Long-chain polyunsaturated fatty acids, gestation duration and birth size:
a Mendelian randomization study using *FADS* variants

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**Sources of Support:** This work was supported by the Singapore National Research Foundation under its Translational and Clinical Research (TCR) Flagship Programme and administered by the Singapore Ministry of Health’s National Medical Research Council (NMRC) [NMRC/TCR/004-NUS/2008, NMRC/TCR/012-NUHS/2014]; Singapore Institute for Clinical Sciences; Agency for Science Technology and Research (A\*STAR) and Nestec. The study sponsors were not involved in study design, data collection, analysis and interpretation of data, manuscript writing, and the decision to submit the article for publication. PCC and KMG are supported by the National Institute for Health Research through the NIHR Southampton Biomedical Research Centre and KMG is supported by the European Union's Seventh Framework Programme (FP7/2007-2013), project EarlyNutrition [grant agreement number 289346].

**Short running head:** *FADS* variants and gestation duration: a MR study.

**Abbreviations:**

GUSTO, growing up in Singapore towards healthy outcomes; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; PUFA, polyunsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; *FADS1*, fatty acid desaturase 1; *FADS2*, fatty acid desaturase 2; *FADS3*, fatty acid desaturase 3; SNP, single nucleotide polymorphism; MR, Mendelian randomization.

**Clinical Trial Registration Number**: NCT01174875 on clinicaltrials.gov.

**Abstract**

*Background*: In randomized trials, supplementation of omega-3 (ω3) long-chain polyunsaturated fatty acids (LC-PUFA) during pregnancy has resulted in increased size at birth, attributable to longer gestation.

*Objective*: We examined this finding using a Mendelian randomization approach utilizing fatty acid desaturase (*FADS*) gene variants affecting LC-PUFA metabolism.

*Design*: As part of a tri-ethnic mother-offspring cohort in Singapore, 35 genetic variants in *FADS1*, *FADS2* and *FADS3* were genotyped in 898 mothers and 1,103 offspring. Maternal plasma ω3 and ω6 PUFA levels at 26-28 weeks gestation were measured. Gestation duration was derived from a dating ultrasound scan in early pregnancy and birth date. Birth length and weight were measured. Eight *FADS* variants were selected through tagging-SNP approach and examined in association with PUFA levels, gestation duration among spontaneous labors and birth size, using ethnicity-adjusted linear regressions and survival models that accounted for the competing risks of induced labor and pre-labor caesarean section.

*Results*: Maternal *FADS1* variant rs174546, tagging for 8 other variants located on *FADS1* and *FADS2*, was strongly related to plasma ω6 but not ω3 LC-PUFA concentrations. Offspring and maternal *FADS3* variants were associated with gestation duration among women who had spontaneous labor: each copy of rs174450 minor allele C was associated with a shorter gestation by 2.2 (95% CI: 0.9, 3.4) and 1.9 (0.7, 3.0) days for maternal and offspring variants, respectively. In survival models, rs174450 minor allele homozygotes had reduced time to delivery after spontaneous labor compared with major allele homozygotes (hazard ratio (95% CI): 1.51 (1.18, 1.95) and 1.51 (1.20, 1.89) for maternal and offspring, respectively).

*Conclusions:* Using a Mendelian randomization approach, we observed associations between *FADS* variants and gestation duration. This suggests potential role of LC-PUFAs in gestation duration.

**Keywords:** pregnancy, birth size, fatty acids, cohort studies, Mendelian randomization.

**Introduction**

The omega-3 (ω3) and omega-6 (ω6) long-chain polyunsaturated fatty acids (LC-PUFAs), such as docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (AA, 20:4ω6) are required by the fetus for normal development of the brain, retina and central nervous system (1-3). Experimental and clinical evidence shows that the placenta, a tissue of fetal origin, transfers maternal LC-PUFAs to the fetal circulation (4).

The ω3 and ω6 LC-PUFAs can be provided through the diet or synthetized from their respective precursors, alpha-linolenic (ALA, 18:3ω3) and linoleic (LA, 18:2ω6) acids. ALA and LA cannot be synthesized in mammals and are required in the diet (5). LC-PUFA synthesis from their precursors involves desaturation and elongation (**Supplemental Figure 1**), which are known to be inefficient in humans, especially for ω3 PUFAs (6). LC‑PUFA synthesis is more efficient in women than in men, and may be enhanced during pregnancy (7-9). It is controlled by the rate-limiting enzymes delta-5 and delta-6 desaturases, which are encoded by the *FADS1* and *FADS2* genes, respectively (10, 11). Located on the same gene cluster, *FADS3* is presumed to have a role in PUFA metabolism; its encoded protein and exact function remain unknown (11, 12). Single nucleotide polymorphisms (SNP) within the *FADS* cluster are related to plasma, erythrocyte and breast milk PUFA content and to child health outcomes (11, 13-16).

