	0.60	(0.22, 1.65)	23 (25.6)	2.21	(0.97, 5.04)	14 (16.1)	1.22	(0.48, 3.10)	0.17
Eczema[a]	7 (8.3)	Reference	12 (14.6)	2.54	(0.87, 7.43)	11 (12.2)	1.32	(0.45, 3.92)	7 (8.0)	0.97	(0.29, 3.26)	0.64
Asthma[a]	8 (9.5)	Reference	5 (6.1)	0.70	(0.20, 2.46)	12 (13.3)	1.38	(0.47, 4.01)	8 (9.2)	1.20	(0.37, 3.84)	0.53
Otitis media[b]	16 (19.0)	Reference	12 (14.6)	1.05	(0.42, 2.62)	17 (18.9)	1.27	(0.54, 3.01)	16 (18.4)	1.49	(0.60, 3.73)	0.34
Male (n=169)												
Food allergy[a]	7 (17.9)	Reference	5 (11.6)	0.59	(0.15, 2.41)	11 (23.9)	1.55	(0.47, 5.18)	7 (17.1)	1.04	(0.27, 3.96)	0.54
Eczema[a]	3 (7.7)	Reference	10 (23.3)	5.96	(1.24, 28.68)	3 (6.5)	1.10	(0.18, 6.78)	4 (9.8)	1.39	(0.23, 8.46)	0.50
Asthma[a]	3 (7.7)	Reference	2 (4.7)	0.62	(0.08, 4.55)	6 (13.0)	2.08	(0.40, 10.94)	5 (12.2)	2.48	(0.45, 13.68)	0.13
Otitis media[b]	9 (23.1)	Reference	8 (18.6)	1.42	(0.39, 5.18)	10 (21.7)	1.51	(0.43, 5.36)	9 (22.0)	1.59	(0.42, 6.03)	0.51
Female (n=174)												
Food allergy[a]	5 (11.1)	Reference	3 (7.7)	0.53	(0.11, 2.55)	12 (27.3)	3.31	(0.96, 11.43)	7 (15.2)	1.47	(0.38, 5.67)	0.17
Eczema[a]	4 (8.9)	Reference	2 (5.1)	0.43	(0.06, 3.02)	8 (18.2)	1.58	(0.35, 7.12)	3 (6.5)	0.68	(0.12, 3.83)	0.98
Asthma[a]	5 (11.1)	Reference	3 (7.7)	0.57	(0.08, 3.97)	6 (13.6)	0.86	(0.17, 4.43)	3 (6.5)	0.63	(0.10, 3.74)	0.69
Otitis media[b]	7 (15.6)	Reference	4 (10.3)	0.79	(0.20, 3.17)	7 (15.9)	1.35	(0.39, 4.70)	7 (15.2)	1.35	(0.37, 4.98)	0.51

[a] Logistic regression model adjusted for maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant sex, breast-feeding period, environmental tobacco smoke exposure, day care attendance and blood sampling period.

[b] Logistic regression model adjusted for maternal age, maternal educational level, parity, infant sex, breast-feeding period, environmental tobacco smoke exposure, day care attendance and blood sampling period.

Table6. Adjusted odds ratio (95% CI) for infant allergic and infection disease during the first 18 months of life according to maternal PFOA levels in quartiles.

PFOA (ng/mL)	ND-0.8		0.9-1.3			1.4-1.7			1.8-5.3			p-for
	No. (%)		No. (%)	OR	(95%CI)	No. (%)	OR	(95%CI)	No. (%)	OR	(95%CI)	trend
Overall (n=343)												
Food allergy[a]	11 (14.9)	Reference	17 (19.1)	1.54	(0.65, 3.68)	14 (16.7)	1.44	(0.57, 3.65)	15 (15.6)	1.31	(0.51, 3.34)	0.69
Eczema[a]	7 (9.5)	Reference	11 (12.4)	1.10	(0.37, 3.28)	9 (10.7)	1.44	(0.45, 4.57)	10 (10.4)	1.07	(0.34, 3.37)	0.86
Asthma[a]	6 (8.1)	Reference	12 (13.5)	1.74	(0.56, 5.39)	8 (9.5)	1.84	(0.53, 6.45)	7 (7.3)	1.24	(0.35, 4.42)	0.83
Otitis media[b]	13 (17.6)	Reference	18 (20.2)	1.36	(0.56, 3.32)	11 (13.1)	1.11	(0.41, 2.97)	19 (19.8)	2.09	(0.82, 5.32)	0.16
Male (n=169)												
Food allergy[a]	5 (13.9)	Reference	12 (30.0)	2.83	(0.77, 10.39)	8 (19.0)	1.45	(0.35, 5.99)	5 (9.8)	0.76	(0.17, 3.51)	0.35
Eczema[a]	3 (8.3)	Reference	5 (12.5)	1.26	(0.24, 6.60)	7 (16.7)	2.45	(0.47, 12.74)	5 (9.8)	0.96	(0.18, 5.28)	0.98
Asthma[a]	1 (2.8)	Reference	5 (12.5)	4.40	(0.43, 45.22)	8 (19.0)	12.78	1.25, 130.73)	2 (3.9)	1.51	(0.11, 19.98)	0.82
Otitis media[b]	8 (22.2)	Reference	7 (17.5)	0.54	(0.14, 2.14)	7 (16.7)	1.25	(0.33, 4.77)	14 (27.5)	1.98	(0.57, 6.85)	0.14
Female (n=174)												
Food allergy[a]	6 (15.8)	Reference	5 (10.2)	0.62	(0.16, 2.38)	6 (14.3)	1.17	(0.31, 4.35)	10 (22.2)	2.12	(0.59, 7.59)	0.16
Eczema[a]	4 (10.5)	Reference	6 (12.2)	0.59	(0.12, 2.96)	2 (4.8)	0.39	(0.04, 3.44)	5 (11.1)	1.38	(0.27, 6.93)	0.68
Asthma[a]	5 (13.2)	Reference	7 (14.3)	1.21	(0.22, 6.76)	0 (0.0)	-	-	5 (11.1)	3.21	(0.47, 21.83)	0.43
Otitis media[b]	5 (13.2)	Reference	11 (22.4)	2.66	(0.73, 9.71)	4 (9.5)	1.05	(0.23, 4.90)	5 (11.1)	1.70	(0.37, 7.83)	0.88

[a] Logistic regression model adjusted for maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant sex, breast-feeding period, environmental tobacco smoke exposure, day care attendance and blood sampling period.

