表4. マーカー奇形の発生頻度（2014年1月現在）

JAOG:日本産婦人科医医会先天異常モニタリング 2006-2010年度比較（ICBDSR. Annual Report 2012.）

項目			全分娩 n=18,333		在胎22週以降の分娩 n=18,166		JAOG
			数	北海道スタディ（出産1万対）	数	北海道スタディ（出産1万対）	2006-2010年（出産1万対）
頭部	A1	無脳症	4	2.2	0	-	0.80
	A2	脳瘤	0	-	0	-	5.46
	A3	小頭症	1	0.5	1	0.6	1.76
	A4	水頭症	2	1.1	2	1.1	7.55
	A5	全前脳胞症	2	1.1	2	1.1	1.20
眼部	B1	眼瞼欠損	0	-	0	-	0.40
	B2	小眼球症・無眼球症	0	-	0	-	0.68
	B3	白内障	0	-	0	-	
耳部	C1	小耳症	2	1.1	2	1.1	1.83
	C2	外耳道閉鎖	2	1.1	2	1.1	
	C3	埋没耳	3	1.6	3	1.7	
	C4	耳介低位	6	3.3	4	2.2	
口顔部	D1	口唇裂	9	4.9	9	5.0	
	D2	口蓋裂	11	6.0	11	6.1	5.20
	D3	口唇口蓋裂	16	8.7	16	8.8	
	D4	顔面裂	0	-	0	-	
	D5	先天性歯	1	0.5	1	0.6	
上肢	E1	多指症	15	8.2	15	8.3	
	E2	合指症	8	4.4	7	3.9	
	E3	裂手症	0	-	0	-	
	E4	上肢の減数異常	2	-	2	-	
	E5	上肢先天性絞扼輪症候群	0	-	0	-	
	E6	橈骨側の異常	0	-	0	-	
	E7	尺骨側の異常	0	-	0	-	
体幹	F1	脊髄髄膜瘤（二分脊椎）	3	1.6	2	1.1	5.46
	F2	臍帯ヘルニア	3	1.6	2	1.1	4.05
	F3	腹壁破裂	0	-	0	-	
	F4	その他の腹壁異常	10	5.5	10	5.5	
		横隔膜ヘルニア	6	3.3	6	3.3	6.14
		鼠径ヘルニア	4	2.2	4	2.2	
心臓	G1	先天性心疾患（病名不明含む）	95	51.8	95	52.3	

項目			全分娩 n=18,333		在胎22週以降の分娩 n=18,166		JAOG
			数	北海道スタディ（出産1万対）	数	北海道スタディ（出産1万対）	2006-2010年（出産1万対）
消化器	H1	食道閉鎖	2	1.1	2	1.1	4.49
	H2	直腸肛門奇形	5	2.7	5	2.8	
	H3	小腸閉鎖	5	2.7	5	2.8	7.41
	H4	十二指腸閉鎖	3	1.6	3	1.7	
泌尿器・生殖器	I1	水腎症	16	8.7	15	8.3	
	I2	異形成腎	2	1.1	1	0.6	
	I3	尿道下裂 ＊男児のみ 全9,194人/22週以降9,135人	8	8.7	8	8.8	4.78
	I4	停留精巣・非触知精巣 ＊男児のみ 全9,194人/22週以降9,135人	14	15.2	14	15.3	
	I5	膀胱外反症・ 総排泄腔外反症	1	0.5	1	0.6	0.26
	I6	陰核肥大	0	-	0	-	
	I7	性別不分明	1	0.5	1	0.6	
	I8	膣欠損	0	-	0	-	
下肢	J1	多趾症	9	4.9	9	5.0	
	J2	合趾症	6	3.3	4	2.2	
	J3	裂足症	1	0.5	0	-	
	J4	下肢の減数異常	0	-	0	-	
	J5	下肢先天性絞扼輪症候群	0	-	0	-	
皮膚	K1	6個以上または 巨大な色素異常斑	4	2.2	4	2.2	
	K2	継続する水疱・小水疱 ・びらん形成（先天性表皮水疱症）	2	1.1	2	1.1	
症候群・染色体異常	L1	Down症候群	20	10.9	18	9.9	12.02
	L2	軟骨無形成症	0	-	0	-	
	L3	Apert症候群	0	-	0	-	
	L4	先天性多発性関節拘縮症	0	-	0	-	
	L5	trisomy 18	3	1.6	2	1.1	9.39
	L6	trisomy 13	1	0.5	1	0.6	2.00
結合双生児	M1	結合双生児	0	-	0	-	

表5. 先天性心疾患の内訳 (2014年1月現在)
JAOG:日本産婦人科医医会先天異常モニタリング 2006-2010 年度比較
(ICBDSR. Annual Report 2012.)