Meta-analyses of randomized controlled trials suggest that supplementation of ω3 LC-PUFAs or fish oil (rich in ω3 LC-PUFAs) during pregnancy leads to a reduced risk of early preterm delivery and small increases in birth weight and length attributable to a longer gestation (17-19). Most, but not all, observational studies reported similar findings with maternal fish consumption as exposure (20-22). Yet, published RCTs and observational studies have limitations preventing from definitive inferences about causality (23, 24). The published RCTs were carried out in Western populations only, and less is known in other populations where both genetic background and LC-PUFA intake differ. Observational studies, mostly carried out in Western populations as well, are subject to confounding and reverse causality. Varying study designs and approaches may help overcome inherent pitfalls and strengthen the evidence base (25). Mendelian randomization (MR) has been proposed as a method to remove the risk of reverse causality and confounding, by using genetic variants as instrumental variables for the exposure of interest (26).

Using data from the multiethnic mother-offspring cohort Growing Up in Singapore Towards healthy Outcomes (GUSTO), we examined whether maternal and offspring *FADS* gene variants, used as instrumental variables for maternal and antenatal LC-PUFA status, are related to gestation duration and size at birth.

**Subjects and Methods**

*Study population*

Between June 2009 and September 2010, the GUSTO study recruited pregnant women of Chinese, Malay and Indian ethnicities and attending before the 15th week of pregnancy their first ultrasound scan at two public maternity units in Singapore. Women were ineligible for the study if they had non-homogeneous ethnic background (up to the four grandparents of the offspring), had no intention of delivering in the study centers or not staying in Singapore for the 5 years following delivery. The recruitment protocol has been described in detail (27). All participants signed informed written consent at enrolment. The study received ethical approval from the National Healthcare Group Domain Specific Review Board and the SingHealth Centralised Institutional Review Board. From the 1237 pregnant women recruited, 1171 singleton newborns were included. Twins were excluded from the current analysis (Supplemental Figure 2). Information on gestation duration, whether the labor was spontaneous or induced, and offspring sex were abstracted from birth records.

*Maternal PUFA levels*

Fasting blood samples were collected at 26-28 weeks’ gestation. Blood lipid extraction was undertaken with chloroform/methanol (Fisher Scientific, Hampton, NH, USA). Plasma phosphatidylcholine (PC) was separated by solid-phase extraction. Fatty acid methyl esters were prepared by reaction with methanolic sulphuric acid and then separated by gas chromatography (BPX-70 column mounted on a Hewlett-Packard HP6890) and detected by flame ionization before quantification as μg/mL of plasma. Inter- and intra-assay variation coefficients for all fatty acids identified in plasma PC were lower than 6% and 3%, respectively. Eleven PUFAs were identified (Supplemental Figure 1) and expressed as % of total fatty acids. Additional details have been published previously (28).

*Genotyping and selection of FADS variants*

Maternal and offspring DNA were extracted from maternal blood and umbilical cord, respectively. Samples were genotyped using Illumina Omniexpress + exome arrays (Illumina Inc., San Diego, CA, USA). DNA hybridization arrays and subsequent scanning were performed by Expression Analysis Inc. (Morrisville, NC, USA). Data were processed in GenomeStudio Genotyping Module v1.0 (Illumina Inc.).

We focused on *FADS* genes since they were reported being the most strongly related to LC-PUFA status, including in Asian populations (11, 13-16, 29, 30). Maternal and offspring genotypes of 35 variants from the *FADS* genes region (chromosome 11, 11q12-13.1) were available on our arrays. All were located on introns between positions 61569830 and 61656117. Data quality control procedures excluded the following: i) rate of missingness >5% in the whole sample (no variants), ii) monomorphism in the overall cohort (13 variants), iii) minor allele frequency <5% by ethnicity (5 variants), or iv) *p*-value for Hardy-Weinberg Equilibrium test by ethnicity <10-3 (no variants). Seventeen variants were retained after this quality control step (**Supplemental Table 1**). Linkage disequilibrium plots in mothers and offspring are shown in **Supplemental Figures 3** and **4**, respectively. Because high linkage disequilibrium was observed between variants, we then used a tagging-SNP approach with Haploview software v4.2 (Broad Institute of MIT and University of Harvard, Boston, MA, USA). Briefly, this method selects variants that are representative of regions in high linkage disequilibrium, and allows examination of a smaller number of variants that are less likely to be inherited together. Pairwise tagging-SNP selection was performed based on r² using a threshold of ≥0.80. The 17 variants were tagged by 8 variants as follows: rs174546 on *FADS1*, rs2727270, rs174593, rs498793 and rs17156506 on *FADS2*, and rs174450, rs1000778 and rs174455 on *FADS3* (Supplemental Table 1).

*Gestation duration and anthropometric measurements at birth*

Gestational age and date of conception were estimated by ultrasonography when women attended their first dating scan during the first trimester of pregnancy (mean gestational age ± SD: 11.7 ± 1.7 weeks, range: 6.6-17.0). Gestation duration was derived from the estimated dates of conception and of delivery, and was reported in days. The offspring’s anthropometric measures were taken in duplicate by trained hospital staff within a few hours of birth and then averaged. Weight was measured to the nearest 1 g using a calibrated mobile digital scale (Seca 334, Seca GmBH & Co Kg, Hamburg, Germany). Length was measured in the recumbent position to the nearest 1 mm using a mobile infant mat (Seca 210). All research staff were unaware from the participants’ *FADS* genotype and maternal mid-pregnancy PUFA status. Gestational age-specific weight and length z-scores at birth for Singaporeans were derived from a previous reference based on our cohort (31).