[b] Logistic regression model adjusted for maternal age, maternal educational level, parity, infant sex, breast-feeding period, environmental tobacco smoke exposure, day care attendance and blood sampling period.

平成 20～22 年度厚生労働科学研究費補助金（化学物質リスク研究事業）
分担研究報告書

母体血中のダイオキシン類(PCDDs, PCDFs)濃度と *CYP1B1* 遺伝子多型が出生体重におよぼす影響の検討

研究代表者 岸 玲子 北海道大学環境健康科学研究教育センター センター長・特任教授
研究分担者 梶原 淳睦 福岡県保健環境研究所 保健科学部生活化学課 課長

研究要旨

ダイオキシン類(PCDDs, PCDFs)は胎児発育に負の影響を与えることが示唆されているが，まだ一致した結果は得られていないことから，曝露濃度の違いや遺伝的感受性がその要因として考えられる。本研究では，低用量曝露の妊婦を対象として母体血中ダイオキシン類濃度とその代謝に関与する遺伝子多型が出生時体重におよぼす影響を検討した。喫煙妊婦のダイオキシン類濃度が High 群で *CYP1B1* 遺伝子(C>G, Leu 432 Val)が CG/GG 型では，ダイオキシン類濃度が Low 群で *CYP1B1* 遺伝子が CC 型に比べて，出生時体重が減少する傾向が認められた。非喫煙妊婦ではこのような関連がみられなかったことから，喫煙妊婦における出生体重の低下には母体血中ダイオキシン類濃度と *CYP1B1* 遺伝子多型が関与している可能性が示唆された。

研究協力者

小林 澄貴，佐々木 成子，湯浅 資之，吉岡 英治，小西 香苗，鷲野 考揚，宮下 ちひろ，岡田 恵美子
(北海道大学大学院医学研究科 予防医学講座公衆衛生学分野)
吉村 健清，中川 礼子，平川 博仙，堀 就英，芦塚 由紀，村田 さつき，松枝 隆彦，飛石 和大，安武 大輔
(福岡県保健環境研究所)

A．研究目的

ダイオキシン類は，胎児発育に負の影響を与えることが示唆されているが[1-3]，母体血の血清中 PCBs 濃度と出生時体重との間に関連は認められなかった報告もあり[4]，まだ一致した結果は得られていない。この理由として，曝露濃度の違いや遺伝的感受性などの要因が考えられる。

ダイオキシン類曝露と，これらの代謝に関わる Cytochrome P450 1A1(*CYP1A1*)遺伝子と *CYP1B1* 遺伝子の多型と子宮内膜症発生との関連が報告されているが[5]，胎児発育にどのように影響を与えるのか検討した報告はこれまで全くない。本研究では母体血中ダイオキシン類濃度と代謝に関与する遺伝子多型が出生体重におよぼす影響について検討した。

B．研究方法

札幌市内 1 産科病院外来を受診し同意を得た妊娠23～35週の妊婦を対象に，前向きコホート研究を実施した。対象者の基本的属性は自記式調査票，出生時所見は病院記録から得た。双胎，妊娠高血圧症候群，糖尿病および胎児心不全は除外して 421 名を解析した。福岡県保健環境研究所で母体血中ダイオキシン類濃度(PCDDs 7 種，PCDFs 10 種，non-ortho PCBs 4 種，mono-ortho PCBs 8 種の合計 29 種)を異性体別に高分解能ガスクロマトグラフ・高分解能マススペクトロメーター(HRGC/HRMS)で測定した。ダイオキシン類の代謝に関与する遺伝子として，

CYP1A1 遺伝子(A>G, Ile462Val)および *CYP1B1* 遺伝子(C>G, Leu432Val)の多型をリアルタイム-PCR 法で，*CYP1A1* 遺伝子(T>C, MspⅠ)をPCR-RFLP 法で解析した。出生時体重をアウトカムとして，ダイオキシン類濃度は 3 分位にし，中・高の分位群(High)と低分位群(Low)の2群と遺伝子多型を組み合わせて検討した。児の性別，在胎週数，世帯収入，出産経歴，母の喫煙の有無，母の非妊娠時 BMI，採血時期，近海魚摂取，遠洋魚摂取，妊娠中カフェイン摂取量で調整後，重回帰分析を行った。統計解析には SPSS 15.0J を使用した。