	全分娩 n=18,333		在胎22週以降の分娩 n=18,100		JAOG
	数	北海道スタディ (出産1万対)	数	北海道スタディ (出産1万対)	2006-2010年 (出産1万対)
先天性心疾患全体	95	51.8	95	52.3	
心室中隔欠損症	35	19.1	35	19.3	
心房中隔欠損症	10	5.5	10	5.5	
肺動脈（弁）狭窄症	7	3.8	7	3.9	
ファロー四徴症	5	2.7	5	2.8	6.49
動脈管開存症	7	3.8	7	3.9	
大動脈縮窄症	5	2.7	5	2.8	5.98
肺動脈閉鎖症	2	1.1	2	1.1	
大血管転位症	4	2.2	4	2.2	4.19
単心室	1	0.5	1	0.6	
単心房単心室	2	1.1	2	1.1	
大動脈（弁）狭窄症	1	0.5	1	0.6	4.19
心内膜床欠損症	4	2.2	4	2.2	
左室低形成症	2	1.1	2	1.1	
右室低形成症	1	0.5	1	0.6	
両大血管右室起始	2	1.1	2	1.1	
右胸心	1	0.5	1	0.6	
総肺静脈還流異常症	1	0.5	1	0.6	
動脈管動脈瘤症	1	0.5	1	0.6	
三尖弁逆流症	1	0.5	1	0.6	
心室内結節	1	0.5	1	0.6	
大動脈逆流弁	1	0.5	1	0.6	
不明	29	15.8	29	16.0	

表6. その他の先天奇形の発生頻度（2014年1月現在）

項目			全出産 n=18,333	
			数	北海道スタディ（出産1万対）
頭部	1	無頭蓋骨	2	1.1
	2	脳室上衣下嚢胞	1	0.5
	3	小脳低形成	1	0.5
	4	透明中隔欠損、脳梁低形成	1	0.5
	5	頭蓋骨形成不全	1	0.5
	6	頭部陥没	1	0.5
	7	側脳室拡大（疑い）	1	0.5
顔頸部	8	眼球異常（網膜欠損ほか）	1	0.5
	9	耳瘻孔	2	1.1
	10	副耳	10	5.5
	11	鰓丘症候群	3	1.6
	12	声門狭窄	1	0.5
	13	耳介低形成	1	0.5
	14	耳介水平	1	0.5
	15	耳形状・位置の左右差	1	0.5
	16	先天性聴力障害（疑い）	2	1.1
	17	先天性真珠腫	1	0.5
皮膚	18	先天性頭部皮膚欠損	2	1.1
	19	先天性皮膚欠損症	1	0.5
	20	無爪症	1	0.5
	21	爪欠損	3	1.6
	22	胎児後頸部浮腫	2	1.1
	23	胸水貯留	1	0.5
	24	頭頂部に水腫瘍突起物	1	0.5
	25	手指腫瘍	1	0.5
	26	脂肪腫（背部）	1	0.5
	27	いぼ（側胸部）	1	0.5
	28	仙尾部奇形腫（疑い）	2	1.1
	29	前上腸骨棘付近の腫瘍	1	0.5
	30	腹部の腫瘤	1	0.5
	31	リンパ管腫（背部）	1	0.5
	32	リンパ管腫（頸部）	1	0.5
	33	外陰部脂肪腫（外陰腫瘤）	1	0.5
	34	嚢胞性ヒグローマ	3	1.6
	35	先天性魚鱗癬症	1	0.5
	36	血管腫	3	1.6
	37	母斑	1	0.5
	38	脂性（腺）母斑	1	0.5
	39	Skene腺のう症	1	0.5
	40	羊膜索症候群	1	0.5
手指	41	関節拘縮	1	0.5
	42	手指形態異常	1	0.5
	43	拇指屈指症	1	0.5

項目			全出産 n=18,333	
			数	北海道スタディ（出産1万対）
体幹	44	単一臍帯動脈	3	1.6
	45	肺低形成	1	0.5
	46	内臓逆位	1	0.5
	47	脊椎側弯	1	0.5
	48	脊髄髄膜瘤に伴う麻痺性の変形	1	0.5
	49	胎児水腫・胎児腹水	9	4.9
消化管	50	消化管穿孔	1	0.5
	51	下部消化管通過障害（疑い）	1	0.5
	52	胆のう拡張	1	0.5
	53	腸管重複症（疑い）	1	0.5
	54	ヒルシュスプルング病	1	0.5
泌尿器・生殖器	55	腎のう胞（疑い）	2	1.1
	56	多のう胞腎	1	0.5
	57	腎（腎盂）拡張	2	1.1
	58	腎盂拡大	1	0.5
	59	腎盂尿管移行部狭窄	1	0.5
	60	精索水瘤・陰嚢水腫	4	4.4
	61	陰茎短小	1	1.1
	62	外陰のう胞	1	1.1
	63	卵巣のう腫（疑い含む）	3	3.3
	64	尿膜管遺残症	3	1.6
	65	プルーンベリー症候群（疑い）	2	1.1
下肢	66	脊椎側弯	1	0.5
	67	大腿骨短縮	1	0.5
	68	内反足	3	1.6
	69	外反足	2	1.1
	70	四肢短縮	7	3.8
	71	先天性骨形成不全	1	0.5
	72	大腿骨、上腕骨が長い	1	0.5
	73	先天性下肢変形	1	0.5
	74	下肢低形成	3	1.6
	75	趾変形	1	0.5
	76	屈曲肢異形成症	1	0.5
染色体	77	染色体異常（46,XX,t(9;18)(q32;	1	0.5
	78	染色体異常（45X）	1	0.5
	79	染色体異常（47XYY）	2	1.1
	80	染色体異常（45X/47XXX）	1	0.5
	81	Camptomelic dysplasia	1	0.5
	82	ターナー症候群	1	0.5
	83	ピエールロバン症候群（疑い）	1	0.5
	84	レックリングハウゼン氏病	1	0.5
	85	筋ジストロフィー（ドュシャンヌ型）	1	0.5
	86	クラインフェルター症候群	1	0.5
	87	クルーゾン症候群	1	0.5
	88	胎児常染色体劣性多発性のう胞	1	0.5
	89	不明（疑い）	1	0.5
詳細不明	90		5	2.7