*Mendelian randomization (MR)*

This study relies on a MR design, a design proposed to remove reverse causality and confounding (26). **Figure 1** shows the directed acyclic graph (DAG) (24) that illustrates the MR design as applied to our study to estimate the causal effect of LC-PUFA status (exposure E) on gestation duration (outcome O) using *FADS* genetic variants (G) as instrumental variables for E. If G causes E (at least partly), has no effect on O other than through its effect on E, and is not associated with any E→O confounder U, then the relationship E→O can be estimated from the relationship G→O. Although maternal LC-PUFA intake (V) is a major determinant of E, it is not a confounder of the E→O and G→O relationships because it is unrelated to *FADS* variants and must not be controlled for (*FADS* genetic variants do not affect dietary LC-PUFA intake, and vice-versa). Potential confounding in estimating G→O may, however, arise from ethnicity (W), which affects G, E, O, and also V, and therefore should be controlled for.

*Statistical analyses*

As a preliminary analysis, we assessed the validity of using maternal FADS variants as instrumental variables within our population of Asian pregnant women by examining the associations of maternal *FADS* variants with maternal PUFA levels and ratios. This was carried out by linear regression: variants were first introduced into the models as categorical variables; since a codominant effect was observed in heterozygotes, we then used an additive genetic model by introducing variants as ordinal variables. We therefore report regression coefficients for each additional copy of the minor allele (major allele homozygous as reference). PUFA variables were standardized (mean=0, SD=1) to facilitate comparisons of regression coefficients across tests.

Associations of maternal and offspring *FADS* variants with gestation duration were examined by linear regression among the mother-offspring pairs who experienced spontaneous labor (excluding births after labor induction and pre-labor cesarean delivery). In a complementary approach, we examined the associations between *FADS* variants and gestation duration by using competing risks survival models for time to birth. In these models, spontaneous delivery after spontaneous labor was considered as the event of interest, while induced labor and pre-labor cesarean delivery were competing events. Indeed, *FADS* gene variants were associated with type of labor (**Supplemental Table 2**); not accounting for competing events in survival models may result in bias (32). We therefore used both cause-specific proportional hazards models and the Fine–Gray proportional subdistribution hazards models, yielding cause-specific hazard ratios (csHRs) and subdistribution hazard ratios (sdHRs), respectively (32). We reported the HRs for both the event of interest (spontaneous labor) and the competing events (induced labor and pre-labor caesarian delivery) (33). As a prerequisite, we assessed the proportional hazards assumption by plotting the Schoenfeld residuals (cause-specific hazard model) and the cumulative incidence function (subdistribution hazard model). Since potential departures from that assumption were observed, we performed sensitivity analyses by allowing a time-varying effect by adding the time\**FADS* interaction into the models. We plotted the cumulative incidence function when associations of *FADS* variants with gestation duration were observed. Finally, the associations of *FADS* variants with gestational age-adjusted birth size (length and weight) among offspring with birth after spontaneous labor were analyzed by linear regression.

All analyses were carried out both in the full study sample (with adjustment for ethnicity) and after stratification by ethnicity. Previous publication based on genome-wide data in GUSTO reported excellent agreement between self-reported ethnicity and genetic ancestry clustering (34). All statistical analyses were performed with SAS 9.4 (SAS Institute Inc, Cary, NC, USA).

**Results**

The flow diagram for study participation is shown in Supplemental Figure 1. Maternal (*n*=897) and offspring (*n*=1100) genotype frequencies for the 17 variants that passed quality control are shown in **Table 1**. Important frequency differences in *FADS1* and *FADS2* variants were observed by ethnicity; major alleles in Chinese and Malay participants were opposite to those in Indian participants (*p*-value χ² test=10-100 and 10-18 for rs174546 and rs174593, respectively). In Indian participants, the frequency of minor allele homozygotes was generally lower than 1%. *FADS3* variant frequencies were more balanced across ethnicity but remained significantly different (*p*-value=10-10 for rs174450).

Most maternal plasma LC-PUFA levels differed by ethnicity (**Supplemental Table 3**). This was particularly striking for ω6 LC-PUFA levels, which were higher in Indian women, and for ω3 LC-PUFA levels, which were higher in Chinese women. The preliminary analysis comparing maternal plasma PUFA levels by maternal variants confirmed the validity of using *FADS* variants as instrumental variables (**Supplemental Table 4**). Strong associations (*p*-values <10-7) were seen between plasma ω6 PUFA levels and maternal *FADS1* rs174546 and *FADS2* rs2727270 (both being in negative linkage disequilibrium). Minor allele carriers had higher and lower levels of plasma ω6 LC-PUFA levels, respectively. The associations were less strong for the variants located on *FADS3;* minor allele carriers ofrs174450, rs1000778 and rs174455 had lower plasma ω6 LC-PUFA levels (*p*-values between 10-3 and 10-7). In contrast, no association between *FADS* variants and ω3 PUFA levels reached a significance level <10‑3. These associations were, overall, similar by ethnicity (**Supplemental Table 5**).