（倫理面への配慮）

北海道大学大学院医学研究科医の倫理委員会および研究協力施設の研究倫理委員会に諮り，承認を得たうえで実施した。

C．研究結果

在胎週数(p<0.001)，母の身長(p=0.013)，母の妊娠前体重(p=0.020)，児の性別(p=0.007)で出生時体重との関連がみられた(表 1)。

母体血中のダイオキシン類濃度の total TEQ 値(98)は先行研究で報告されている濃度と比較して低かった[6](表 2)。

妊娠中の喫煙の有無および遺伝子多型によるダイオキシン類濃度を検討した。喫煙妊婦は非喫煙妊婦と比べるとダイオキシン類濃度が低い傾向が認められた。(表 3)。

次に妊娠中の喫煙状況で分類し，ダイオキシン類濃度と *CYP1B1* 遺伝子多型を組み合わせて検討した。妊娠中の喫煙状況を考慮しない場合，ダイオキシン類濃度や *CYP1B1* 遺伝子多型の違いによる出生時体重低下には有意な差は認められなかった。しかし，非喫煙妊婦と喫煙妊婦で比較すると喫煙妊婦ではダイオキシン類濃度(PCDDs，PCDFs および PCDDs/Fs)が High 群で *CYP1B1* 遺伝子が CG/GG 型では，ダイオキシン類濃度が Low 群で *CYP1B1* 遺伝子が CC 型に比べて，出生時体重が減少する傾向が認められた。非喫煙妊婦ではこのような関連はみられなかった(図 1-3)。

D．考察

母乳中ダイオキシン類濃度は非喫煙者よりも喫煙者で低いという報告があり[7]，本研究の結果と一致している。喫煙者では化学物質の代謝能が亢進している可能性があるため，喫煙妊婦のダイオキシン類曝露量は今回の測定値よりも高いことが考えられる。

ダイオキシン類は胎盤に蓄積することが報告されていることから[8]，ダイオキシン類やその代謝物が胎盤へ移行することにより，胎盤機能に障害を与えて胎児発育を阻害し，出生体重が低下すること考えられるが，喫煙妊婦においては，母親の *CYP1B1* 遺伝子多型がその影響を修飾することが示唆された。

E．結論

喫煙妊婦のダイオキシン類濃度が High 群で *CYP1B1* 遺伝子が CG/GG 型では，ダイオキシン類濃度が Low 群で *CYP1B1* 遺伝子が CC 型に比べて，出生時体重が減少する傾向が認められた。非喫煙妊婦ではこのような関連がみられなかったことから，喫煙妊婦における出生時体重の低下には母体血中ダイオキシン類濃度と *CYP1B1* 遺伝子多型が関与している可能性が示唆された。

F．研究発表

1.論文発表

なし

2.学会発表

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G．知的財産権の出願・登録状況

該当なし

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●表1. 母児の属性と出生時体重との関連

		(n=421)			出生時体重(g)		
			Mean±SD	Median(Min.-Max.)	Mean±SD	r	p value
在胎週数(週)			38.9±0.1			0.487	<0.001***[a]
母の妊娠年齢(年)			30.8±4.7			-0.058	0.237[a]
母の身長(cm)			158.2±0.3			0.121	0.013*[a]
母の妊娠前体重(kg)			53.2±0.4			0.113	0.020*[a]
母の妊娠前BMI(kg/m²)			21.2±0.2			0.070	0.149[a]
出生時体重(g)			3065.0±18.6				
妊娠中カフェイン推定摂取量(g/day)				117.3(1.5-646.3)		-0.093	0.057[a]
		No.	%	Median(Min.-Max.)			
妊娠中アルコール摂取	あり	128	30.4		3047.1±398.6		
	なし	293	69.6		3105.6±339.2		0.148[b]
妊娠中飲酒者アルコール推定摂取量(g/day)				1.2(0.3-51.8)		-0.029	0.743[a]
		No.	%		Mean±SD		p value
児の性別	男	201	47.7		3116.8±392.9		
	女	220	52.3		3017.4±366.4		0.007**[b]
出産経歴(回)	0	204	48.5		3045.3±380.1		
	1以上	217	52.5		3083.2±383.9		0.309[b]
妊娠中喫煙	妊娠初期までにやめた	349	82.8		3070.1±393.6		
	妊娠中期以降も喫煙した	72	17.2		3039.5±322.0		0.537[b]
教育レベル(年)	12年以下	177	42.0		3048.7±391.9		
	13年以上	244	58.0		3076.6±375.2		0.459[b]
世帯収入(万円)	500万円未満	277	65.8		3080.2±375.7		
	500万円以上	144	34.2		3035.4±393.8		0.255[b]
近海魚摂取	週1回未満	230	54.6		3086.4±352.8		
	週1回以上	191	45.4		3039.0±414.1		0.205[b]
遠洋魚摂取	週1回未満	194	46.1		3067.8±387.5		
	週1回以上	227	53.9		3062.4±378.3		0.885[b]
採血時期	出産前採血	293	69.6		3071.9±389.2		
	出産後採血	128	30.4		3048.7±366.2		0.568[b]
CYP1B1 (C>G, Leu432Val)	CC	317	75.3		3070.9±392.7		
	CG	95	22.6		3036.3±350.7		
	GG	9	2.1		3154.0±327.2		0.579[c]
	CG/GG	104	24.7				
CYP1A1(T>C, *Msp I*)	TT	176	41.8		3086.2±338.9		
	TC	201	47.7		3028.6±404.0		
	CC	44	10.5		3145.3±429.6		0.116[c]
	TC/CC	245	58.2				
CYP1A1(A>G, Ile462Val)	AA	253	60.1		3057.4±373.1		
	AG	150	35.6		3082.2±376.8		
	GG	18	4.3		3025.1±542.5		0.743[c]
	AG/GG	168	39.9				

