20142801 2A（別冊）

厚生労働科学研究費補助金
化学物質リスク研究事業

前向きコーホート研究に基づく先天異常、免疫アレルギーおよび小児発達障害のリスク評価と環境化学物質に対する遺伝的感受性の解明

平成26年度　総括・分担研究報告書

別冊　【　研究成果の刊行物　】

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平成27（2015）年3月

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地球社会の環境ビジョン

—これからの環境学—

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力財団

9784990498955

1920240007500

ISBN978-4-9904989-5-5
C0240 ¥750E

定価：750円＋税

市民協力による環境疫学研究

——我国で初の本格的出生コーホート研究の経験から学ぶ——

岸 玲子

環境疫学（市民協力による科学的エビデンスの収集）の重要性

疫学とは、人集団における病気の分布と頻度、およびそれらに影響を与える要因に注目して、健康障害の原因について〝人びと〟を直接対象にしてその原因を解明する科学的な方法である。古くは1854年にロンドンで起こったコレラの対策から、チェルノブイリでの放射線被ばくとがんの関係まで、すべて疫学という方法論が用いられてきた。

19世紀から20世紀の半ばまでは、当時の流行病（感染症）の原因や予防法の発見に疫学研究が応用された。一方、20世紀半ば以降はあらゆる疾病と健康障害の原因の解明に用いられ、証拠を見出すようになった。特に、20世紀後半にはがんや環境化学物質や放射線など低濃度環境汚染と人の健康への影響の研究が進み、また、Evidence based medicineの普及から臨床疫学の発展が目覚ましい。このように疫学は私たちの生活に密着した健康リスクとその原因の解明に用いられるようになった。近年は、遺伝子解析研究が進み、健康障害を引き起こすリスクの高い人に対して、そのリスクを軽減できるように働きかけるための科学的な知識を蓄積することもなされている。その結果、水質汚染や大気・土壌の汚染など環境が引き起こす健康障害を予防し、がん、心臓や呼吸器の病気や感染症などの病気の発症を予防し、発症を遅らせ、あるいは病気の悪化を防ぎ、良好な健康維持に役立てることができるようになった。

環境と健康に関わる様々な疫学研究があるが、私自身は対象を既に特定の病気を有している患者さんではなく、むしろ、予防医学の視点から地域に住む一般の人々や産業職場で働く人々として生活や労働の場で研究を進めてきた。そうした意味で、疫学研究によって確立しようとする科学的なエビデンスは、一人一人の市民や働く人の参加と協力によって成し得た成果ともいえる。

これまで実施した数多くの環境疫学研究の中から、ここでは妊婦と子どもを対象とした我が国で初の本格的な前向き出生コーホート研究（環境と子どもの健康に関する北海道スタディ：先天異常・発達・アレルギー）を紹介する。

出生コーホート研究が盛んになった背景

環境要因が子どもの健康に与える影響、とりわけ環境化学物質の胎児期曝露による影響についての世界的な関心の背景として、１９９６年発刊のColbornらによる「奪われし未来（Our Stolen Future）」において、環境化学物質の内分泌撹乱作用の影響は胎児期が最も感受性が高いとされたこと、翌年１９９７年の8カ国環境大臣会合において「マイアミ宣告」が採択され、子どもの環境保健は環境問題の最優先事項であり、政策の実施が緊急の課題となったことがあげられる。一方、〝疾病の胎児期起源説（Fetal origins hypothesis）〟として循環器疾患やⅡ型糖尿病などへの罹患しやすさが胎児期の低栄養等の影響を受けるというBarker仮説（１９８６）によれば胎児期に過酷な環境に適応し「倹約型」体質にプログラミングされ小児期の肥満や成人期疾患につながる懸念があることが指摘されている。さらに、「Developmental Origins of Health and Diseases（DOHaD）」概念に発展し、胎児期から成人期までライフコースアプローチによる生涯を通じた疫学研究が大きな関心を持たれるようになったことも挙げられる（Kishi, Sasaki, Yoshioka, 2010）。現在、子どもの健康に与える環境要因を解明するために、世界中の国と地域で出生コーホート研究が実施されている。

北海道コーホート研究と環境省エコチル調査の関係

我が国では、１９９０年代には出生後の乳幼児を追跡する調査が幾つかの地域で実施されていたが、胎児期曝露に焦点をあて、出生前から追跡した研究はほとんど存在しなかった。そこで著者らは厚生労働省および文部科学省の研究助成を受け、２００１年から「環境と子どもの健康に関する北海道研究（北海道スタディ）」（Kishi et al, 2010）を立ち上げた。胎児期の母親の血液、分娩時の臍帯血などを長期保存し、先天異常、出生時体格、神経発達、アレルギー疾患など種々のアウトカムについて、環境化学物質の実測値に基づく暴露リスク評価をこれまで約10年間にわたって調査してきた。この北海道スタディは、①５００人規模の札幌市内産科コーホートと、②北海道全域での2万人の大規模コーホートの二つで、地域の母と子のサンプルから多くの科学的な成果が生まれ、海外でも評価されるコーホートとなっている。

２０１１年の2月から全国で始まった環境省の「子どもの健康と環境に関する全国調査（エコチル調査）」の基本設計では、その先駆け的な研究として北海道コーホートから多くの関係資料を提供し、北海道コーホートはその原型（計画のモデル）ともなった。そこで、次に、北海道スタディの概要と最近の科学的知見を紹介し、最後に地域における子どもの健康と環境に関する今後の調査研究のあり方や課題についても述べることとする。

「環境と子どもの健康に関する北海道研究」の概要

(1)研究デザインの特徴

「環境と子どもの健康に関する北海道研究：先天異常、発達、免疫アレルギー（北海道スタディ）」（英語正式名称 The Hokkaido Study of Environment and Children's Health, malformation, development & allergy）の一つは行動発達を詳しく調べる小規模コーホートで、他の一つは先天異常あるいはＡＤＨＤなど比較的発生頻度の少ない疾病を調べる大規模コーホートで構成される。特徴は、1)低濃度の環境要因の影響解明に焦点を当てたこと、2)前向き研究として母体血および臍帯血の採取保存により、器官形成期など胎児期の環境要因について曝露測定を行ったこと、3)先天異常、体格、神経行動発達、甲状腺機能、免疫機能など種々のアウトカムを対象に、4)リスク評価を行い予防対策に結びつけ、さらに、5)個人の感受性素因に着目し、環境と遺伝の交互作用を解明する目的で、化学物質代謝酵素・Ahレセプター・神経伝達物質受容体等の遺伝子多型も考慮したハイリスク群の発見とEpigenetics（後天的遺伝子修飾）の検討を行っていることである。

