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学 位 論 文 題 名

EFFECTS OF MATERNAL SECONDHAND SMOKE EXPOSURE AND GENE POLYMORPHISMS OF *CYP1A1, EPHX1* AND *NAT2* ON INFANT BIRTH SIZE

(妊婦の受動喫煙曝露と*CYP1A1, EPHX1*, *NAT2*遺伝子多型が出生時体格に及ぼす影響)

学位論文内容の要旨

BACKGROUND: The recent high prevalence of low birth weight (LBW, defined as birth weight <2500g) in Japan compared to other developed countries is a major public health concern. Nonsmoking pregnant women are at risk of secondhand smoke (SHS) exposure especially at home due to the high prevalence of smoking among Japanese men. Results of the effects of secondhand smoke (SHS) exposure on infant birth weight observed in previous studies are inconclusive. Also, not all mothers who were prenatally exposed to SHS gave birth to LBW babies. This suggests that genetic susceptibility may be playing a role.

OBJECTIVE: The main aim of this study was to investigate the moderating role of maternal genetic susceptibility in the association of secondhand smoke exposure with infant birth size

METHODS: A prospective cohort study involving 1336 nonsmoking native Japanese pregnant women enrolled for antenatal care at hospitals within Hokkaido Prefecture from 2003 to 2007 was conducted. Self-administered questionnaires and birth records were used for gathering information on maternal and infants' characteristics and lifestyle behaviors. Cotinine measurements were carried out by analyzing biochemically maternal blood specimen using enzyme-linked immunosorbent assay (ELISA) technique. Genetic analyses included extraction of genomic DNA from maternal blood specimen collected at delivery and genotyping using real-time polymerase chain reaction (PCR) technique. Descriptive statistics, analysis of variance (ANOVA), ROC curve and multiple linear regression models were used for the statistical analyses. All statistical analyses were performed using SPSS for Windows, version 16.0.

RESULTS: Exposure to SHS had a marginal association with reductions in the mean birth weight (-32g, SE, 21; $P = 0.07$) and birth length (-0.4cm, SE, 0.2; $P = 0.09$) after adjusting for the confounding variables. Independently, *Tyr/His113* and *His/His113*

genotypes of *EPHX1 Tyr113His* had an adverse effect on the mean birth weight (-59g, $P = 0.039$ and 92g, $P = 0.003$ respectively). However, in the presence of SHS exposure, *CYP1A1*2C* variant genotype conferred an adverse effect on the birth size of the infants of the exposed mothers (mean birth weight reduction = -88g, SE, 37; $P = 0.019$ and birth length reduction = -0.9cm, SE, 0.4; $P = 0.025$). The exposed women with *EPHX1 Tyr113His* homozygous recessive genotypes also had the lowest mean birth weight and birth length (-154g, SE, 42; $P < 0.001$ and 1.1cm, SE, 0.4; $P = 0.010$) respectively. For NAT2*7, the mean reduction in birth weight was 51g (SE, 24; $P = 0.034$) for the exposed slow acetylators.

EPHX1 Tyr113His and *NAT2*7* had a combined effect on the birth weight, birth length and head circumference (145g, SE, 48; $P = 0.003$; 1.1cm, SE, 0.5; $P = 0.003$; 0.9cm, SE, 0.5; $P = 0.052$ respectively) among the exposed *EPHX1* homozygous recessive group and *NAT2*7* slow acetylation genotype. Also, SHS exposure with a combination of *EPHX1 His/His 113* and *NAT2*7* fast acetylation genotype groups reduced birth weight by 180g (SE, 70; $P = 0.010$). Interaction of both genes with SHS exposure had a stronger effect on birth weight. The unexposed showed no statistically significant association between any of the genotypes and birth size reduction.

DISCUSSION: The consistent vulnerability of CYP1A1*2C variant genotypes (A/G +G/G), EPHX1 Tyr113His His/His113 and NAT2*7 slow alleles to the negative effects of tobacco smoke implies a relationship between metabolic genes and SHS exposure. *CYP1A1* is a phase 1 gene whereas *EPHX1* and *NAT2* perform dual functions (activation and detoxification) in xenobiotic metabolism depending on the substrate. The ability of an individual to convert the toxic metabolites of cigarette smoke into less harmful compounds is crucial for reducing their adverse effects on birth outcomes. The missense mutation which occurred in *EPHX1 Tyr113His* at codon 113 of exon 3, however, may lead to a decrease in enzyme activity while that of *CYP1A1*2C* variant genotypes may increase enzyme activity.