表7.　2歳までに新たに報告された先天異常数（主な疾患）

	0歳	1歳	2歳
心室中隔欠損症	35	46	8
心房中隔欠損症	10	17	1
肺動脈(弁)狭窄症	7	16	0
停留精巣・非触知精巣	14	35	13
尿道下裂	8	2	0

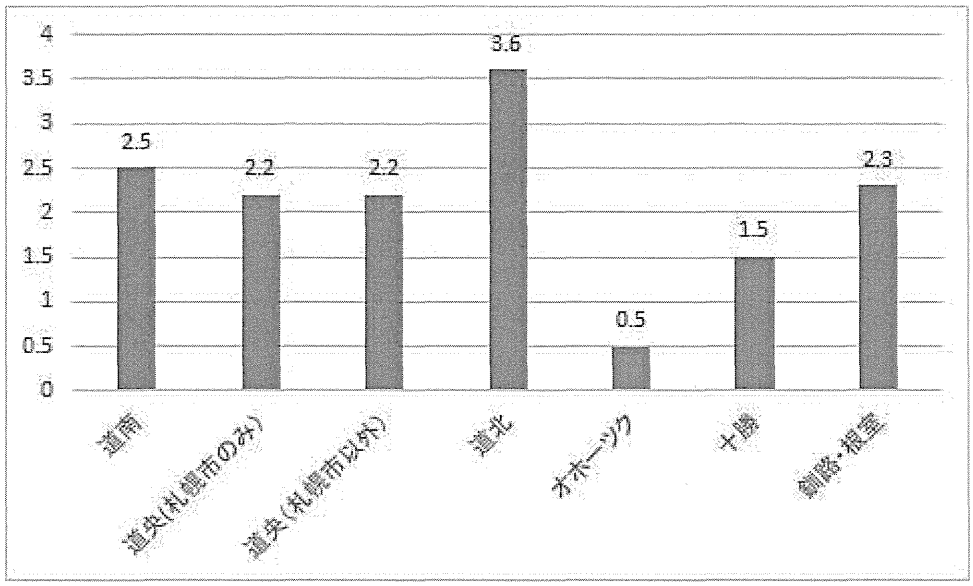

図3. 北海道医療圏別の出産報告件数による先天異常の発生頻度

血液中のビスフェノールA（BPA）フリー体の分析

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研究要旨
昨年度までに検討し、開発した血液中のビスフェノールA（BPA）の測定分析方法をもとにBPAのフリー体の分析方法を開発した。この方法を用い保存中の血液試料についてBPAフリー体の濃度を確認し、コンタミネーション(汚染)の状況を確認した。分析した試料からは、BPAは検出されなかったため、試料採取から保存までの操作における試料のコンタミネーションはなかったと考えられた。

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A．研究目的

ビスフェノールA（以降BPA）はポリカーボネート製のプラスチックを製造する際、モノマーや、エポキシ樹脂の原料として使用されている化学物質であり、摂取によりエストロゲン受容体が活性化され、エストロゲンに類似した生理作用を表すことが報告されている。低用量仮説の提唱によって注目を受けたが、ヒトに対する健康影響評価に関しては現在も諸説の報告例があり継続して研究が行われているところである。また、近年、BPAについては尿道下裂との関連性について幾つか報告がなされているが、血中濃度は極低濃度であり、試料間の有意な濃度差を観測するためには精確な測定値が必要と考えられる。

BPAの体内負荷量を評価するにはヒト血液中における濃度データが必要であるが、存在量は極低く、また、様々な化成品に含まれていることからBPAの分析においてはブランク値や試料採取から保存中におけるコンタミネーション(汚染)の低減、把握する事が必要となる。本研究ではヒト血液中のBPAを議論可能な濃度レベルで精確に測定できていることを確認するため、コンタミネーションの状況を把握する事を目的とした。

血液中に存在するBPAのほとんどがグルクロン酸と結合し、抱合体の形で存在することに着目し、BPAフリー体の測定方法について開発し、試料採取から保存におけるコンタミネーション状況の確認を行う事とした。

B．研究方法

これまでに血液中のBPAの測定を行うため同位体希釈-液体クロマトグラフ/タンデム型質量分析法（以降ID-LC-MS/MS）を開発し、ヒト血液試料（母体血及び臍帯血）に適用した。この方法は、酵素を用いてBPAグルクロン酸抱合体の脱抱合化を行い、血液中の総BPAを評価する方法であるが、今回は、脱抱合処理を行わず、BPAフリー体を測定する事で、血液試料のコンタミネーションの状況を確認した。

今回の測定に用いた分析フローを図 1に、LC-MS/MSの測定条件を表1に示す。

C．研究結果・考察

(1)　分析法の確認

純水、又は血液試料を用いBPAフリー体の分析法の確認を行った。純水と血液試料それぞれに 0.5ng/mL となるようにBPAを添加し、回収率の確認を行った。純水に添加した場合は、回収率 97%、血液試料に添加した場合は、回収率 101%で

あった。

(2) 操作ブランク値及び分析法の検出下限値（MDL：Method Detection Limit）

血液試料 20 検体（母体血 10 検体及び臍帯血 10 検体）の分析と操作ブランク試験を 5 回実施した。操作ブランク試験の結果及びそれらの結果より計算された MDL を表 2 に示す。操作ブランクの平均値は 0.036ng/mL であり、操作ブランク試験の結果より計算された MDL は 0.037ng/mL であった。これまでの総 BPA の分析法と同様に、操作ブランク及び MDL は、0.1ng/mL 未満であった。