具体的には「札幌市内1産院コーホート」では、妊娠週数23～35週の妊婦５１４人とその出生児を前向きに追跡し、児の神経発達への影響を測るために詳細な対面調査を実施している。ＢＳＩＤ－Ⅱを6ヶ月時と18ヶ月時に実施、Fagan testを7ヶ月時に、日本版ＤＤＳＴを18ヶ月時に行い、42ヶ月時には日本版Ｋ－ＡＢＣと母親のＷＡＩＳ－Ｒを、43ヶ月時にはＣＢＣＬを実施し、就学時以降は児の行動発達調査を実施している。臍帯血IgEや出生後の感染症、アレルギーなど免疫系への影響を調べている。環境要因としてはＰＣＢ・ダイオキシン類、ＰＦＯＳ・ＰＦＯＡ、水銀の測定を行い、農薬、ビスフェノールＡ、ＯＨＰＣＢなどの測定も進み、アウトカムとの関係を既に報告している。

「大規模コーホート」は、全道の30の産科施設に協力をいただき器官形成期にあたる時期に母体血の採取と質問票の回収を行い、臍帯血を採取し、マーカー奇形55種を調べ、生後は、発育とアレルギー、行動発達に関し環境要因との関係を追跡している。２０，０００人を目標に研究を進め、２０１２年4月には妊婦約２０，８００名が参加し、現在も縦断的な調査を継続している。

(2)これまで得られた成果

環境化学物質の次世代影響

1)ＰＣＢダイオキシン類と出生時体格

ＰＣＤＤｓ、ＰＣＤＦｓおよびＰＣＢｓは親油性、かつ、難分解性の有機塩素化合物で、環境中に広範囲に分布し、おもに食物連鎖を介してヒトの体内に蓄積される。生体内の半減期が長く体内に長期に蓄積され、また、母乳および臍帯を介して母親から児へ移行する。高精度のＧＣ／ＭＳ分析を用

いて、世界で初めて母体血中のＰＣＤＤｓ、ＰＣＤＦｓの同族異性体分析およびdioxin-like PCBs濃度を測定し、また、ＷＨＯが設定したＴＥＦを用いてダイオキシン類（29種類）の毒性等価量（ＴＥＱ）の算出を行い、交絡要因を調整した結果、総ＰＣＤＦｓ濃度、総ＰＣＤＦｓ／ＴＥＱ濃度と出生体重との間に有意な負の関連を認めた。男児では総ＰＣＤＤｓ濃度、総ＴＥＱレベルが高いほどリスクを上げ出生時体重が低かった（log10-unit: β=-256.4g, 95%CI: -448.6 to -64.2）。性差があり女児ではそのような傾向は認められなかった（Konishi et al, 2009）。

2)有機フッ素系難燃剤ＰＦＯＳ、ＰＦＯＡと出生時体格

１９５０年頃から難燃剤として世界で使用されてきたＰＦＯＳ、ＰＦＯＡの濃度と出生体重との関連を調べたところ、母体血清ＰＦＯＳ濃度は出生体重との間に負の関連を認めた（log10-unit: β=-148.8g, 95%CI: -297.0 to -0.5）。しかし、ＰＦＯＡの影響は見られなかった。ＰＦＯＳ、ＰＦＯＡはＰＯＰｓ条約に含まれ規制がはじまり、現在、代替物質について影響を子細に検討している。

3)ダイオキシン・ＰＣＢ類の児の神経発達に与える負の影響

児の神経発達に与える負の影響が示された。ＰＣＤＦとＰＣＤＤの異性体濃度が高くなると生後６ケ月時のＢＳＩＤ-Ⅱの得点が低くなる負の関連が、特に、運動発達に顕著に見られた。Total TEQ値もＢＳＩＤ-Ⅱの得点と有意な負の関連が運動発達で見られ低い得点と有意に関連した（Nakajima, Saijo, Kato et al, 2006）。

4)免疫・アレルギーへの影響

母体血中ダイオキシン類濃度が高いほど臍帯血ＩｇＥレベルが低下した。18ヶ月までのアレルギー症状および感染症との関連を検討したところ、アレルギーとの有意な関係は認められなかったが、ダイオキシンレベルは中耳炎と関連が認められた。ＴＥＱ値はＰＣＤＦｓが１増加すると中耳炎オッズ比が１・36倍と有意に増加した。男児のみ母体血中ダイオキシンレベル増加に伴い中耳炎ＯＲの有意な増加が認められ、女児では有意な関連が認められなかった。

遺伝的感受性素因によるハイリスク群の存在

母親のＣＹＰ１Ａ１遺伝子、ＡｈＲ遺伝子、およびＧＳＴＭ１についてみると、ＡｈＲ遺伝子とＣＹＰ１Ａ１遺伝子の特定の組み合わせで体重への影響がマイナス３１５ｇと最も低かった。ＣＹＰ１Ａ１遺伝子ＴＣ／ＣＣ型ではＴＴ型よりも酵素活性が上昇しているため、中間代謝物であるジオールエポキシドなどの発がん性物質の生成が促進され影響に個体差がみられたと考えられる。

発癌物質ニトロソアミン類代謝活性化に関与するＮＱＯ１遺伝子の多型の検討も行った。喫煙母親のＮＱＯ１遺伝子多型がＣＴ／ＴＴ型では体重が77ｇ低いのに対して、ＣＣ型では体重低下がマイナ

ス199gであった。NQO1遺伝子の多型は身長と頭囲にも影響がみられ、より大きな低下を示した。妊娠初期に禁煙した場合は非喫煙の母親と変わらなかった。

北海道コーホートの今後の予定

以上まとめると、北海道コーホートでは、世界ではじめて同属異性体レベルでPCB・ダイオキシン類の胎児期曝露影響が詳細に検討され、生下時体重に負の影響が示された。ダイオキシン類では生後発達への影響や感染症罹患リスクを上げる可能性も示唆された。総PCDDs濃度、総PCDFs／TEQ濃度と出生体重との関連には性差が認められ男児のほうが感受性は高かった。性差の原因については、Ah受容体とエストロゲン受容体の両者の作用で引き起こされる可能性が示唆されている。しかし、詳細なメカニズムは不明であるので、今後、種々の角度から検討を進める。

PFOS・PFOAについては既にPOPS条約に入れられたが、我が国ではまだ規制が弱い。北海道（小規模）コーホートでは出生体重への影響が認められ、甲状腺機能に対しても影響が認められた。今後、大規模コーホートでさらに検討を進める予定で準備をしている。特に、今後、生後発達への影響についても解析する必要がある。

胎児期に参加した子どもが学童期を迎えた今、1)アレルギーやADHDなどがより明確になる学童期以降までの追跡、軽度発達障害の研究を始めている。今後は、2)複合曝露による影響の評価や3)生後曝露の測定と評価、4)エピジェネテック作用の検討が重要になる。受動喫煙をコチニン量で評価し、また、葉酸値を全員について測定しているので、受動喫煙、葉酸サプリメントや葉酸値そのものと児の胎内成長や先天異常なども解析を進めている。