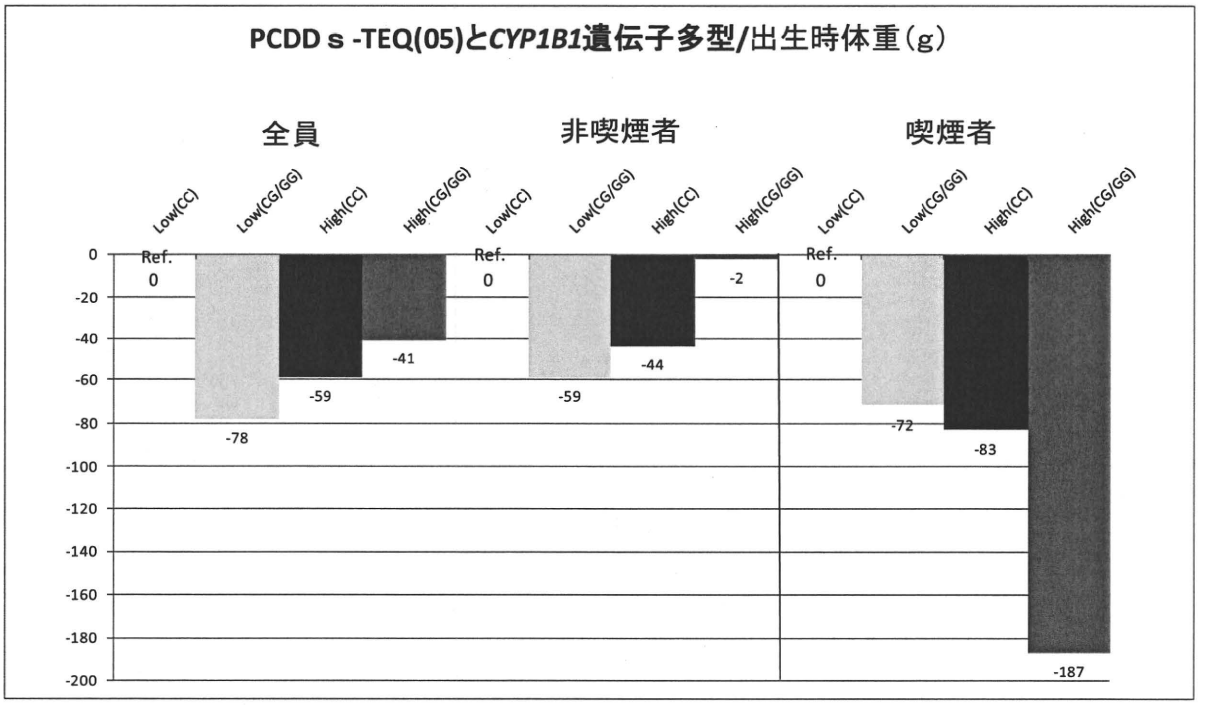
[a]Pearson's correlation test, [b]Student's t-test, and [c]ANOVA: *p<0.05, **p<0.01, ***p<0.001

●表2. 母体のダイオキシン類・PCB類血中濃度

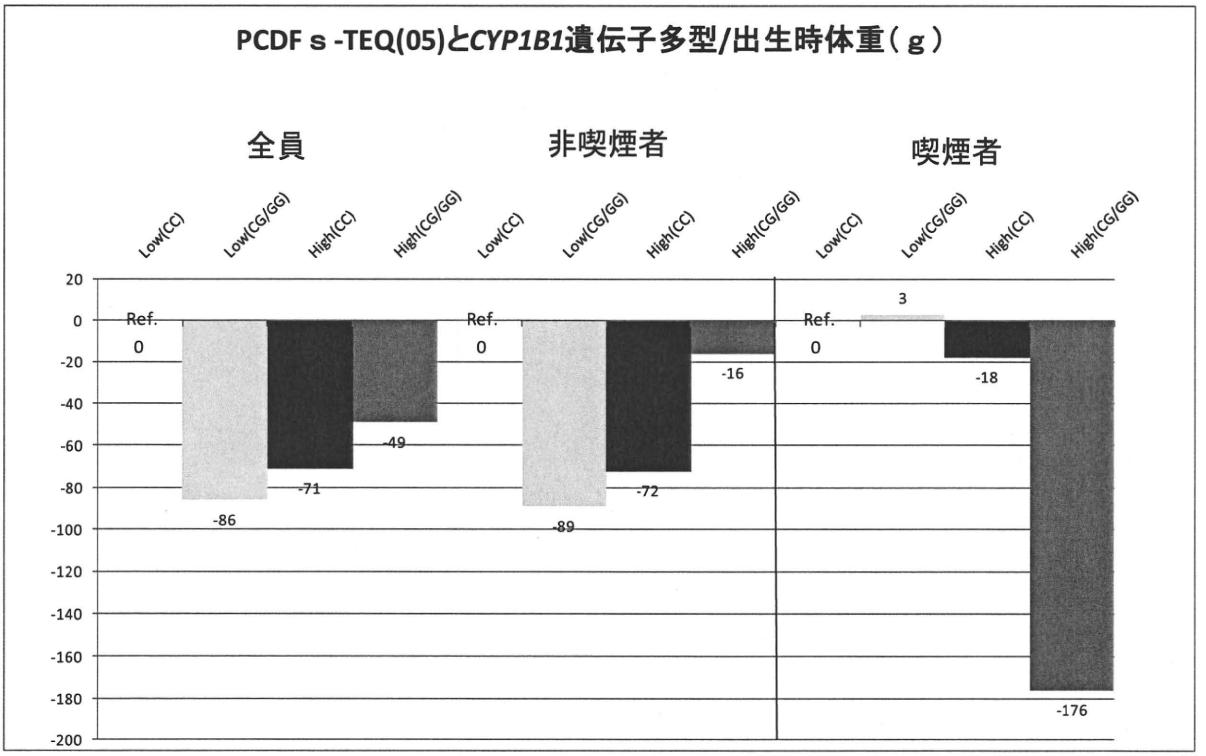
(n=421)	Mean±SD	Minimum	Median	Maximum	Geometric mean
<Total> (pg/g lipid)					
Total PCDDs	507.1±10.7	92.7	457.3	1602.4	467.6
Total PCDFs	20.2±0.6	9.5	18.1	192.4	18.8
Total PCDD/Fs	527.4±11.0	103.2	476.3	1637.5	487.5
Total non-ortho PCBs	81.0±2.1	20.0	75.4	553.6	72.6
Total mono-ortho PCBs	12472.9±321.4	1724.3	11302.3	49632.0	10919.7
Total coplanar PCBs	12553.9±323.0	1744.3	11375.8	49813.4	10993.2
Total Dioxins	13081.3±327.7	1847.6	11876.7	50477.5	11539.1
<WHO-98> (TEQ pg/g lipid)					
Total PCDDs-TEQ(98)	7.2±0.2	1.6	6.7	29.2	6.6
Total PCDFs-TEQ(98)	3.7±0.1	0.8	3.5	11.8	3.4
Total PCDD/Fs-TEQ(98)	11.0±0.2	2.5	10.3	36.8	10.0
Total non-ortho PCBs-TEQ(98)	4.1±0.1	0.6	3.7	22.3	3.4
Total mono-ortho PCBs-TEQ(98)	2.4±0.1	0.3	2.2	10.1	2.1
Total coplanar PCBs-TEQ(98)	6.5±0.2	0.9	6.0	26.4	5.6
Total Dioxins-TEQ(98)	17.4±0.4	3.4	16.5	51.2	15.9
<WHO-05> (TEQ pg/g lipid)					
Total PCDDs-TEQ(05)	7.3±0.2	1.7	6.8	29.3	6.7
Total PCDFs-TEQ(05)	2.6±0.1	0.6	2.4	7.8	2.4
Total PCDD/Fs-TEQ(05)	9.9±0.2	2.5	9.2	34.4	9.1
Total non-ortho PCBs-TEQ(05)	4.6±0.1	0.7	4.2	23.2	3.9
Total mono-ortho PCBs-TEQ(05)	0.4±0.0	0.1	0.3	1.5	0.3
Total coplanar PCBs-TEQ(05)	4.9±0.1	0.7	4.6	23.9	4.2
Total Dioxins-TEQ(05)	14.8±0.3	3.2	13.9	42.9	13.5