(3) 血液中の BPA フリー体の分析

試料は、以前、BPA の分析を行い、濃度の確認の取れているものを用いた。ヒト血液試料 20 検体（母体血 10 検体及び臍帯血 10 検体）について BPA フリー体の測定分析を行った。結果を表 3 に示す。血液中の BPA フリー体の濃度は、すべての試料で ND であった。なお、表 3 における BPA 濃度は、操作ブランク値を差し引いた値で、ND は、操作ブランクの 5 回測定より求めた検出下限値（MDL）未満であることを示す。

(4) 回収率

検体の測定における各試料の回収率（クリーンアップスパイク内標準物質(BPA d-16)/シリンジスパイク内標準物質(BPA d-4)）の値を用い、回収率を計算した。結果を表 3 に示す。全試料において回収率は 66～112%の範囲であった。なお、本分析方法は内標準法であるので回収率の数値は結果に影響を与えない。

D. 結論

本研究で開発した分析方法によって血液中の BPA フリー体が評価可能な濃度レベルでデータ取得可能となった。過去の測定で BPA が検出された試料について BPA フリー体の測定を行い採血から保存中における BPA のコンタミネーションの状況の確認を行ったが、BPA のフリー体は検出されなかった。このため、本研究における BPA のコンタミネーションの影響は軽微であり、これまで報告を行った試料の分析値に与える影響は、ほとんどないものと考えられた。

E. 健康危険情報

該当なし

F. 研究発表

1) 論文発表

なし

2) 学会発表

なし

G. 知的財産権の出願・登録状況(予定を含む。)

1. 特許取得
   該当なし
2. 実用新案登録
   該当なし
3. その他
   該当なし

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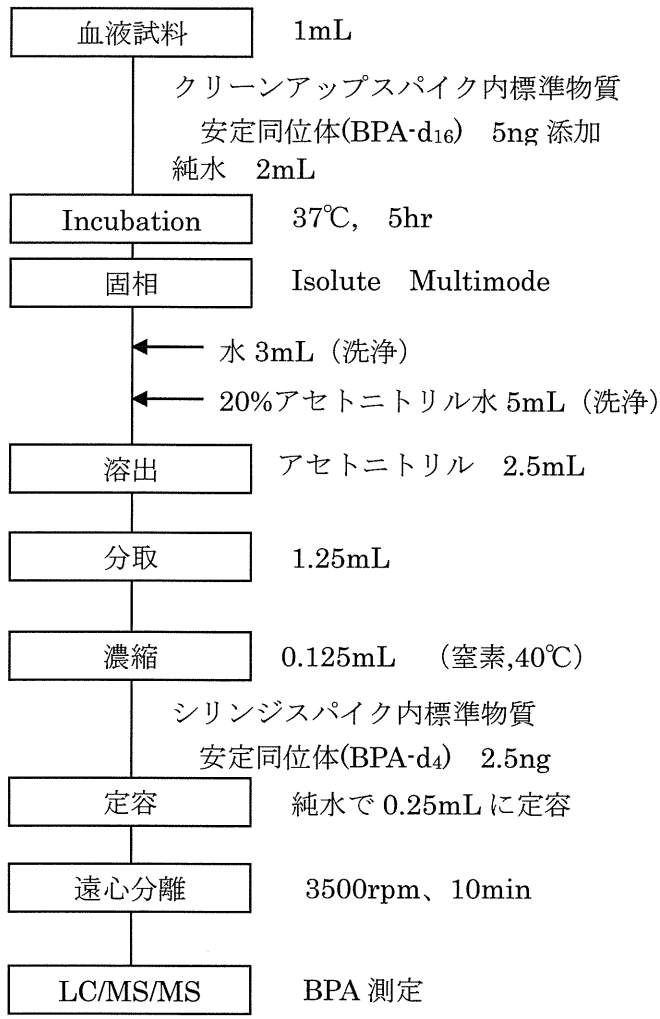

図 1. 血液中の BPA フリー体の分析フロー

表 1. BPA 分析における LC/MS/MS 測定条件

測定装置	LC：Agilent-1100 MS：API-4000 Q Trap
分析カラム	Waters ACQUITY UPLC BEH C18 2.1×50mm,1.7μm
溶離液	A：水 B：アセトニトリル
グラジエント(B)	20%(0min)→20%(1min)→60%(7min)→99%(7.1min)→99%(13min) →20%(13.1min)→20%(19min)
注入量	20μL
カラム温度	40℃
モード	ESI-Negative
m/z	227.0＞132.9(BPA) 241.0＞142.0(BPA-d_{16}) 231.0＞134.9(BPA-d_4)

表 2 操作ブランク試験の結果及び MDL.

試料名	BPA 濃度(ng/mL)
Blank 1	0.042
Blank 2	0.040
Blank 3	0.033
Blank 4	0.022
Blank 5	0.042
平均値	0.036
標準偏差	0.00865
t 値(危険率 5%、片側)	2.132
MDL	0.037

【注釈】
MDL= (標準偏差) × t 値 × 2

表 3 母体血及び臍帯血中の BPA フリー体の濃度及び各検体における回収率.