さらに、「社会的要因」の重要性を指摘し、地域における子どもたちの健康と安全の問題に、これまで以上に積極的な関与と情報発信が不可欠であると考えている。2006年にはOECD統計によれば我が国が先進国の中ではアメリカについで第2位で貧困層の率が高く、13人に1人が貧困と言われている。北海道スタディでは、今後、それら社会的環境要因を含めた調査や解析によって健康障害や疾病のリスク要因を明らかにしていくことになる。

せっかくの疫学研究の成果を環境リスクマネジメントにどう活用するのか

日本の場合、農薬等のみならずダイオキシン類はその取り込み源は9割が食品、特に、魚介類である。また、フッ素系化学物質では6割から8割が食事由来で、そのほか飲用水からも比率が高い。従って一般のお母さんたちへの影響は非常に大きい。しかし、残念なのは、せっかく上述の新しい知見が日本の国民を対象にしたデータから過去10年、数多く出てきており、海外のトップジャーナルに発表

されているのにもかかわらず、それらを利活用し、リスクマネジメントや環境化学物質対策に利用することをまだ十分できていないことである。我が国では研究データの発見や知見の蓄積と、それらへの予防的な諸対策の間の距離が非常に大きいように感じられる。

一方、環境省エコチル研究は、日本で初めての出生コーホートのように伝えられているが、そうではなく、我々の北海道スタディや東北コーホートは既に過去10年の蓄積がある。出生コーホート研究は甚大なエネルギーと費用、時間がかかるが、国家財政が今後、非常に厳しいことを考えると、国（環境省）の政策としては、たとえ他の省庁（厚生労働省と文部科学省）の補助金で実施されていたとしても、環境リスク評価として既に科学的に何がわかってまだ何がわかっていないのかを十分整理しながら、課題を絞って実施するような方向性が国としては本来、大事なのではないだろうか？

(1) Kishi et al, Cohort Profile: The Hokkaido Study on Environment and Children's Health in Japan, Int. J. Epid, 2010.
(2) Konishi et al, Prenatal Exposure to PCDDs/PCDFs and dioxin-like PCBs in relation to birth weight, Environ. Res, 2009.
(3) Nakajima et al, Effects of Prenatal Exposure to Polychlorinated Biphenyls and Dioxins on Mental and Motor Development in Japanese Children at 6 Months of Age, Environ. Health Persp, 2006.

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専門：公衆衛生学・環境

Chemosphere 133 (2015) 13–21

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan

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HIGHLIGHTS

- PCDDs/PCDFs and DL-PCBs, and PFOS decreased with maternal smoking history.
- NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal alcohol consumption during pregnancy.
- Total hair Hg increased with household income.
- Beef and fish/seafood intake may be important exposure sources of NDL-PCBs.
- Chemical exposure and elimination rate may be related to lifestyle factors.

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 13 February 2015
Accepted 26 February 2015
Available online 28 March 2015

Handling Editor: Andreas Sjodin

Keywords:
Persistent organic pollutants
Mercury
Pregnant women
Biomarkers
POPs

ABSTRACT

Persistent organic pollutants and mercury are known environmental chemicals that have been found to be ubiquitous in not only the environment but also in humans, including women of reproductive age. The purpose of this study was to evaluate the association between personal lifestyle characteristics and environmental chemical levels during the perinatal period in the general Japanese population. This study targeted 322 pregnant women enrolled in the Hokkaido Study on Environment and Children's Health. Each participant completed a self-administered questionnaire and a food-frequency questionnaire to obtain relevant information on parental demographic, behavioral, dietary, and socio-economic characteristics. In total, 58 non-dioxin-like polychlorinated biphenyls, 17 dibenzo-p-dioxins and -dibenzofuran, and 12 dioxin-like polychlorinated biphenyls congeners, perfluorooctane sulfonate, perfluorooctanoic acid, and mercury were measured in maternal samples taken during the perinatal period. Linear regression models were constructed against potential related factors for each chemical concentration. Most concentrations of environmental chemicals were correlated with the presence of other environmental chemicals, especially in the case of non-dioxin-like polychlorinated biphenyls and, polychlorinated dibenzo-p-dioxins and -dibezofurans and dioxin-like polychlorinated biphenyls which had similar exposure sources and persistence in the body. Maternal smoking and alcohol habits, fish and beef intake and household income were significantly associated with concentrations of

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http://dx.doi.org/10.1016/j.chemosphere.2015.02.062
0045-6535/

environmental chemicals. These results suggest that different lifestyle patterns relate to varying exposure to environmental chemicals.

1. Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and -dibezofurans (PCDDs/PCDFs), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA)—categorized as persistent organic pollutants—and mercury (Hg) are known environmental chemicals that have been detected ubiquitously in animal samples and the environment. Exposure to environmental chemicals during prenatal and neonatal periods, which are considered windows of vulnerability for fetuses, may cause various toxicities including carcinogenicity, teratogenicity, endocrine, immune, and reproductive disruption, and neurobehavioral effects (Clarkson and Magos, 2006; Wigle et al., 2008; Olsen et al., 2009; Todaka et al., 2010).

Epidemiological studies of Asian, European, and US populations have revealed that environmental chemical levels measured in maternal samples were associated with demographic, behavioral, dietary, and socioeconomic characteristics. Fish and seafood are the main dietary sources of PCB and PCDDs/PCDFs exposure in Japan, Taiwan, Nordic countries, and Italy (Arisawa et al., 2011); whereas, meat products, dairy products and fish are the main dietary sources in the US, The Netherlands, and Germany (Larsen, 2006). Potential exposure sources of PFOS and PFOA were reported to be fish and marine mammals, red meat, animal fat, tap (drinking) water, and household dust in Spain, Norway, and Denmark (D'Hollander et al., 2010; Haug et al., 2011). Many reports to date have also found fish/seafood consumption responsible for bioaccumulated methylmercury in humans (Clarkson and Magos, 2006; Kim et al., 2008; Ramon et al., 2008). Consequently, it is plausible that the presence of exposure sources and their contribution to whole body burden levels of environmental chemicals would vary according to the specific characteristics of populations in different countries or regions (Glynn et al., 2007; Halldorsson et al., 2008; Kim et al., 2008; Ramon et al., 2008; Sonneborn et al., 2008; Brauner et al., 2011; Ibarluzea et al., 2011).

The elimination rate of toxic substances as a reflection of internal metabolism is an effective way to detect body burden levels of environmental chemicals. Tobacco smoking and alcohol habits are considered behavioral factors related to altered elimination rates of environmental chemicals. For example, tobacco smoking induces increased expression of dioxin-metabolizing enzymes, such as cytochrome P450 (CYP) 1A2, leading to enhanced elimination of PCDDs/PCDFs and dioxin-like PCBs (DL-PCBs) (Milbrath et al., 2009). Animal and human studies have also demonstrated that fluorinated organic compounds can regulate CYP enzymes (Ishibashi et al., 2008; Narimatsu et al., 2011).