●表3. 喫煙の有無、*CYP1A1*、*CYP1B1*遺伝子多型によるダイオキシン類濃度

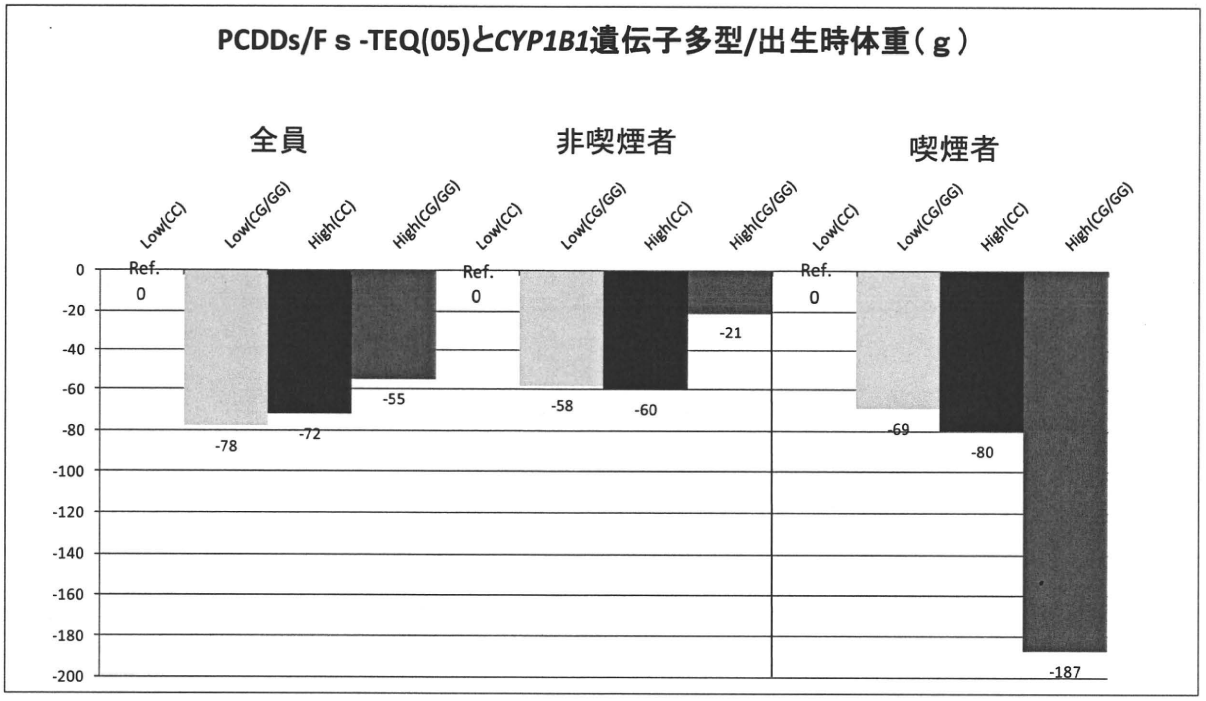
		No.	Total PCDDs-TEQ(05) Mean±SD (TEQ pg/g lipid)	Median	Total PCDFs-TEQ(05) Mean±SD (TEQ pg/g lipid)	Median	Total PCDD/Fs-TEQ(05) Mean±SD (TEQ pg/g lipid)	Median
妊娠中喫煙	妊娠初期までにやめた	349	7.38±3.04	7.00	2.58±1.06	2.48	9.96±4.00	9.56
	妊娠中期以降も喫煙した	79	6.99±4.02	6.16	2.45±1.15	2.12	9.43±5.04	8.36
CYP1B1 (C>G, Leu432Val)	CC	317	7.31±3.10	6.83	2.57±1.10	2.35	9.88±4.10	9.14
	CG/GG	104	7.31±3.61	6.93	2.55±1.01	2.58	9.85±4.46	9.57
CYP1A1(T>C, *Msp I*)	TT	176	7.59±3.39	6.95	2.64±1.01	2.41	10.23±4.30	9.42
	TC/CC	245	7.11±3.09	6.75	2.51±1.12	2.36	9.61±4.10	9.04
CYP1A1(A>G, Ile462Val)	AA	253	7.28±3.28	6.72	2.57±1.06	2.36	9.86±4.22	9.14
	AG/GG	168	7.35±3.15	7.05	2.55±1.11	2.49	9.90±4.15	9.37