試料名		BPA 濃度	回収率
	SRL	(ng/mL)	(%)
臍帯血	124	ND	72
	234	ND	106
	241	ND	78
	198	ND	88
	289	ND	88
	252	ND	84
	283	ND	90
	196	ND	95
	227	ND	112
	265	ND	102
母体血	390	ND	85
	395	ND	106
	397	ND	76
	519	ND	66
	520	ND	86
	526	ND	81
	528	ND	87
	85	ND	70
	110	ND	92
	222	ND	86

【注釈】
BPA 濃度は、ブランク値を差し引いた値.
ブランク試験結果から求めた MDL(0.037ng/mL)未満の試料については、『ND』で表記.
回収率は、クリーンアップスパイク内標準物質(BPA d-16)の応答/シリンジスパイク内標準物質(BPA d-4)の応答を用い、(試料液)/(標準液の平均) × 100 で算出した値.

Prenatal exposure to bisphenol A and child neurodevelopment: The Hokkaido Study

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研究要旨

Background: Prenatal bisphenol A (BPA) exposure may affect early child thyroid function and neurodevelopment.

Objective: To evaluate the associations between cord blood BPA levels and child mental and psychomotor development at 6 and 18 months of age. Additionally the association with thyroid stimulation hormone (TSH) and free thyroxine (FT4) of newborn were assessed. **Methods:** Cord blood samples collected from the Hokkaido study participants were analyzed for BPA levels. Child neurodevelopment was assessed using mental and psychomotor development indexes (MDI and PDI) from a Bayley Scales of Infant Development II at 6 and 18 months of age (N = 121, 86, respectively). The associations between cord blood BPA levels and child neurodevelopment were estimated using linear regression models adjusted for potential confounders. Data of TSH and FT4 were obtained from mass screening test for endocrine disorders conducted by Sapporo City Institute of Public Health. **Results:** Overall, there were no statistical significant associations between cord blood BPA levels and child neurodevelopment at 6 and 18 months of age. Among female, MDI score at 6 month of age and the TSH levels was inversely associated with cord blood BPA levels with borderline significance. **Conclusion:** This study added the evidence that relatively lower levels of prenatal BPA exposure may not affect early child neurodevelopment or levels of thyroid hormones of newborn over all. Further studies of investigating sex specific effects of BPA exposure are needed.

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A. 研究目的

Bisphnol A(BPA) is anendocrine-disrup ting chemical used in the manufacture of plastics and resins including food and drink containers, and as an additive in thermal paper, dental sealant, medical equipment and flame retardant (Biedermann et al. 2010; Geens et al. 2011). BPA exposure is nearly ubiquitous in developed countries. The predominant source of BPA exposure for general adult population is diet. According to previous study, pregnant women who regularly consume canned food have higher urinary BPA concentrations compared with women without the habit (Braun et al. 2011). BPA has a weak estrogenic properties (Akingbemi et al. 2004; Lee et al. 2003). Experimentally, BPA has shown to interact with estrogen signaling pathways through binding to the estrogen receptors (Naciff et al. 2002; Vandenberg et al. 2009; Wetherill et al. 2007) and also act as a thyroid hormone agonist (Zoeller et al. 2005). In animal studies, the association between prenatal BPA exposure and neurobehavioral effects such as anxiety (Cox et al. 2010; Xu et al. 2011), cognitive deficit (Tian et al. 2010; Viberg and Lee 2012) and social behavior (Wolstenholme et al. 2011) have indicated. Studies also have shown loss of sex differences in animal behavior (Cox et al. 2010; Patisaul et al. 2006; Rubin et al. 2006). There are limited data of BPA exposure effects on neurodevelopment in humans. Epidemiological studies have investigated the effects of prenatal BPA exposure on child neurobehavior at several different ages using different assessment scales (Braun et al. 2009, 2011, 2014; Perera et al. 2012; Miodovnik et al. 2011; Harley et al. 2013; Yolton et al. 2011). The scales used in these studies were varied such as Behavior Rating Inventory of Executive Function-Preschool (BRIEF), Child Behavior Checklist (CBCL), NICU Network Neurobehavioral Scale (NNNS), Behavioral Assessment System for Children (BASC), Conners' ADHD/DSM-IV Scales (CADS) and Social Rating Scale (SRS). Some findings from epidemiological studies may suggest maternal BPA exposure's adverse effects on child neurobehavior, on the other hand, others did not show any evidence of adverse effects of prenatal BPA exposure. Additionally several random clinical trials of dental restorations found that there was a significant reduction in scores on memory tests in children with composite fillings containing BPA at ages 6 and 10 (Bellinger et al. 2007; Bellinger et al. 2008), children with composite fillings reported significantly increased anxiety, depression, social stress, and interpersonal-relation problems at ages 11 and 16 (Maserejian et al. 2012). Among these epidemiological investigations, we did not find any published studies using Bayley Scales of

Infant Development (BSID), which is a standard series of measurements to assess the development of infants. The BSID-II mental scale assesses the age-appropriate children's level of cognitive, language, and personal/social development. The motor scale assesses fine and gross motor development. Our group have reported prenatal exposure to several isomers of dioxins may affect the motor development of 6 month-old infants (Nakajima et al. 2006).

Thyroid hormones play an essential role in pre and postnatal brain development. Several epidemiological studies including prospective cohort and cross-sectional studies have investigated the association between BPA levels and thyroid function of adults and children and showed suggestive inverse associations with TSH and T4 and positive associations with T3 (Bucker-Davis et al. 2011; Chevrier et al. 2013; Wang et al. 2012; Meeker and Ferguson, 2011; Wang et al. 2013), however, there is no human studies on BPA exposure and neonatal thyroid hormone levels along with child neurodevelopmental assessment.