To date, limited epidemiological studies have been conducted in Japan among pregnant women with no history of accidental poisoning in Japan. Some studies found that PCBs and PCDDs/PCDFs in maternal samples increased with maternal age, alanine aminotransferase levels and alcohol intake, as well as decreased with maternal history of delivery and smoking (Tajimi et al., 2005; Nakamura et al., 2008; Arisawa et al., 2011). However, no study has assessed maternal smoking and alcohol habits during the pre-pregnancy periods, which is considered an important period because chemicals that have a long half-life could be influenced by lifestyle factors during the entire perinatal period. There have also been no current studies to evaluate associations between background exposure levels of environmental chemicals even though certain chemical levels could be correlated with the presence of other chemicals in the human body. This information could help in estimating the magnitude of body burden levels after exposure to various chemicals.

Thus, the purpose of this study was to evaluate associations between concentrations of individual chemicals including non-dioxin-like PCBs (NDL-PCBs), PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg and the potential factors responsible for their varied elimination rates and exposure sources in the general Japanese population.

2. Materials and methods

2.1. Study population

We enrolled 514 Japanese women at 23–35 weeks gestation who were visiting the Sapporo Toho Hospital to take part in the Hokkaido Study on Environment and Children's Health Study (Kishi et al., 2011) between July 2002 and September 2005 (Supplementary Fig. 1). In their last trimester, the subjects filled out a self-administered questionnaire regarding the following parental information: tobacco smoking and alcohol habits during pre- and post-pregnancy; frequency of food consumption during pregnancy of items such as shoreline fish (e.g., saury, Pacific herring, mackerel), pelagic fish (e.g., tuna, bonito, salmon), beef, pork, chicken, milk, and eggs; education level; and household income. Estimated intake value for alcohol (g d^{-1}) was calculated from a modified self-administered questionnaire about frequency and type of alcohol consumption (Washino et al., 2009).

From enrollment to delivery, 10 subjects dropped out because of intrauterine growth retardation (2), hospital transfer (1), or voluntary withdrawal (7). The medical records for the remaining 504 mother–newborn pairs were used to obtain data on maternal height and weight before pregnancy. To obtain information on maternal fish intake throughout pregnancy, we contacted subjects within 5 d of delivery. They completed part of a food-frequency questionnaire (FFQ) and provided information about intake frequency and portion size for 28 fish and seafood items and their estimated total fish intake (g d^{-1}) was calculated as previously described (Yasutake et al., 2003) (Supplementary Table 1). We were not able to contact 74 subjects because of poor health conditions immediately after delivery. Subjects also provided a sample of their hair for Hg measurements and information on their past history of having their hair permed. This study was conducted with written informed consent from all subjects and was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.2. Experimental and exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein in the last trimester, except in those subjects with pregnancy-related anemia, from whom blood samples were taken immediately after delivery. All blood samples were stored at −80 °C. NDL-PCBs (Supplementary Table 2) and, PCDDs/PCDFs and DL-PCBs levels (Supplementary Table 3) in maternal blood were detected by high-resolution gas chromatography/high-resolution mass spectrometry equipped with a solvent-cut large-volume injection system at the Fukuoka Institute of Health and

Environmental Sciences as previously described (Iida and Todaka, 2003; Todaka et al., 2003, 2008). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels were adjusted by total lipid content (pg g^{-1} lipid) (Todaka et al., 2003). Toxic equivalent (TEQ) values were calculated by multiplying the concentration of each individual congener of PCDDs/PCDFs and DL-PCBs by its specific toxic equivalency factor value (Van den Berg et al., 2006). PFOS and PFOA levels in maternal serum were detected by column-switching liquid chromatography–tandem mass spectrometry at Hoshi University as previously described (Inoue et al., 2004a,b; Nakata et al., 2005a,b). Values below the detection limit were assigned as 50% of the detection limit. The remaining samples were not analyzed owing to unavailable or insufficient sample volumes for measurement. Total Hg levels were detected in the 1-cm hair fiber closest to the scalp (0.7–1.2 mg) by the oxygen combustion-gold amalgamation method using an MD-1 atomic absorption detector (Nippon Institute, Co., Ltd., Osaka) at the National Institute for Minamata Disease as previously described (Yasutake et al., 2003). Total hair Hg concentration is a convenient biomarker for methylmercury exposure because >90% of total hair Hg is methylmercury, which covalently binds to cysteines in hair proteins (Clarkson and Magos, 2006). Finally, 58 NDL-PCBs, 12 DL-PCBs and 17 PCDDs/PCDFs congeners—were detected in 426 blood samples. PFOS and PFOA, and total Hg were detected in 447 sera samples and 430 hair samples, respectively.

Table 1
Subject characteristics ($n = 322$).

		Mean ± SD	n (%)
Maternal characteristics			
Age at delivery (years)		30.63 ± 4.70	
BMI before pregnancy (kg m^{-2})		21.12 ± 3.21	
Parity	⩾1		170 (52.8)
Blood sampling period	<28 weeks		19 (5.9)
	28 to < 36 weeks		144 (44.7)
	⩾36 weeks		70 (21.2)
	After delivery		99 (30.7)
Education level (years)	>12		191 (59.3)
Tobacco smoking history	Yes		168 (52.2)
Tobacco smoking during pregnancy	Smoker		55 (17.1)
Alcohol consumption history	Yes		237 (73.6)
Alcohol consumption during pregnancy	Drinker		97 (30.1)
Alcohol intake (g d^{-1}) during pregnancy		0.00 (0.00, 0.46)[a]	
Fish intake (g d^{-1}) during pregnancy		50.00 (30.00, 50.00)[a]	
Frequency of food consumption during pregnancy			
Shoreline fish	⩾once/week		155 (48.1)
Pelagic fish	⩾once/week		178 (55.3)
Beef	⩾once/week		86 (26.7)
Eggs	⩾once/week		322 (100)
Milk	⩾once/week		285 (88.5)
Paternal characteristics			
Tobacco smoking history	Yes		279 (86.7)
Tobacco smoking during their partner's pregnancy	Smoker		225 (69.9)
Annual household income (million yen)	⩾5		110 (34.2)

BMI: body mass index.
[a] Median (minimum, maximum).

2.3. Statistical analysis

In total, 322 subjects that had complete data about concentration of environmental chemicals and personal characteristics were included in the statistical analyses. Subjects were divided into four categories for each of maternal age, BMI, blood sampling period, and fish intake during pregnancy as shown in Table 1. Spearman's rank test was used to determine correlations between concentrations of environmental chemicals. The Mann–Whitney U-test and Kruskal–Wallis test were used to evaluate simple associations between subject characteristics and the concentrations of each environmental chemical. Linear regression analyses were performed to evaluate associations between concentrations of environmental chemicals and subject characteristics. Because of skewed distributions in these concentrations, $\log_{10}$-transformed values were used for linear regression analysis. Linear regression models were constructed for explanatory variables that had previously been reported as related to concentrations of environmental chemicals or that were significantly associated with these concentrations by bivariate analysis in this study. Backward stepwise regression was used to eliminate those variables with a p-value >0.1.