Among mother-offspring pairs who experienced birth by spontaneous labor, all three maternal *FADS3* variants were associated with gestation duration (**Table 2**). For rs174450, each additional copy of the minor allele C (associated with lower LC-PUFA levels) was associated with a shorter gestation by 2.2 (95% CI: 0.9, 3.4) days, with comparable effect sizes across ethnicity (*P* for interaction=0.92). When considering offspring variants, similar results were observed for *FADS3*; each additional copy of the minor allele of the variant rs174450 was associated with a shorter gestation by 1.9 (0.7, 3.0) days. This association was driven by the Chinese and Indian subgroups, although the test for interaction with ethnicity was not significant (*P* for interaction=0.42). Offspring, but not maternal, *FADS1* variant rs174546 (associated with higher LC-PUFA levels) was also associated with longer gestation duration [1.7 (0.3, 3.0) days per additional copy of the minor allele G], and this association was driven by the Chinese subgroup [2.4 (0.8, 4.1) days]. The other *FADS1* variants and the *FADS2* variants (maternal and fetal) were not associated with gestation duration.

In the cause-specific hazard model considering induced labor and pre-labor caesarean section as competing events of spontaneous labor, mothers homozygous for the minor allele C of rs174450 (decreasing LC-PUFA levels) had an increased cause-specific hazard ratio (csHR (95% CI): 1.51 (1.18, 1.95)) of delivery compared to mothers homozygous for the major allele A (**Table 3**). This was consistent across ethnic groups: 1.44 (1.03, 2.03), 1.46 (0.90, 2.39) and 1.48 (1.08, 2.02) in Chinese, Malay and Indian, respectively. When considering the offspring variants, comparable results were found with *FADS3* variants in the overall population (1.51 (1.20, 1.89) in minor allele homozygotes for rs174450; **Figure 2**); this association was, however, driven by the Chinese subgroup [1.80 (1.31, 2.48)]. No associations were observed with *FADS1* and *FADS2* variants in non-ethnic-stratified analyses. The Fine–Gray subdistribution hazard models yielded comparable results (**Supplemental Table 6**), although with this model, the assumption of proportional hazards was mildly violated; in sensitivity analyses allowing for time-varying effects resulted in similar associations (not shown). Overall, there was no evidence of associations between *FADS* variants and the competing events, with the exception of induced labor which was associated with maternal *FADS2* rs17156506 [csHR (95%): 2.90 (1.19, 7.08)] (**Supplemental Table 7**).

Finally, *FADS1* and *FADS3* genes variants were associated with crude birth length and weight among participants who experienced spontaneous labor. However, this association was almost entirely removed after accounting for gestation duration (**Table 4**).

**Discussion**

In our tri-ethnic mother-offspring cohort, maternal *FADS* variants were associated with maternal ω6 but not ω3 PUFA levels. Among the mothers who experienced spontaneous labor, *FADS3* variants rs174450, rs1000778 and rs174455 were associated with gestation duration, with similar magnitudes across ethnicities. Among offspring born after spontaneous labor, *FADS1* variant rs174546 and all *FADS3* variants were also associated with gestation duration, but less consistently so across ethnicities. In survival models considering induced labor and pre-labor caesarean section as competing events, *FADS3* variants were similarly related to time to delivery after spontaneous labor. Finally, maternal and offspring *FADS1* variant rs174546 and *FADS3* variants were associated with length and weight at birth, but these associations were almost entirely removed after accounting for gestation duration.

Our study includes several novel features. First, ours is the first MR study of associations between genetic variants involved in PUFA metabolism and gestation duration and birth size. In offspring, minor allele of *FADS1* variant rs174546, which is in high linkage disequilibrium with eight other variants located on *FADS1* and *FADS2* (Supplemental Table 1), was associated with longer gestation duration. This finding supports our initial hypothesis and adds new evidence to published meta-analyses of RCTs and observational studies reporting that exposure to LC-PUFAs during pregnancy is associated with duration of gestation (17-19). Our findings also suggest that most of the effect observed on birth size is mediated by gestation duration. However, our study falls short in demonstrating a causal role of DHA on gestation duration because *FADS1* variants were not associated with plasma DHA levels in our cohort, which is in line with recently published genome-wide association studies in Chinese populations (29, 30). A strength of the MR design is to limit potential residual confounding that could arise from the different genetic background and dietary pattern between the three ethnic groups. A second strength of MR is to avoid potential reverse causality issues, although we cannot exclude the possibility that *FADS* variants lead to differences in growth very early in gestation and thus to a differential estimate of gestational age on the dating scan (35).

A second novel aspect of our study is the consistent association of *FADS3* variants with pregnancy outcomes. The function of *FADS3* remains largely unknown but is presumed to be involved in PUFA metabolism (11, 12). It is expressed in many brain areas in baboons and may be expressed in the human placenta (36, 37). It is well known that LC-PUFAs are required by the developing brain. During *Homo sapiens*’s speciation process, an evolutionary trade-off may have occurred to balance the constraints imposed by bipedalism (shorter maternal pelvic region) and encephalization (larger newborn head), thereby increasing maternal mortality. One of these trade-offs may have been shortening gestation and hence reducing the physiological maturity of the offspring at birth compared to other primates, thus leading to increased infant mortality (38). In this context, it is probable that the *FADS* variants maximizing survival of both the mother and the infant have been positively selected throughout human evolution. The fact that human breast milk contains higher levels of the ω3 LC-PUFA DHA than that of other mammals, including the other primates, corroborates such an evolutionary hypothesis (39). Other population genetics studies have shown the *FADS* gene cluster has been under strong selection pressure and may even have played a key role in evolutionary adaptation of modern humans (40-44). We speculate that the *FADS* genes cluster, including *FADS3*, could play enhanced roles during pregnancy, either via placental mechanisms or direct effects on the developing fetus. Mechanistic studies on placental and fetal tissues are required to further explore these speculations.