●図 1. PCDDs-TEQ 値と *CYP1B1* 遺伝子多型の組合せによる出生時体重の変化



●図 2. PCDFs-TEQ 値と *CYP1B1* 遺伝子多型の組合せによる出生時体重の変化



●図 3. PCDDs/Fs-TEQ 値と *CYP1B1* 遺伝子多型の組合せによる出生時体重の変化

平成 20～22 年度厚生労働科学研究費補助金（化学物質リスク研究事業）
分担研究報告書

出生時体重に影響を及ぼす妊婦の喫煙と葉酸代謝酵素遺伝子多型との関連

(Effects of Maternal 5,10-Methylenetetrahydrofolate Reductase C677T or A1298C Polymorphisms and Tobacco smoking on Infant Birthweight in a Japanese Population)

研究代表者 岸 玲子 北海道大学環境健康科学研究教育センター センター長・特任教授
研究分担者 吉岡 英治 北海道大学大学院医学研究科予防医学講座公衆衛生学分野 助教

研究要旨

Folate is very essential for fetal growth and development. Having known that 5,10-methylenetetrahyrdofolate reductase (MTHFR) 677TT homozygosity and tobacco smoking are associated with low folate status, we investigated whether maternal smoking in the presence of 5,10-*MTHFR* C677T and A1298C polymorphisms may adversely reduce offspring's birthweight.

Results showed that maternal 5,10-*MTHFR* 1298AA was associated with low folate status. Smokers with 5,10-*MTHFR* 1298AA genotypes had reduced mean infant birthweight by 107g (95%CI, -180 to -34, p = 0.004) and the reduction was more in male infants by 117g (95%CI, -218 to -15, p = 0.025).

We concluded that maternal 5,10-*MTHFR* 1298AA may be associated with low folate status and more reduction in infant birthweight among tobacco smokers, especially in male infants.

研究協力者

Yila Thamar，金澤 文子，坂 晋，鷲野 考揚，小西 香苗，馬場 俊明，宮下 ちひろ，Braimoh Titilola，樫野 いく子，岡田 恵美子，小林 澄貴，大竹 裕子，伊藤 久美子，Mariko Limpar
(北海道大学大学院医学研究科予防医学講座公衆衛生学分野)

A．研究目的

Maternal smoking in pregnancy is a well established risk factor for preterm delivery (PTD), intrauterine growth restriction (IUGR), low birthweight (LBW), small for gestational age (SGA) and other adverse pregnancy outcomes. More recently, smoking has been associated with nutritional deficiencies including folate[1].

Intracellular folate hemostasis depends on 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene which is located on chromosome 1p36. The 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. So far, fourteen rare mutations in *MTHFR* have been described and these are associated with decrease in *MTHFR* enzyme activities. The two most common single nucleotide polymorphisms (SNPs) identified are 5,10-*MTHFR* C677T (dbSNP ID: rs1801133), a missense mutation in exon 4, characterised by alanine to valine substitution on codon 222 and 5,10-*MTHFR* A1298C (dbSNP ID: rs1801131), a point mutation in exon

7, characterised by a glutamate to alanine substitution on codon 429[2].

Biochemically, 5,10-*MTHFR* C677T polymorphism is associated with thermolability and reduced enzyme activity. The metabolic consequences are relative folate deficiency and mild hyperhomocysteinemia, a risk factor for thrombotic vascular diseases. Several studies have identified maternal 5,10-*MTHFR* C677T polymorphisms as obstetric genetic risk factors for spina bifida, placenta-related vasculopathies, spontaneous fetal loss, PTD, LBW, SGA, neurodevelopmental delays and other congenital anomalies[3]. However, several other investigators have reported that no such associations exist[4]. Such confusing body of literature may be explained by the fact that phenotypic expression of this genetic trait is dependent on folate-related nutritional status and other environmental factors which do vary across geographical and ethnic diversities.

Although the 5,10-*MTHFR* A1298C variant is poorly characterised in terms of functional consequences, so far, it has been recognised as an additional risk factor for neural tube defects.

In Japan, only a few folate-related genetic association studies in relation studies in relation to obstetric events have been documented, and none considered infant birthsize. We therefore hypothesized that maternal smoking in the presence of 5,10-*MTHFR* C677T and/or A1298C polymorphisms may adversely reduce offspring's birthweight.

B．研究方法

Participants were 1784 native Japanese mother-child pairs recruited between February 2003 to March 2006 from the ongoing "Hokkaido study on Environment and Children's Health". Data were acquired from baseline self-administered questionnaires, infants' hospital birth records and post–partum self-administered questionnaires. In addition, first trimester whole blood specimens were obtained for serum folate assays. Folate concentrations were assayed using an automated competitive protein binding (CPB) chemiluminescent enzyme immunoassay (CLEIA) technique by SRL, Inc. Tokyo, Japan. DNA amplifications were performed in batches of 96-well micro-amp reaction plates using validated Taqman® probes for *MTHFR* C677T (Assay ID: C_1202883_20) and A1298C (Assay ID: C_ 850486_20) on a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). End-point allelic discrimination (AD) assay was carried out on the 7300/7500 Real time PCR System and the fluorescence was read on a sequence detection software (SDS) version 1.2.1. (Applied Biosystems, Foster City, CA, USA).