Subgroup analyses were performed to confirm significant associations between maternal smoking history and alcohol consumption during pregnancy and concentrations of each

Table 3
Correlation coefficients between individual environmental chemicals ($n = 322$).

	PCDDs/PCDFs and DL-PCBs	PFOS	PFOA	Hair Hg
NDL-PCBs	0.80**	0.07	0.10	0.38**
PCDDs/PCDFs and DL-PCBs		0.24**	0.14*	0.30**
PFOS			0.25**	0.12*
PFOA				0.03

* $p < 0.05$ by Spearman's rank correlation.
** $p < 0.01$ by Spearman's rank correlation.

Table 2
Concentrations of environmental chemicals in maternal samples ($n = 322$).

	Geometric mean	Minimum	Percentile			Maximum
			25th	50th	75th	
NL-PCBs (ng g^{-1} lipid)	94.0	16.0	66.0	95.1	130	445
DL-PCBs and PCDDs/PCDFs (TEQ pg g^{-1} lipid)[a]	13.5	3.17	9.86	13.8	18.3	42.9
PFOS (ng mL^{-1})	4.78	1.30	3.20	5.00	6.98	14.7
PFOA (ng mL^{-1})	1.20	0.25	0.80	1.30	1.80	5.30
Hair Hg (μg g^{-1})[b]	1.35	0.24	0.96	1.39	1.89	7.55

[a] TEQs were calculated from the individual congener toxic equivalency factor values (Van den Berg et al. (2006)).
[b] >90% Methylmercury.

environmental chemical. Duration of maternal smoking (years) was used as a continuous explanatory variable in subgroup analyses of subjects with a history of smoking. Alcohol intake levels ($g\,d^{-1}$), after categorization into four groups according to their quartile distribution, were used in subgroup analyses of a subjects' alcohol consumption during pregnancy. Presence of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs congeners were examined in subgroup analyses among alcohol drinkers during pregnancy. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS for Windows version 19.0 J (SPSS, Inc., USA).

3. Results

Parental characteristics based on the self-administered questionnaire and the FFQ are shown in Table 1. Approximately half (52.2%) of the mothers had a history of tobacco smoking; 17.1% of mothers

Table 4
Maternal environmental chemical levels in relation to characteristics ($n = 322$).

Characteristics		NDL-PCBs ($ng\,g^{-1}$ lipid)	PCDDs/PCDFs and DL-PCBs (TEQ $pg\,g^{-1}$ lipd)	PFOS ($ng\,mL^{-1}$)	PFOA ($ng\,mL^{-1}$)	Hair Hg ($\mu g\,g^{-1}$)
Age at delivery (years)	<25	60.78**	9.80**	5.0	1.4	1.4
	25 to <30	86.0	13.7	5.3	1.2	1.4
	30 to <35	101.3	14.4	5.0	1.4	1.3
	⩾35	136.4	16.9	4.2	1.2	1.7
BMI at delivery ($kg\,m^{-2}$)	<18.5	89.7	13.7	5.4	1.4	1.3
	18.5 to <25	97.2	13.9	5.0	1.3	1.4
	25 to <30	108.4	13.4	4.4	1.4	1.2
	⩾30	77.3	12.8	4.3	1.2	1.1
Parity	0	101.0	14.6**	5.50**	1.50**	1.4
	⩾1	90.8	13.3	4.6	1.0	1.4
Timing of blood sampling	<28 weeks	114.6	16.7	6.4**	1.8**	
	28 to <36 weeks	108.6	13.9	5.6	1.5	
	⩾36 weeks	102.1	13.8	4.6	1.2	
	After delivery	108.2	13.5	3.8	1.2	
Education level (years)	⩽12	89.4	12.8	4.8	1.3	1.4
	>12	99.2	14.1	5.3	1.4	1.4
Tobacco smoking history	No	101.0	15.2**	5.30**	1.4	1.5
	Yes	87.8	12.6	4.7	1.2	1.3
Tobacco smoking during pregnancy	Nonsmoker	96.9	14.1	5.0	1.3	1.4
	Smoker	84.8	12.2	4.8	1.2	1.4
Alcohol consumption history	No	86.0	13.3	4.8	1.2	1.3
	Yes	101.0	14.0	5.0	1.4	1.4
Alcohol consumption during pregnancy	Non-drinker	92.1	13.8	5.0	1.3	1.4
	Drinker	101.0	13.8	5.1	1.3	1.4
Alcohol intake ($g\,d^{-1}$) during pregnancy	Quartile 1 (<0.73)	0.1	0.0	0.0	0.0	0.1
	Quartile 2 (0.73 to <1.52)	96.0	12.3	4.1	1.2	1.3
	Quartile 3 (1.52 to <3.52)	117.3	13.7	5.6	1.2	1.6
	Quartile 4 (⩾3.52)	100.4	15.7	6.2	1.4	1.4
Fish intake ($g\,d^{-1}$) during pregnancy	Quartile 1 (<25)	101.5*	14.0	4.6	1.3	1.5**
	Quartile 2 (25 to <38.75)	84.8	13.0	5.3	1.4	1.3
	Quartile 3 (38.75 to <50)	101.3	13.8	4.9	1.4	1.4
	Quartile 4 (⩾50)	104.1	14.3	5.0	1.2	1.7
Frequency of food consumption during pregnancy						
Shoreline fish	<once/week	87.2	13.3	4.7	1.2	1.28*
	⩾once/week	101.0	14.5	5.3	1.3	1.5
Pelagic fish	<once/week	94.8	13.4	4.7	1.3	1.25**
	⩾once/week	95.6	14.2	5.4	1.3	1.5
Beef	<once/week	95.0	13.7	5.0	1.3	1.34*
	⩾once/week	100.0	14.1	5.2	1.3	1.5
Egg	<once/week	92.6	13.6	4.10**	1.3	1.28**
	⩾once/week	95.1	13.8	5.0	1.3	1.4
Milk	<once/week	66.2	11.0*	4.3	1.2	1.3
	⩾once/week	99.2	14.1	5.0	1.3	1.4
Paternal characteristics						
Tobacco smoking history	No	97.5	15.1	5.4	1.3	1.3
	Yes	94.7	13.7	5.0	1.3	1.4
Tobacco smoking during their partner's pregnancy	Non-smoker	102.0	15.1*	5.4	1.4	1.4
	Smoker	92.5	13.1	4.8	1.3	1.4
Annual household income (million yen)	<5	91.8**	13.3**	4.7	1.2	1.27*
	⩾5	113.0	15.8	5.5	1.5	1.5

Values shown are medians. BMI: body-mass index.
* $p < 0.05$ by the Mann–Whitney U-test and Kruskal–Wallis test.
** $p < 0.01$ by the Mann–Whitney U-test and Kruskal–Wallis test.

smoked during the pregnancy. There was a history of alcohol consumption in 73.6% of the mothers, and 30.1% of the mothers consumed alcohol during pregnancy. Median concentrations of NDL-PCBs, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA, and hair Hg were 95.1 ng mL^{-1} lipid, 13.8 TEQ pg g^{-1} lipid, 5.00 ng mL^{-1}, 1.30 ng mL^{-1}, and 1.39 μg g^{-1}, respectively (Table 2). Table 3 shows the correlations between concentrations of individual environmental chemicals. The strongest correlation was found between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs ($r = 0.80$, $p < 0.01$). In univariate analyses of maternal levels of environmental chemicals in relation to maternal characteristics, levels of environmental chemicals were significantly associated with maternal age at delivery, parity, blood sampling period, education level, smoking and alcohol habits, fish intake, frequency of food consumption, and annual household income ($p < 0.05$; Table 4).