Given very limited research on human thyroid function and neurobehavior in association with prenatal exposure to BPA, the aim of this study was to investigate the association between cord blood BPA levels and newborn thyroid hormone levels and child mental and psychomotor development at two distinct time points of ages 6 and 18 months.

B. 研究方法

Study population

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Child Health (Kishi et al. 2011; Kishi et al. 2013). Briefly we recruited pregnant women at 23-35 weeks of gestation between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All subjects were resident in Sapporo City or surrounding areas. The participants completed the self-administered questionnaire survey after the second trimester during their pregnancy. The questionnaire contained baseline information including their dietary habits, exposure to chemical compounds in their daily life, home environment, smoking history, alcohol consumption, caffeine intake, family income, educational levels of themselves and partners. The prenatal information of the mothers and their children was collected from their medical records. This study was conducted with the informed consent of all participants in written forms. The protocol used in this study was approved by the Institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environment and Health Sciences.

Measurement of Bisphenol A

Cord blood was obtained at delivery. All samples were stored at -80 ℃ until analysis. The concentration of BPA in cord blood was measured by using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC/MS/MS) at IDEA Consultants, Inc. (Shizuoka, Japan). 1.0 mL whole blood was spiked with BPA-d16 as an internal standard. After addition of 0.2 M acetate buffer (pH 5.0) and β-glucuronidase, the sample was held in an incubator at 37°C for 5 hours. The diluted sample was applied to a solid-phase extraction column. BPA was extracted using acetonitrile. Then, BPA-d4 was added to the extract as an internal standard. The organic extract was concentrated and the sample was analyzed by ID-LC/MS/MS. The limit of detection (LOD) of BPA was 0.048 ng/ml.

Data from mass screening test

We obtained blood samples data of thyroid stimulating hormone (TSH), free thyroxine (FT4) from Sapporo City Institute of Public Health which conducted the mass screening test for endocrine disorders. A heel-prick blood sample of newborns was obtained as spots on a filter paper for the Guthrie card. The blood samples were obtained from infants between 4 and 7 days age of after birth. Blood samples were applied to 0.3 cm filter disks and TSH and FT4 levels were measured using Enzyme-Linked Immuno Sorbent Assay (ELISA) (TSH: Enzaplate N-TSH, Bayer Co., Tokyo, Japan; FT4: Enzaplate N-FT4, Bayer Co.). The FT4 values of all samples were detected, and for samples with TSH levels below the detection limit (0.50 μU/ml), we used a value of half the detection limit.

Developmental measurements

We used BSID-II (Bayley. 1993) to assess the infant mental and psychomotor development at age 6 and 18 months. The BSID-II is an infant developmental test tool used between 0 to 3 years of age. The BSID-II mental scale assesses the age-appropriate children's level of cognitive, language, and personal/social development. The motor scale assesses fine and gross motor development. Mental and motor raw scores were converted to a normalized scale with a mean of 100 and standard deviation of 15. Home Observation for Measurement of the Environment (HOME) was used to investigate the caregiving environmental conditions of children at 6 and 18 months of age (Anme et al. 1997).

Data analysis

We used the following eligibility for criteria for analyses of subjects; no serious illness or complications during pregnancy and delivery, singleton babies born at term (37 to 42 weeks of gestation), Apgar score of > 6 at 1 minute, babies without congenital anomalies or diseases, and BSID-II completed at ages between 166 and 195 days for 6 months

examination. Among all 514 participants of Sapporo Cohort Study, 286 cord blood samples for BPA measurements were available. For the final analyses, 121 and 86 children at 6 months and at 18 months, respectively, were included.

Since the distributions of cord blood BPA concentrations were right skewed, these variables were transformed by the natural logarithms (ln) to improve their linear relation with MDI and PDI scores. BPA concentrations below the LOD was assigned the value of one-half of the LOD, 0.024 ng/ml. To examine the relation between cord blood BPA levels and child neurodevelopment, linear regression models were used. Then models were stratified by child sex. To select covariates to include in multivariable models, risk factors known or suspected of being associated with the BPA concentrations and/or child neurodevelopment were reviewed in the literatures (Kim et al. 2011; Polanska et al. 2014). The covariates used in this study were maternal education, HOME score, annual income and child sex. Additionally, caffeine intake during pregnancy was used for the analyses of 6 month as the correlation between PDI scores at 6 month was significant. In our previous study (Nakajima et al. 2006), gestational age and maternal smoking status were used as covariates, however, these covariates were not used in this study as the correlations were not significant. Results were considered significant at $p < 0.05$. All analyses were conducted using SPSS (Version 22.0; SPSS, Chicago, IL, USA).

C. 研究結果

Table 1 shows basic characteristics of participants. Compared to the Sapporo Cohort full profile data from our previous report (Kishi et al. 2011), no significant differences were observed (data not shown) in maternal age (30.7 ± 4.9 vs. 30.9 ± 4.9 years old), maternal education (55.6% vs. 61.2%, > 12 years), annual income (31.0% vs. 37.2%, ≧5M), smoking status during pregnancy (18.6% vs. 10.7%, smoker) birth weight (3065 ± 375 vs. 3158 ± 316 g) and gestational age (39.0 ± 1.4 vs. 39.7 ± 1.0 weeks). Duration of breast feeding was used as a covariates in previous reports (Kim et al. 2011; Tellez-Rojo et al. 2013), however, 34.7 % of data were missing in our study, and thus duration of breast feeding was not used as covariate for adjustment. Table 2 shows the characteristics of exposure and outcomes of participants. The median level of cord blood BPA was 0.059 ng/ml. Cord blood BPA level was detected in 73.8% of samples and the range of cord blood BPA levels was from below LOD to 0.217 ng/ml. The median TSH and FT4 levels of newborn were 1.90μU/ml and 2.00ng/ml, respectively.