Our results related to *FADS* variants add new insights into how LC-PUFAs may affect gestation duration. Previous studies have found that ω3 LC-PUFA supplementation prolongs gestation duration. Our findings suggest an additional role of ω6 LC-PUFAs. AA is the precursor of prostaglandins, especially prostaglandin E2, which is involved in cervical ripening and labor initiation and is often administered vaginally to induce labor (45). Animal models report that supplementation with EPA- and DHA-rich food reduces AA metabolite synthesis and might thereby delay labor onset (46). A balance of ω3 and ω6 LC-PUFAs may influence the pathways involved in optimal gestation duration. In our study, LC-PUFA status was measured at mid-pregnancy, which may be a poor proxy for LC-PUFA status at delivery. ω3 LC-PUFA status is also more influenced by dietary intake than by *FADS* variants, thereby weakening those variants as causal instruments in the MR design, particularly in Chinese populations as compared to European populations (29). Using the formula from Brion et al. (47), we calculated that >300,000 participants would been necessary to detect an effect of DHA on gestation duration using rs174546 instrument in our study population. Future longitudinal measurements of AA metabolites in maternal blood, placenta and cord blood should provide a better understanding of the mechanisms differentially regulated by ω3 and ω6 LC-PUFAs. An additional area of investigation will be the competition between the mother and the fetus for sharing limited resources of LC-PUFA. This will help address the issue of supply and demand and its impact on gestation duration and fetal growth.

The main limitation of our study is that *FADS* variants were, in our setting, poor causal instruments of ω3 LC-PUFA status. Additional limitations must be noted. First, MR assumption that there is no horizontal pleiotropy cannot be assessed (48). *FADS* variants might have effects on gestation duration through pathways other than LC-PUFA status. The known determinants of gestation duration are, however, unlikely to be caused by variation in *FADS* genes (49). Second, our sample size was small compared to current standards in genetic epidemiology, and we must therefore lack statistical power, especially when stratifying by ethnic subgroup. Nonetheless, we found no interactions with ethnicity in the full sample, and most observed associations were consistent across ethnic subgroups, thus supporting an ethnic-adjusted pooled approach. Another limitation is that the assumption of proportional hazards was mildly violated in the Fine–Gray subdistribution hazard models. However, results from Fine–Gray analyses, even when misspecified, still provide a useful summary measure approximating the true effect size (50). Furthermore, the results with and without time-varying effects were consistent with those obtained by linear regression restricted to women who experienced spontaneous labor.

Implications of our study are both etiological and clinical. Our findings add further evidence to pre-existing demonstrations of a causal effect of LC-PUFAs on gestation duration. This knowledge may also help improve clinical practices to reduce the risks related to short gestation. Minor allele carriers of *FADS3* variants appear to have an earlier delivery, with an average effect size of 4 (2 to 6) days between major allele homozygotes and minor allele homozygotes. Prolonging gestation to this extent can be of clinical importance for fetal development, and hence for postnatal care and health care costs.

In conclusion, using an MR approach for *FADS* cluster variants, we add new evidence that maternal LC-PUFA status during pregnancy affects gestation duration. In agreement with other studies, we also found that the observed larger size at birth is mainly attributable to longer gestation. We found consistent associations of *FADS3* variants with gestation duration, which are novel and suggest a role for the *FADS3* gene in placental function and/or fetal development. Our study calls for replication of MR design in larger independent epidemiological studies. Further mechanistic work is needed to understand the underlying biological mechanisms.

**Acknowledgement**

The GUSTO study group includes Allan Sheppard, Amutha Chinnadurai, Anne Eng Neo Goh, Anne Rifkin-Graboi, Anqi Qiu, Arijit Biswas, Bee Wah Lee, Birit F.P. Broekman, Boon Long Quah, Borys Shuter, Chai Kiat Chng, Cheryl Ngo, Choon Looi Bong, Christiani Jeyakumar Henry, Cornelia Yin Ing Chee, Yam Thiam Daniel Goh, Doris Fok, Fabian Yap, George Seow Heong Yeo, Helen Chen, Hugo P S van Bever, Iliana Magiati, Inez Bik Yun Wong, Ivy Yee-Man Lau, Jeevesh Kapur, Jenny L. Richmond, Jerry Kok Yen Chan, Joanna D. Holbrook, Joshua J. Gooley, Keith M. Godfrey, Kenneth Kwek, Kok Hian Tan, Krishnamoorthy Niduvaje, Leher Singh, Lin Lin Su, Lourdes Mary Daniel, Lynette P Shek, Marielle V. Fortier, Mark Hanson, Mary Foong-Fong Chong, Mary Rauff, Mei Chien Chua, Michael Meaney, Mya Thway Tint, Neerja Karnani, Ngee Lek, Oon Hoe Teoh, P. C. Wong, Peter D. Gluckman, Pratibha Agarwal, Rob M. van Dam, Salome A. Rebello, Seang-Mei Saw, Shang Chee Chong, Shirong Cai, Shu-E Soh, Sok Bee Lim, Chin-Ying Stephen Hsu, Victor Samuel Rajadurai, Walter Stunkel, Wee Meng Han, Wei Wei Pang, Yap-Seng Chong, Yin Bun Cheung, Yiong Huak Chan and Yung Seng Lee.