The linear regression models in Fig. 1 show the potential relationship between various factors and maternal blood concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS, and PFOA, and total hair Hg. Significant positive associations with each log_{10}-transformed concentration of environmental chemicals were observed for maternal age, maternal alcohol consumption during pregnancy, fish intake, pelagic fish intake, beef intake, and household income (Supplementary Table 4). Significant negative associations with each log_{10}-transformed concentration of environmental chemicals were observed for multiparous subjects, smoking history, and blood sampling period (Supplementary Table 4). In the subgroup analyses of the 168 subjects with a history of smoking, the duration of tobacco smoking was inversely associated with log_{10}-transformed PFOS values (Fig. 2; −0.08 (−0.15, −0.02)). With additional adjustment for maternal age, statistical significance remained. In the subgroup analysis of the 97 subjects who reported drinking alcohol during their pregnancy, there was no significant association between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs and their congeners with alcohol intake for any quartile as well as across quartiles (Table 5; Supplementary Table 5).

4. Discussion

4.1. Correlations between concentrations of environmental chemicals

NDL-PCBs and, PCDDs/PCDFs and DL-PCBs are reported to have high lipophilicities and the biological half-life of most their congeners ranges from a few years to approximately 20 years (Todaka et al., 2010). Perfluoroalkyl acids (PFAAs) are reported to distribute mainly in blood serum and the liver as a result of protein fraction binding (Karrman et al., 2010), and the half-lives of PFOS and PFOA are estimated to be 3.8 and 5.4 years, respectively (Olsen et al., 2009). Methylmercury binds to hemoglobin in the blood, and its half-life is estimated to be 2 months (Clarkson and

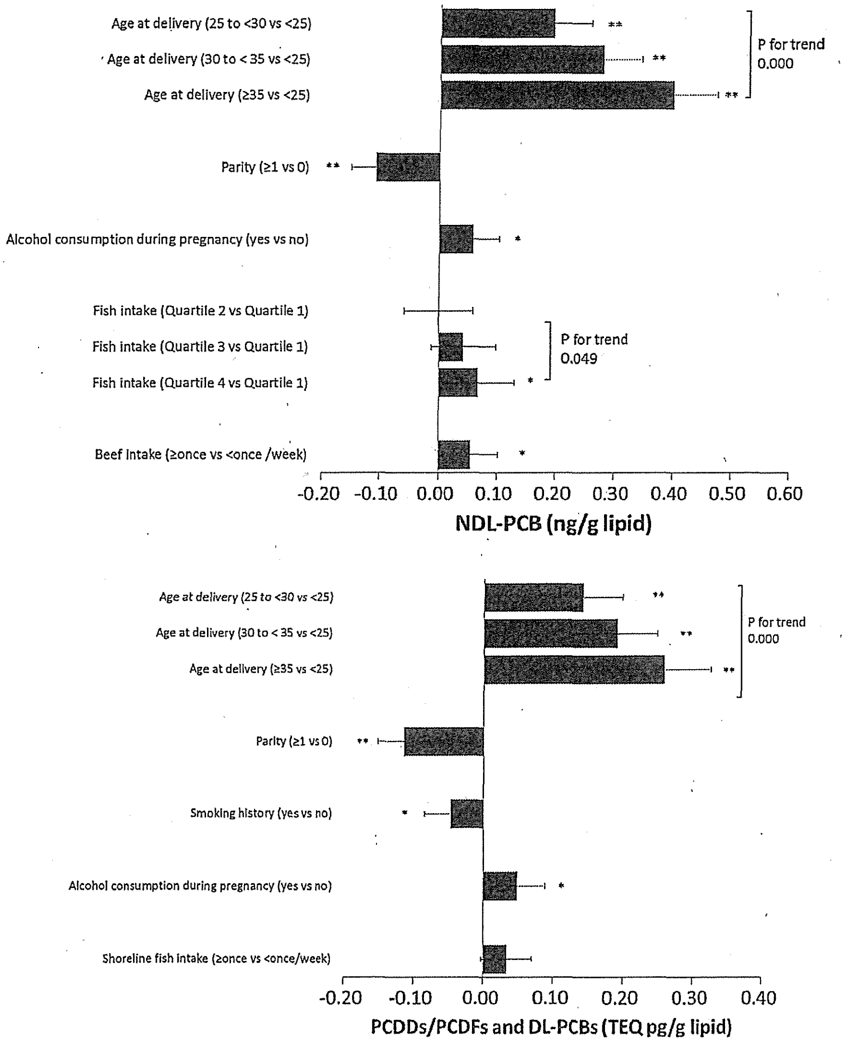

Fig. 1. Partial regression coefficients of factors related to environmental chemical levels. The bar graph denotes B (95%CI) for factors related to the concentrations of environmental chemicals in linear regression analyses, adjusted for mutually related factors.