Overall smoking status was classified using both self-reported active tobacco smoking and passive exposure to environmental tobacco

smoke (ETS) at home into three categories. Non-smokers were those who neither actively smoked nor passively inhaled at home. Non-smokers and quitters with passive exposure were classified as the passive smoking group, while smokers consisted of active smokers irrespective of ETS exposure status. Non-exposed quitters during the first trimester had mean infants' birthweights similar to nonsmokers hence added to nonsmoking group while quitters during the second and third trimesters were added to the active smoking group. 5,10-*MTHFR* C677T and A1298C genotypes were categorized based on AD assay's allele calling as dominant homozygous, heterozygous and recessive homozygous genotypes (677CC, 677CT and 677TT; 1298AA, 1298AC and 1298CC) respectively. These were abbreviated as CC, CT and TT; AA, AC and CC genotypes respectively in the tables.

Statistical tests included univariate ANOVA with multiple comparison tests to explore main effects of maternal 5,10-*MTHFR* C677T and A1298C polymorphisms and smoking on serum folate levels and ANCOVA for interactive association between smoking and 5,10-*MTHFR* C677T and A1298C polymorphisms in relation to folate and infant birthweight. In the final regression analyses, known major predictors of infant birthweight were adjusted for (infant gender, gestational age at delivery, maternal age, maternal pre-pregnancy weight, maternal height, parity, and alcohol intake during pregnancy). In the preliminary analysis, folic acid supplement use highly positively correlated with serum folate levels, therefore, we included it as a covariate. Sub-groups with less than a 15-member sample size were excluded. *MTHFR* 677CC and 1298AC were the reference categories in the regression because the groups represented those with the desirable high folate status. Assessments of *MTHFR* C677T and A1298C genotypes for deviation from Hardy-Weinberg's equilibrium and other data quality check parameters were generated from Haploview version 4.2 Software. All other analyses were performed using SPSS version 16.00 for WINDOWS (SPSS Inc., Chicago, IL., USA). Level of statistical significance was set at < 0.05.

（倫理面への配慮）

This study was conducted with the informed consent of all subjects. The study protocol was approved by the institutional ethical board for human gene and genome studies of the Hokkaido University Center for Environmental and Health Sciences and the Hokkaido University Graduate School of Medicine.

C．研究結果

Maternal mean serum folate was 16.4 ±1.5 nmol/L and mean infant birthweight was 3040±374 g. Prevalence of active smoking in pregnancy was 15.9% while that of passive smoking was 53.0%.

Prevalence of folate deficiency (<6.8 nmol/L) status was very low (0.3%). The distributions of 5,10-*MTHFR* C677T and A1298C genotypes did not deviate from Hardy–Weinberg equilibrium (p=0.546 and 0.909) respectively. Frequencies of *MTHFR* 677CC, 677CT and 677TT were 37.3%, 46.7% and 16.0% respectively while that of *MTHFR* 1298AA, 1298AC and 1298CC were 62.7%, 33.1% and 4.2% respectively. A strong linkage disequilibrium (D'=0.943) between MTHFR C677T and A1298C was also observed and minor allele frequencies were 0.392 and 0.205 respectively (Table 1). These were similar to previous documentations from Japanese population.

Carrying the T allele was associated with a decrease in mean serum folate levels with the lowest score (14.1nmol/L) among the 677TT homozygous group. Tukey's honestly significant differences of 1.0nmol/L (p=0.008) and 3.8nmol/L (p <0.0005) were observed between 677CC versus 677CT and between 677CC versus 677TT respectively. On the other hand, carrying the 1298C allele was associated with increase in mean serum folate levels. Tukey's honestly significant difference of 1.5nmol/L (p<0.0005) was observed between 1298AA versus 1298AC. Mean serum folate scores were generally lower in smokers across 5,10-*MTHFR* C677T genotypes with the lowest among 677TT homozygotes (11.8nmol/L, p for interaction =0.031). The lowest mean folate scores were identified among smokers with 1298AA homozygous genotypes, p for interaction = 0.046 (Figures 1, and 2).

To investigate whether the presence of maternal 5,10-*MTHFR* C677T and A1298C polymorphisms could be relevant in the determination of offspring's birthweight, we conducted a multiple regression analysis with adjustments for major known predictors of birthweight. For 5,10-*MTHFR* C677T, 677CT heterozygous genotype was observed to be associated with an increase in mean infant birthweight by 36.4g, (95%CI, 2.6 to 70.3,
p=0.035). Polymorphism in 5,10-*MTHFR* A1298C showed no independent evidence of statistical significance in relation to offspring's birthweight. Infants of active tobacco smokers were 85.0g (95% CI, 133.3 to -36.8, p=0.001), lower than those born to nonsmokers (Table 2).

Further interactive analyses with tobacco smoking status, 5,10-MTHFR 677CT genotypes had male newborns with higher mean birthweight of 90.7g (95%CI, 6.0 to 175.5, p=0.036) (Table 3). 5,10-MTHFR 1298AA genotype was generally associated with decrease in mean infant birthweight in smokers by 107.0g (95%CI, -180.0 to -33.9, p=0.004). After stratification by infant gender, the effect was more pronounced in male infants with a reduction of 117.0g (95%CI, -218.6 to -14.7, p=0.025) (Table 4). In both cases, female infants never demonstrated any statistically significant changes in their mean birthweight.