**Conflict of Interest Statement:** LS, YSC, PCC and KMG have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. They are part of an academic consortium that has received research funding from Abbott Nutrition, Nestle, and Danone. The other authors have no potential conflicts of interest to disclose.

**Authors’ Contributions**:

JYB, FY, KHT, LS, YSC, PDG, KMG, MFFC, MSK, NK and YSL designed research; HP, IMA, SES and PCC conducted research; JYB, HP, IMA, SES and MFFC provided essential materials; JYB and MMB performed statistical analysis, JYB, MMB and MSK wrote paper; JYB had primary responsibility for final content.

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Table 1. Maternal and offspring *FADS* genotype frequencies by ethnicity in the GUSTO cohort.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SNPs | Position (kb) | Alleles(M/m)1 | Maternal allele frequency by ethnicity(MM / Mm / mm) | Offspring allele frequency by ethnicity(MM / Mm / mm) |
|   |   |   | Chinese*n*=516 | Malay*n*=231 | Indian*n*=151 | Chinese*n*=628 | Malay*n*=277 | Indian*n*=198 |
| *FADS1* |  |  |  |  |  |  |  |  |
| **rs174546** | **61569830** | **A/G** | **0.37 / 0.46 / 0.17** | **0.56 / 0.38 / 0.05** | **0.01 / 0.23 / 0.77** | **0.33 / 0.51 / 0.16** | **0.57 / 0.38 / 0.05** | **0.01 / 0.19 / 0.81** |
| rs174547 | 61570783 | G/A | 0.37 / 0.46 / 0.17 | 0.56 / 0.38 / 0.05 | 0.01 / 0.23 / 0.77 | 0.33 / 0.51 / 0.16 | 0.57 / 0.38 / 0.05 | 0.01 / 0.19 / 0.81 |
| rs174548 | 61571348 | C/G | 0.35 / 0.47 / 0.18 | 0.51 / 0.40 / 0.07 | 0.01 / 0.22 / 0.77 | 0.32 / 0.52 / 0.16 | 0.52 / 0.41 / 0.07 | 0.00 / 0.17 / 0.83 |
| rs174550 | 61571478 | G/A | 0.37 / 0.46 / 0.17 | 0.56 / 0.37 / 0.05 | 0.01 / 0.23 / 0.77 | 0.33 / 0.51 / 0.16 | 0.57 / 0.38 / 0.05 | 0.01 / 0.19 / 0.81 |
| *FADS2* |  |  |  |  |  |  |  |  |
| rs174570 | 61597212 | A/G | 0.36 / 0.46 / 0.18 | 0.55 / 0.37 / 0.06 | 0.00 / 0.15 / 0.85 | 0.32 / 0.52 / 0.16 | 0.53 / 0.40 / 0.07 | 0.01 / 0.13 / 0.86 |
| rs1535 | 61597972 | G/A | 0.37 / 0.46 / 0.17 | 0.57 / 0.37 / 0.05 | 0.01 / 0.21 / 0.78 | 0.33 / 0.51 / 0.16 | 0.57 / 0.38 / 0.05 | 0.01 / 0.19 / 0.81 |
| **rs2727270** | **61603237** | **G/A** | **0.29 / 0.50 / 0.21** | **0.26 / 0.55 / 0.14** | **0.87 / 0.13 / 0.00** | **0.29 / 0.54 / 0.18** | **0.29 / 0.48 / 0.24** | **0.91 / 0.09 / 0.00** |
| rs174576 | 61603510 | A/C | 0.37 / 0.46 / 0.17 | 0.58 / 0.35 / 0.05 | 0.01 / 0.23 / 0.76 | 0.33 / 0.51 / 0.16 | 0.57 / 0.38 / 0.04 | 0.01 / 0.19 / 0.80 |
| rs174577 | 61604814 | A/C | 0.37 / 0.46 / 0.17 | 0.58 / 0.36 / 0.05 | 0.01 / 0.23 / 0.77 | 0.33 / 0.51 / 0.16 | 0.57 / 0.38 / 0.05 | 0.01 / 0.19 / 0.80 |
| rs174583 | 61609750 | A/G | 0.37 / 0.45 / 0.17 | 0.58 / 0.35 / 0.05 | 0.01 / 0.28 / 0.72 | 0.34 / 0.51 / 0.16 | 0.58 / 0.38 / 0.05 | 0.02 / 0.19 / 0.79 |
| rs2851682 | 61616012 | A/G | 0.31 / 0.49 / 0.20 | 0.26 / 0.56 / 0.13 | 0.88 / 0.12 / 0.00 | 0.29 / 0.54 / 0.17 | 0.29 / 0.48 / 0.23 | 0.91 / 0.09 / 0.00 |
| **rs174593** | **61618831** | **A/G** | **0.73 / 0.25 / 0.03** | **0.45 / 0.46 / 0.06** | **0.77 / 0.22 / 0.01** | **0.72 / 0.26 / 0.02** | **0.48 / 0.41 / 0.11** | **0.79 / 0.20 / 0.01** |
| **rs498793** | **61624705** | **G/A** | **0.83 / 0.16 / 0.01** | **0.66 / 0.30 / 0.03** | **0.40 / 0.49 / 0.11** | **0.84 / 0.16 / 0.01** | **0.69 / 0.27 / 0.04** | **0.40 / 0.46 / 0.13** |
| rs174611 | 61627881 | A/G | 0.97 / 0.03 / 0.00 | 0.88 / 0.11 / 0.01 | 0.87 / 0.13 / 0.00 | 0.98 / 0.02 / 0.00 | 0.85 / 0.14 / 0.01 | 0.89 / 0.11 / 0.00 |
| rs174618 | 61629322 | A/G | 0.96 / 0.04 / 0.00 | 0.82 / 0.16 / 0.01 | 0.52 / 0.42 / 0.06 | 0.96 / 0.04 / 0.00 | 0.78 / 0.20 / 0.03 | 0.57 / 0.39 / 0.05 |
| **rs17156506** | **61632913** | **G/A** | **0.85 / 0.13 / 0.01** | **0.77 / 0.21 / 0.01** | **0.95 / 0.05 / 0.00** | **0.85 / 0.15 / 0.00** | **0.78 / 0.21 / 0.01** | **0.96 / 0.04 / 0.00** |
| *FADS3* |  |  |  |  |  |  |  |  |
| **rs174450** | **61641542** | **A/C** | **0.34 / 0.49 / 0.16** | **0.17 / 0.47 / 0.32** | **0.27 / 0.53 / 0.20** | **0.35 / 0.50 / 0.15** | **0.21 / 0.45 / 0.35** | **0.28 / 0.46 / 0.25** |
| **rs1000778** | **61655305** | **G/A** | **0.49 / 0.42 / 0.09** | **0.43 / 0.43 / 0.10** | **0.31 / 0.48 / 0.21** | **0.49 / 0.43 / 0.08** | **0.46 / 0.39 / 0.15** | **0.30 / 0.52 / 0.18** |
| **rs174455** | **61656117** | **A/G** | **0.37 / 0.47 / 0.16** | **0.20 / 0.47 / 0.28** | **0.26 / 0.50 / 0.24** | **0.35 / 0.51 / 0.14** | **0.22 / 0.44 / 0.33** | **0.27 / 0.50 / 0.23** |
| 1 Major (M) and minor (m) alleles were defined based on allele frequencies in the whole study.Tagged SNPs are in bold. |