This is the first study to demonstrate a significant association between *CYP1A1*2C, EPHX1 Tyr113His* and *NAT2*7* mutant alleles and SHS exposure with infant birth size in the Japanese population.

CONCLUSION: This study has demonstrated that maternal genetic factor (*EPHX1 Tyr113His, NAT2*7* and *CYP1A1*2C*) play an important modifying role in the association between maternal exposure to SHS during pregnancy and birth size among Japanese subjects.

学位論文審査の要旨

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申請者は（I）日本人妊婦の受動喫煙曝露状況（II）妊婦の受動喫煙曝露が出生時体格に及ぼす影響（III）妊婦の受動喫煙曝露とたばこ煙中化学物質の代謝に関与する cytochrome P-450 1A1（*CYP1A1*2C*），epoxide hydrolase 1（*EPHX1* Tyr113His）および N-acetyl transferase 2（*NAT2*6*, *NAT2*7*）遺伝子多型が出生時体格に及ぼす影響を検討した。最初に申請者は論文の構成および第 1 章から第 3 章までの各研究の概要を紹介して，胎児発育に影響を及ぼす妊婦の受動喫煙曝露と遺伝的感受性の関連について述べた。

本研究では，母の *CYP1A1*2C* 遺伝子多型 *A/G+G/G* 型，*EPHX1* 遺伝子多型 *His/His* 型および *NAT2*7* 遺伝子多型 *slow* 型と受動喫煙曝露の交互作用により出生時体格が低下したことが認められ，これら遺伝子多型による代謝の違いが DNA 損傷を引き起こし，胎児発育を阻害したことが示唆された。

引き続き行われた質疑応答では，まず佐藤副査から出生アウトカムに悪影響を及ぼすたばこ煙中化学物質とその代謝に関与する酵素は血糖値など，他の代謝にも影響を与えるのかという質問があった。申請者は，チトクローム P450 やエポキシド加水分解酵素は多環芳香族炭化水素(PAHs)や芳香族アミンなどたばこ煙中化学物質の代謝に関与するが，糖の代謝との関係はないことが考えられると説明した。次いで，藤田副査からたばこ煙中化学物質の代謝を考える場合，これらの中間代謝物は母体と胎児とどちらに影響を与えるのか。もし，母体であれば，代謝物の影響は胎児に影響を及ぼすほど長期なのかという質問があった。申請者は本研究で検討したたばこ煙中化学物質は発がん性が非常に強く，先行研究

で胎盤移行が指摘されている物質であることから，母体のみならず胎児へ影響も強いことが考えられると説明した。有賀副査からは，妊婦の受動喫煙曝露指標としてコチニン値を測定しているが，曝露量と出生時体格に関連はあったのかという質問があった。申請者は母の血漿中コチニン濃度が高くなると，出生時体格が低下する関連が認められたことを説明した。最後に玉城主査から出生時体重，出生時身長と出生時頭囲との間には関連はあったのか。もし関連があったのなら，胎児発育指標として，出生時体重だけを検討しなかったのかという質問があった。申請者は，出生時体重は出生時身長や出生時頭囲と関連しているが，出生時頭囲が小さいと，出生後の児の神経行動発達に影響を与えることが示唆されていることから，妊娠期の受動喫煙曝露が児の健康にどのような影響を与えるかを検討するうえで，必要な指標と考えていることを説明した。

この論文は，妊娠期の能動喫煙のみならず受動喫煙および母の遺伝的感受性が胎児発育に影響を及ぼすことを明らかにした。わが国では欧米諸国と比較して，男性や子育て世代となる若い女性の喫煙率がいまだ高いことが報告されており，今後の禁煙施策の方向性を示すともに，予防医学的研究への発展が期待される。

審査員一同は，これらの成果を高く評価し，大学院課程における研鑽や取得単位なども併せて申請者が博士（医学）の学位を受けるのに充分な資格を有するものと判定した