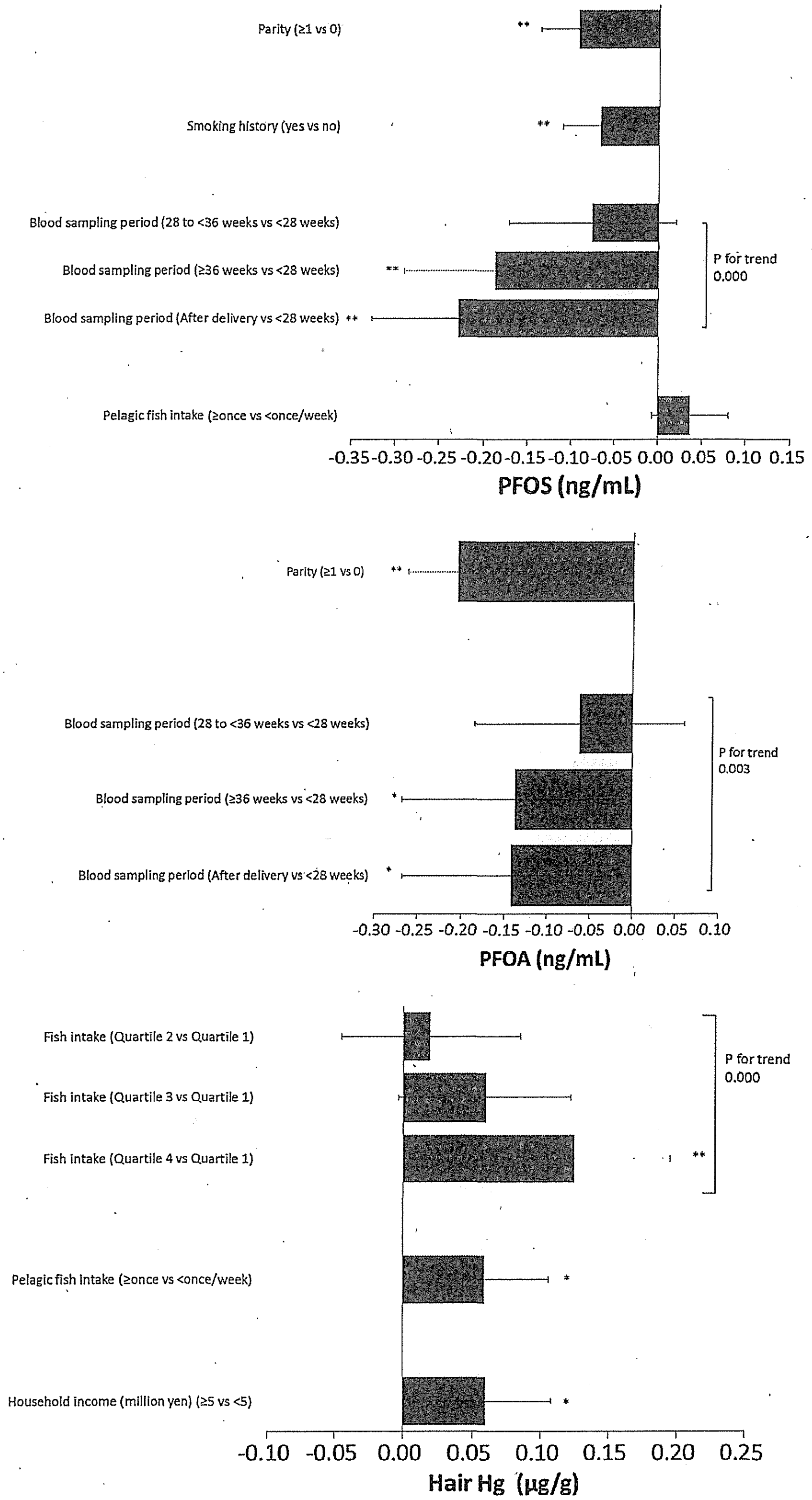

Fig. 1 (*continued*)

Magos, 2006). The degrees of correlations between environmental chemicals could correspond to differences in their exposure sources as well as their individual pharmacokinetics. Almost all of the environmental chemicals, with the exception of relationships between PFOS and PCBs, PFOA and PCBs, and Hg and PFOA, were significantly correlated, implying that concentrations of certain environmental chemicals could be used to estimate the magnitude of exposure among the general population in Japan to other environmental chemicals, especially those from similar exposure sources and with similar persistence in the body.

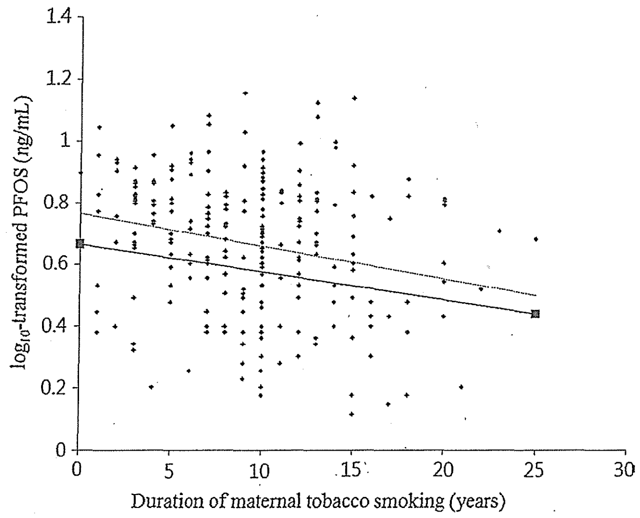

Fig. 2. Association between duration of maternal tobacco smoking (years) and log_{10}-transformed PFOS concentrations (ng mL^{-1}) among subjects with smoking history. The solid line denotes the predicted fit from the multivariate regression model, adjusted for maternal parity, blood sampling period, and pelagic fish intake ($n = 168$). The broken line denotes the predicted fit from the univariate regression model.

4.2. Tobacco smoking

Maternal smoking history was significantly related to a decline in concentrations of PCDDs/PCDFs and DL-PCBs, and PFOS in this study. A previous study reported that tobacco smoking lead to a decrease in PCDDs/PCDFs and DL-PCBs levels because of increased expression of dioxin-metabolizing enzymes after activation of the aryl hydrocarbon receptor (Milbrath et al., 2009). PFAAs are known to activate peroxisome proliferator-activator receptor (PPAR), and a study in wild animals suggested the possibility that a signaling pathway exists between receptor PPAα and CYP that promotes elimination of PFAAs from the body after PFAA exposure (Ishibashi et al., 2008). Previous epidemiological studies reported inconsistent relationships between PFAA levels and smoking status among pregnant women when categorized by history or current smoking status (Halldorsson et al., 2008, 2012; Jain, 2013; Ode et al., 2013). In a Swedish study, maternal cotinine levels among current smokers were not associated with PFOA and PFOS plasma levels; in fact, PFOA and PFOS plasma levels were significantly lower than those of subjects who had never smoked (Ode et al., 2013). Ode discussed that these results could reflect differences in lifestyle patterns between smokers and non-smokers that were associated with sources of PFOA and PFOS exposure or an enhanced elimination rate of these environmental chemicals in smokers. Our study is the first study to indicate an inverse association between PFOS concentrations and the duration of tobacco smoking using a linear regression model adjusted for confounding factors. This result supports previous studies and suggests that a smoking habit may lead to enhanced elimination rate of not only PCDDs/PCDFs and DL-PCBs but also PFOS through activation of PPAα and CYP.

4.3. Alcohol consumption

Our results showed that mothers who drank alcohol during pregnancy had higher blood concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs. In a previous Japanese study that included women of reproductive age, two possible explanations for this positive association between PCDDs/PCDFs and DL-PCBs and alcohol consumption were proposed. The first is that alcohol intake likely affects hepatic drug-metabolizing enzymes, which could result in slowed elimination of these environmental chemicals. The second is that alcohol intake may indicate a greater likelihood of rich, fatty food consumption, which may result in increased PCDDs/PCDFs and DL-PCBs levels (Arisawa et al., 2011). In this study, no significant associations were found across alcohol-intake quartiles and concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, and congeners among alcohol drinkers during pregnancy. However, concentrations NDL-PCBs and, PCDDs/PCDFs and DL-PCBs in maternal drinkers were higher than those of women who did not drink during pregnancy. Because of their long half-lives, NDL-PCBs and, PCDDs/PCDFs and DL-PCBs could be influenced by drinking in the pre-pregnancy period as well as that of pregnancy, on the assumption that maternal alcohol intake could affect the elimination rate of these chemicals. However, in this study, history of alcohol consumption had no association with NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels. Therefore, maternal alcohol consumption may reflect subsequent lifestyle patterns during pregnancy that increase concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, rather than indicating an effect on hepatic drug-metabolizing enzymes.