Table 2. Mean differences in gestation duration according to *FADS* gene variants in mother-offspring pairs from the GUSTO cohort.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| SNPs | Minor allele | All ethnic subgroups2 | Chinese | Malay | Indian | *p*-value for interaction3 |
| Mothers |  | *n*=502 | *n*=284 | *n*=150 | *n*=68 |  |
| rs1745464 (*FADS1*) | G | 0.8 (-0.5, 2.1) | 1.4 (-0.3, 3.1) | -0.6 (-2.6, 1.5) | 0.2 (-6.5, 6.8) | 0.43 |
| rs2727270 (*FADS2*) | A | -0.5 (-1.8, 0.8) | -1.3 (-3.0, 0.4) | 1.1 (-0.8, 3.0) | 0.1 (-8.8, 9.0) | 0.24 |
| rs174593 (*FADS2*) | G | 0.3 (-1.3, 1.9) | 0.5 (-1.8, 2.9) | -0.8 (-2.8, 1.2) | 4.3 (-1.7, 10.3) | 0.23 |
| rs498793 (*FADS2*) | A | -0.1 (-1.8, 1.6) | 1.4 (-1.6, 4.4) | -0.5 (-2.6, 1.7) | -2.0 (-6.0, 2.1) | 0.33 |
| rs17156506 (*FADS2*) | A | 0.3 (-1.8, 2.4) | -0.5 (-3.4, 2.5) | 0.7 (-2.1, 3.4) | 6.4 (-3.9, 16.6) | 0.35 |
| rs174450 (*FADS3*) | C | -2.2 (-3.4, -0.9)\*\*\* | -2.1 (-3.8, -0.4)\* | -2.1 (-4.0, -0.3)\* | -2.9 (-7.2, 1.4) | 0.92 |
| rs1000778 (*FADS3*) | A | -1.6 (-2.8, -0.3)\* | -1.7 (-3.4, 0.1) | -1.4 (-3.2, 0.4) | -1.6 (-5.4, 2.3) | 0.99 |
| rs174455 (*FADS3*) | G | -1.9 (-3.1, -0.8)\*\* | -1.9 (-3.6, -0.3)\* | -1.8 (-3.6, -0.1)\* | -2.4 (-6.3, 1.6) | 0.97 |
| Offspring |  | *n*=601 | *n*=337 | *n*=174 | *n*=90 |  |
| rs1745464 (*FADS1*) | G | 1.7 (0.3, 3.0)\* | 2.4 (0.8, 4.1)\*\* | 0.3 (-1.7, 2.4) | -0.3 (-7.4, 6.8) | 0.31 |
| rs2727270 (*FADS2*) | A | -1.0 (-2.3, 0.3) | -2.0 (-3.7, -0.3)\* | 0.6 (-1.1, 2.2) | -1.1 (-13.7, 11.6) | 0.18 |
| rs174593 (*FADS2*) | G | -0.7 (-2.1, 0.8) | -1.5 (-3.6, 0.6) | -0.8 (-2.5, 0.9) | 3.3 (-2.5, 9.2) | 0.14 |
| rs498793 (*FADS2*) | A | 0.6 (-1.1, 2.2) | 0.3 (-2.4, 2.9) | 0.7 (-1.4, 2.9) | 0.7 (-3.8, 5.2) | 0.97 |
| rs17156506 (*FADS2*) | A | 0.8 (-1.3, 3.0) | 0.3 (-2.5, 3.1) | 0.8 (-1.9, 3.4) | 6.3 (-5.3, 17.8) | 0.43 |
| rs174450 (*FADS3*) | C | -1.9 (-3.0, -0.7)\*\* | -2.4 (-4.0, -0.9)\*\* | -0.7 (-2.4, 0.9) | -2.2 (-6.1, 1.8) | 0.42 |
| rs1000778 (*FADS3*) | A | -2.0 (-3.3, -0.8)\*\* | -2.4 (-4.1, -0.7)\*\* | -1.1 (-2.9, 0.6) | -2.8 (-7.0, 1.5) | 0.60 |
| rs174455 (*FADS3*) | G | -1.9 (-3.1, -0.8)\*\* | -2.8 (-4.3, -1.2)\*\*\* | -0.5 (-2.2, 1.1) | -2.1 (-6.1, 2.0) | 0.25 |
| 1 Values are mean differences (95% CI) in gestation duration (in days) per copy of the minor allele among participants who experienced a spontaneous labor. Estimates were assessed by linear regression. *p*-values: \*<0.05, \*\*<0.01, \*\*\*<0.001.2 Models in all ethnic subgroups were adjusted for ethnicity.3 *p*-value for interaction of the SNP with ethnicity.4 rs174546 is in negative linkage disequilibrium with the other SNPs. |