D．考察

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

5,10-*MTHFR* 677T allele was associated with low folate status, which agrees with other earlier reports[5] . 677TT homozygosity was associated with low folate status and these values were much lower among active and passive smokers. This observation revealed the independent effects exerted by tobacco smoke and 5,10-*MTHFR* C677T polymorphism on folate status. On the contrary, 5,10-*MTHFR* 1298AA genotype was found to be associated with low serum folate status. Although this SNP has not been fully characterized in terms of its metabolic and clinical impact, various investigators are recently exploring it. In a recent study conducted among Koreans, it was observed that mean plasma homocysteine value was highest among 1298AA homozygotes compared to those who carried 1298C allele[6]. Since serum folate is known to have an inverse correlation with plasma homocysteine, we inferred that our study population might have had similar patterns of plasma homocysteine distribution across genotypes. Another report from Portugal noted that 1298AC heterozygosity was associated with high plasma folate level with the lowest among the 1298CC homozygotes. That we had similar findings with a report from a Korean population sounds genetically plausible because racial, geographic and nutritional disparities may account for the differences in functional behaviors of 5,10-*MTHFR* SNPs.

2. Effects of maternal 5,10-MTHFR A1298C polymorphism and tobacco smoke on infant birthweight

The birthweight reducing effect of maternal 5,10-*MTHFR* 1298AA genotype among tobacco smokers can be related to materno-fetal folate nutrition. Maternal 1298CC was observed to be associated with reduction in the risk of low birthweight than the 1298AA genotype[7]. 1298CC homozygous genotype was also reported to be protective against offspring's IUGR in Canadians[8]. In our study, we could not demonstrate the role of 1298CC genotype because of the low frequency in the population. Having observed the association between 5,10-*MTHFR* 1298AA genotype and lower serum folate status, additional chronic extracellular and intracellular folate deficits consequent to effects of tobacco smoke might have been severe enough to inflict nutritional stress. The consequence of hyperhomocysteinemia might have increased the risk of placental vasculopathies via oxidative stress, endothelial cell dysfunctions and coagulopathies leading to fetoplacental hypoperfusion. Among smokers, there might have been some form of nutritional deficiency including vitamin B_{12}, as a result, the methylation of homocysteine to methionine was further compromised

with a sequel of impaired fetal growth. The minimal folate levels needed to silence the phenotypic expression of 1298AA among smokers was probably not met. Higher exogenous folate may be needed to correct deficits and maintain optimal levels for optimal fetal growth. Owing to the scarcity of literature about 5,10-*MTHFR* A1298C polymorphism especially among Japanese, further research is needed to verify this observation and uncover more biologic mechanisms associated with this SNP.

3. Effects of maternal 5,10-MTHFR C677T polymorphism and tobacco smoke on infant birthweight

Although 5,10-MTHFR 677T allele is known to be associated with low folate and high homocysteine status, we observed that carrying the 677CT genotype was protective against male offspring's birthweight reduction only in the absence of active or passive tobacco smoke. Such observation may be related to the fact that nonsmokers had higher serum folate status. Adequate folate status is said to silence the negative phenotypic expression of 5,10-*MTHFR* 677T allele. This observation underscores the need for smokers carrying the 677T allele modify their smoking lifestyle in order to benefit from the genetic trait.

4. Gender-specific response to MTHFR activities

Depending on the smoking status, only the male infants were more responsive to the gene-environment interplays eitherway. Nutritional needs are inherently higher for males than female fetuses, thus in a background of hyponutrinemia, the male fetus may be worst affected. Alhough the male fetus naturally thrives better under favourable conditions, it may not have withstood additional severe folate deficit among smokers with 1298AA genotype. Poorly understood phenomena on fetal gender-specific signals have been implicated in fetal growth especially in response to glucocorticoid activity which may modify fetal response to stress[9]

Japanese population genetics of 5,10-MTHFR A1298C polymorphism shows that 1298AA genotype frequency is as high as >60.0%. The discovery of its association with low folate status may constitute a great public health burden especially in materno-fetal health.The recent threat of westernization on traditional dietary habits, deliberate dieting among young women to maintain low body mass indices and most importantly the increase in the annual prevalence of tobacco smoking among young Japanese women may adversely interact with this genetic susceptibility leading to increased morbidity and mortality. Healthy lifestyle modification like smoking cessation and encouragement towards targetted use of folic acid supplements could be very important tools of public health interest for this population especially the vulnerable smokers with genetic susceptibility.

E．結論

Maternal 5,10-*MTHFR* 1298AA genotype may be associated with folate impairment. 5,10-*MTHFR* 677CT may be protective in non-smokers while 1298AA may interact with tobacco smoke to decrease offspring's birthweight. Male infants seemed to be more responsive to 5,10-*MTHFR* activities.

F．研究発表

1.論文発表

None

2.学会発表

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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]
Pre-pregnancy 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 birthweight (g)	3040 (374)[e]
Infant gender	
Males	873 (48.9)
Females	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 supplement use	
Nonuser	1601 (89.7)
User	183 (10.3)
Maternal *MTHFR* C677T genotypes[a, b]	
CC	666 (37.3)
CT	833 (46.7)
TT	285 (16.0)
CT / TT	1118 (62.7)
Maternal *MTHFR* A1298C genotypes[c,d]	
AA	1118 (62.7)
AC	591(33.1)
CC	75 (4.2)
AC / CC	666 (37.3)

ETS = Environmental tobacco smoke, [e] Mean (SD)
MTHFR = Methylenetetrahydrofolate reductase gene
[a] HWE = Hardy-Weingberg's Equillibrium p value = 0.5463
[b] MAF = Minor allele frequency = 0.392
[c] HWE = Hardy-Weingberg's Equillibrium p value = 0.9091
[d] MAF = Minor allele frequency = 0.205