4.4. Food intake

We found that meat, especially beef intake, may be an important exposure source of NDL-PCBs in Japan, similar to that in the US and the Europe (Larsen, 2006). In a Japanese food market study, meat provided the second highest contribution to total daily dietary intake of PCDDs/PCDFs and DL-PCBs (Sasamoto et al., 2006). Our results indicated that fish/seafood, especially pelagic fish, may be an important exposure source for Hg. This is supported by another study that showed that large predatory fish were the largest contributor to total hair Hg among pregnant women in Japan (Yaginuma-Sakurai et al., 2009).

4.5. Other related factors

In agreement with previous studies, a history of parity was associated with decreasing concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS and PFOA, suggesting that

Table 5
Partial regression coefficients (95%CI) for environmental chemical concentrations from mothers who consumed alcohol during pregnancy ($n = 97$).

Quartiles by alcohol intake (n, range in g d^{-1})	NDL-PCBs		PCDDs/PCDFs and DL-PCBs	
	B (95%CI)	p for trend[a]	B (95% CI)	p for trend[a]
Quartile 1 ($n = 26$, <0.73)	Reference	0.802	Reference	0.404
Quartile 2 ($n = 27$, 0.73 – 1.52)	0.07 (−0.05, 0.18)		0.06 (−0.03, 0.16)	
Quartile 3 ($n = 24$, 1.52 – 3.52)	0.07 (−0.05, 0.18)		0.08 (−0.02, 0.17)	
Quartile 4 ($n = 20$, ⩾3.52)	0.00 (−0.12, 0.13)		0.04 (−0.07, 0.14)	

B: partial regression coefficient provides the expected change in the log_{10}-transformed environmental chemical concentrations between quartiles in the regression linear model, adjusted for maternal age, parity, smoking history, fish intake, shoreline fish intake, and beef intake.
[a] Quartiles are represented as ordinal variables.

reproductive events could play a role in elimination of environmental chemicals from the maternal body (Milbrath et al., 2009; Olsen et al., 2009). PFOS and PFOA were inversely associated with gestational age at the time of blood sampling, possibly due to the dilutional effect of plasma volume expansion, especially after the last trimester (Glynn et al., 2007). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal age, which could be explained by previous reports that maternal age might be a good marker for the estimated duration of exposure to chemicals with long half-lives (Milbrath et al., 2009). Hg in hair increased with household income in our study, which is supported by a previous report indicating that high socioeconomic status is related to increased fish consumption, dental amalgams and vaccines, which are all associated with increased exposure to Hg (Tyrrell et al., 2013).

4.6. Strengths and limitations

This study provides useful information on associations between demographic, behavioral, dietary, and socioeconomic characteristics and background concentrations of individual chemicals including NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg during the perinatal period by liner regression models. These characteristics may also influence the level of fetal exposure to environmental chemicals through effects on maternal exposure levels. However, we did not collect data during maternal breastfeeding despite indications that this is an important determinant in the body burden of environmental chemicals (Milbrath et al., 2009). Further studies are also needed to evaluate etiological mechanisms of maternal smoking on the elimination rate of PFOS mediated by PPAα and CYP activation.

In conclusion, most concentrations of individual NDL-PCBs, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg were correlated, especially the association between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, which had similar exposure sources and persistence in the body. PCDDs/PCDFs and DL-PCBs and PFOS decreased with maternal smoking history. NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal alcohol consumption during pregnancy. Total hair Hg increased with household income. Beef and fish/seafood intake may be important exposure sources of NDL-PCBs. These results may reflect various lifestyle patterns associated with exposure sources and elimination rates of these environmental chemicals.

5. Conflicts of Interest

The authors declare they have no competing financial interests. This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Health, Labour and Welfare, and from the Japanese Ministry of Education, Culture, Sports, Science & Technology. The funding sources did not play a role in the study design; the collection, analysis and interpretation of the data; the writing of the report; or the decision to submit the article for publication.

Acknowledgements

We would like to express our gratitude to all study participants and staff members at Sapporo Toho Hospital for their generous collaboration.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.02.062.

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RESEARCH ARTICLE

Effects of Prenatal Leydig Cell Function on the Ratio of the Second to Fourth Digit Lengths in School-Aged Children

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Abstract

Prenatal sex hormones can induce abnormalities in the reproductive system and adversely impact on genital development. We investigated whether sex hormones in cord blood influenced the ratio of the second to fourth digit lengths (2D/4D) in school-aged children. Of the 514 children who participated in a prospective cohort study on birth in Sapporo between 2002 and 2005, the following sex hormone levels were measured in 294 stored cord blood samples (135 boys and 159 girls); testosterone (T), estradiol (E), progesterone, LH, FSH, inhibin B, and insulin-like factor 3 (INSL3). A total of 350 children, who were of school age and could be contacted for this survey, were then requested via mail to send black-and-white photocopies of the palms of both the left and right hands. 2D/4D was calculated in 190 children (88 boys and 102 girls) using photocopies and derived from participants with the characteristics of older mothers, a higher annual household income, higher educational level, and fewer smokers among family members. 2D/4D was significantly lower in males than in females ($p<0.01$). In the 294 stored cord blood samples, T, T/E, LH, FSH, Inhibin B, and INSL3 levels were significantly higher in samples collected from males than those from females. A multivariate regression model revealed that 2D/4D negatively correlated with INSL3 in males and was significantly higher in males with <0.32 ng/mL of INSL3 ($p<0.01$). No correlations were observed between other hormones and 2D/4D. In conclusion, 2D/4D in school-aged children, which was significantly lower in males than in females, was affected by prenatal Leydig cell function.

Citation: Mitsui T, Araki A, Imai A, Sato S, Miyashita C, Ito S, et al. (2015) Effects of Prenatal Leydig Cell Function on the Ratio of the Second to Fourth Digit Lengths in School-Aged Children. PLoS ONE 10(3): e0120636. doi:10.1371/journal.pone.0120636

Academic Editor: Bernhard Fink, University of Goettingen, GERMANY

Received: October 15, 2014

Accepted: January 25, 2015

Published: March 6, 2015

Data Availability Statement: Due to ethical restrictions, data are available to qualified researchers on request from the corresponding author.

Funding: The study was supported by the Ministry of Health, Labour and Welfare, Health and Labour Sciences Research Grants, Grants-in Aid for Scientific Research from the Japan Society for the Promotion of Science, and Environment Research and Technology Development Fund (5C-1252) from the Ministry of the Environment, Japan. The funders had no role in study design, data collection and