Table 3 shows BPA levels and MDI, PDI scores at 6 and 18 months in relation to

participants' characteristics. Maternal caffeine intake during pregnancy was negatively correlated with both MDI and PDI scores at 6 month and statistical significance was found only with PDI score ($p = 0.011$). MDI score at 18 month was higher in the group of annual income was above 5 million yen compared to below 5 million yen (81.2 vs. 86.3, respectively, $p = 0.043$). PDI scores at 18 month was higher in the group of higher paternal education compared to lower (83.8 vs. 89.6, respectively, $p = 0.043$). Both MDI and PDI scores at 18 month were higher in female compared to male with statistical significance (86.4 vs. 79.4, $p = 0.005$ for MDI, 91.1 vs. 84.0, $p = 0.006$ for PDI).

Table 5 and 6 show MDI and PDI scores of BSID-II at 6 and 18 months in relation to natural log transformed cord blood BPA levels. Overall, both MDI and PDI scores at 6 months were negatively associated with cord blood BPA levels. MDI and PDI scores at 18 months were negatively associated with cord blood BPA levels without adjustment, however, after the adjustment, the associations became weakly positive. Since there have been reported that BPA may have sex-specific effects, we performed analyses for male and female separately. After stratification by child sex, MDI scores at 6 months showed opposite associations with cord blood BPA levels between male and female. The scores were positively associated in male ($\beta = 1.38$, 95% CI: -1.40, 4.16), contrary, negatively associated in female ($\beta = -1.99$, 95% CI: -4.28, 0.31) and the significance was borderline. For PDI scores, the negative association was stronger in male ($\beta = -3.18$, 95% CI: -7.70, 1.35) compared to female ($\beta = -0.91$, 95% CI: -5.52, 3.70). MDI scores at 18 months showed weak negative association with cord blood BPA levels after adjustment in both sexes. PDI scores at 18 months showed opposite association between sexes, positive association in female ($\beta = 2.28$, 95% CI: -3.10, 7.65) and negative association in male ($\beta = -2.05$, 95% CI: -9.11, 5.01). The borderline significance of negative association between female MDI scores at 6 months and cord blood BPA levels was not found at 18 months. Similarly the negative association found in PDI scores at 6 months in male with cord blood BPA levels became weaker at 18 months.

Table 4 shows the associations between cord blood BPA levels and TSH and FT4 of newborn. Overall, TSH levels were negatively associated with cord blood BPA levels. Further analysis after stratification of child sex, female showed borderline significant negative association ($\beta = -0.232$, $p = 0.089$), contrary male showed weak positive association ($\beta = 0.048$, $p = 0.823$). Cord blood BPA level showed weak positive association with FT4 levels with no

statistical significance.

D. 考察

This is the first published study of examining thyroid hormone levels and child neurodevelopment at 6 and 18 months using BSID-II in relation to cord blood BPA levels. There was borderline significant inverse association between cord blood BPA levels and TSH levels in female. Meeker and Ferguson observed suggestive inverse trends for BPA quintiles and TSH (p trend = 0.14) in cross-sectional study of 1367 adults (Meeker and Ferguson. 2011). However, no association was found with FT4 in smaller study of 167 adult men (Meeker et al. 2010). Our observation on TSH and FT4 agreed with their report. Brucker-Davis et al. (2011) reported weak trend for a negative correlation between BPA and TSH in prospective cohort of 164 newborn boys and Chevrier et al. (2013) reported that maternal BPA was negatively associated with neonatal TSH in boys in CHAMACOS study. These studies found negative associations between BPA and THS levels only in male, and our findings did not agree with these previous reports as we observed stronger negative associations in female rather than in male. A study by Kaneko et al. reported that BPA suppresses TSH release from amphibian pituitary in manner independent of both the thyroid hormone feedback mechanism and the estrogenic activity of BPA (2008) which may explain our observation of negative association between BPA and TSH.

There was no significant association between cord blood BPA levels and child neurodevelopment at 6 and 18 months among all children. The different responses were observed in MDI scores at 6 months; female exhibited decreases in scores and male exhibited increases in scores. PDI scores at 6 months, negative association was stronger in male than in female. At 18 months, the different responses were observed in PDI scores; female exhibited increases in scores and male exhibited decreases in scores. Prenatal BPA exposure may have adverse influences on endocrine or neurotransmitter pathways and cause sexual differentiation of brain and alter behavior in a gender dependent manner (Manson. 2008). Limited observational evidence suggests an association between prenatal BPA exposure and adverse neurobehavioral outcomes in children. Our findings on cord blood BPA levels and child neurodevelopment were compared to the observations from previous human studies. Out of 7 available epidemiological studies regarding BPA exposure and child neurodevelopment, 5 studies suggested prenatal BPA exposure and adverse effects of child neurodevelopment. Braun et al., reported evidences of adverse effect of prenatal BPA exposure predominately in girls using the BASC at 2 years of age

and the BRIEF-P at 3 years of age (Braun et al. 2009, 2011). Perera et al. (2012) used CBCL ages between 3 and 5 years old and suggested that prenatal exposure to BPA may affect child behavior differently among boys and girls. Harley et al. (2013) reported that prenatal urinary BPA concentrations were associated with increased anxiety and depression in boys age at 7 using BASC-2. Contrary, 2 studies, Yolton et al. (2011) and Miodovnik et al. (2011) reported no evidence of an association between prenatal BPA exposure and child neurodevelopment at 5 weeks of age using NNNS and at ages between 7 and 9 years old using SRS, respectively. Those epidemiological results were conflicting and very limited. This could be due to a number of differences between the study designs, and timing and tools of outcome assessment as well as timing of exposure measurements. The assessment tool used in this study, BSID-II assesses developmental domains different from intelligence or executive function. Each unique assessment tool used in different studies had specific purpose; BASC-2 has excellent reliability and validity for assessing adaptive and maladaptive behaviors (Reynolds and Kamphaus 2004), the CBCL measures child behavior problems, the BRIEF-P assess the ability to modulate emotions, the capacity to control behavioral responses, the ability to anticipate and to plan for future events, the capacity to transition to and from events and the ability to hold information in mind for completing a task, the NNNS assesses 13 dimensions of neurobehavior (Lester and Tronick. 2004), the SRS is a scale for detecting and measuring the severity of autistic behavior, and CADS assesses attention and hyperactivity (Conners. 2001), thus these results simply were not able to be compared on the same table. Also noted that most of the studies used the same cohort. In our study, BPA in cord blood was measured as prenatal exposure whereas maternal urine samples were used in the other epidemiological studies for exposure assessment. This difference made it difficult to compare our observations with previous findings. Even studies used urinary BPA as exposure measurements, intra-individual variability of BPA concentrations were moderately correlated (Braun et al. 2009) and accurately characterizing exposure from a single measurement was difficult. On the other hand, using mean concentration of urinary BPA from several measurements would decrease the ability to identify shot time-sensitive window of development (Braun et al. 2011). To improve exposure classification during critical windows of neurodevelopment, the importance of single measurement or summary measurement of BPA concentration should be considered. The cord blood BPA levels in this study was

much lower compared to the previous reports (Aris. 2014; Zhang et al. 2013; Kosarac et al. 2012; Chou et al. 2011; Brucker-Davis et al. 2008 Lee et al. 2008) and this may imply that prenatal BPA exposure levels as low as we observed did not have significant influences on child neurodevelopment.

A recent study suggested that perinatal exposure to low-dose BPA specifically and non-monotonically impairs spatial learning and memory in male offspring rats (Kuwahara et al. 2013). Several mechanisms including epigenetic changes in gene expression in various brain regions via BPA action as weak estrogen receptor agonists and an anti-androgen were suggested from animal studies (Wolstenholme et al. 2011); synaptogenesis decrease in hippocampus and prefrontal cortex of monkeys and rats (Leranth et al. 2008; MacLusky et al, 2005), disruption in cortical development in mice (Nakamura et al. 2006, 2007), alternation in sexually dimorphic brain regions in hypothalamus (Patisaul et al. 2006; Rubin et al. 2006) and reduction of corticotropin-released hormone and DA cell number in midbrain (Funabashi et al. 2004; Tando et al. 2007; Tanida et al. 2009). In BPA exposed animals, multiple genes in tissues were differently methylated (Kundakovnic et al. 2013; Tang et al. 2012), BPA exposure may change expression and DNA methylation of nuclear estrogen receptors and/or signaling via glutamate receptor (Kundakovic et al. 2013; Xu et al. 2010), these studies suggested that BPA may also lead heritable changes in gene expression.

A couple of issues, especially dose and route of exposure, need to be consider when comparing our result to those of animal studies. Many of dose ranges used in animal studies were not relevant to human study. Route of exposure in animal studies were oral, subcutaneous and direct injection at target organs (Li et al. 2008), whereas oral exposure in human studies were predominate.

The limitations of this study need to be considered. First, there was limited statistical power with our sample size. Additionally, there have been concerns whether single drawing of cord blood sample represent the long-term prenatal BPA exposure due to short half-lives of BPA and there might be a possibility of accidental exposure near blood drawing period. Other limitation is that cord blood samples were taken at delivery, thus, the effect of fetal exposure to BPA during the earlier stages of fetal neurodevelopment have not been assessed in this study. There might be a chance of selection bias in this study as we only included participants with available cord blood samples. However, as described the comparison between original cohort profile and the present study profile did not show significant discrepancy. Another limitation is that we were not able to examine

whether postnatal exposure to BPA was associated with childhood neurodevelopment. The strength of our study was that we measured child neurodevelopment outcome at two different times along with the measurement of newborn thyroid hormone levels. Additionally, in our study we used the BPA levels of cord blood, which accurately indicated the exposure of fetus. However, more studies are necessary to confirm adverse effect BPA exposure on child neurodevelopment.

E. 結論

The findings of this study suggested that relatively lower levels of cord blood BPA levels was not notably associated with thyroid hormone levels or neurodevelopment of children. We have observed suggestive negative associations between BPA levels and TSH levels and MDI at 6 month only in female, thus, additional researches investigating sex specific effects are needed.

F. 研究発表

1. 論文発表

In preparation

2. 学会発表

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G. 知的財産権の出願・登録状況（予定を含む。）

1.特許取得

なし

2.実用新案登録

なし

3.その他

なし

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