Table 3. Cause-specific hazard ratios for spontaneous delivery according to *FADS* gene variants in mother-offspring pairs from the GUSTO cohort.1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | All ethnic subgroups2 | Chinese | Malay | Indian |
| SNPs | Minor allele | Mm | mm | Mm | mm | Mm | mm | Mm + mm3 |
| Mothers |  | *n*=897 | *n*=516 | *n*=231 | *n*=150 |
| rs1745464 (*FADS1*) | G | 0.89 (0.73, 1.09) | 0.82 (0.62, 1.08) | 0.82 (0.63, 1.06) | 0.70 (0.50, 0.99)\* | 1.08 (0.77, 1.51) | 1.06 (0.55, 2.05) | 1.06 (0.79, 1.43) |
| rs2727270 (*FADS2*) | A | 1.02 (0.83, 1.26) | 1.14 (0.88, 1.49) | 1.09 (0.83, 1.43) | 1.44 (1.04, 1.99)\* | 0.93 (0.64, 1.35) | 0.78 (0.48, 1.26) | 0.89 (0.60, 1.32) |
| rs174593 (*FADS2*) | G | 1.16 (0.96, 1.41) | 0.97 (0.61, 1.55) | 0.99 (0.76, 1.30) | 1.17 (0.55, 2.48) | 1.36 (0.97, 1.91) | 0.97 (0.54, 1.76) | 1.18 (0.90, 1.55) |
| rs498793 (*FADS2*) | A | 0.94 (0.76, 1.17) | 1.48 (0.90, 2.45) | 0.81 (0.58, 1.12) | 1.85 (0.59, 5.79) | 1.13 (0.79, 1.61) | 3.42 (1.64, 7.11)\*\* | 0.87 (0.68, 1.11) |
| rs17156506 (*FADS2*) | A | 1.17 (0.92, 1.50) | 1.49 (0.66, 3.34) | 1.31 (0.93, 1.84) | 1.97 (0.73, 5.33) | 1.04 (0.71, 1.52) | 1.12 (0.28, 4.56) | 1.04 (0.66, 1.64) |
| rs174450 (*FADS3*) | C | 1.11 (0.90, 1.37) | 1.51 (1.18, 1.95)\*\* | 1.06 (0.82, 1.38) | 1.44 (1.03, 2.03)\* | 0.95 (0.58, 1.53) | 1.46 (0.90, 2.39) | 1.48 (1.08, 2.02)\* |
| rs1000778 (*FADS3*) | A | 1.04 (0.86, 1.25) | 1.35 (1.03, 1.78)\* | 0.93 (0.73, 1.20) | 1.46 (0.98, 2.18) | 1.09 (0.77, 1.54) | 1.25 (0.77, 2.04) | 1.38 (1.05, 1.83)\* |
| rs174455 (*FADS3*) | G | 1.08 (0.88, 1.33) | 1.42 (1.11, 1.82)\*\* | 1.04 (0.80, 1.35) | 1.33 (0.95, 1.85) | 0.97 (0.63, 1.49) | 1.40 (0.89, 2.2) | 1.46 (1.08, 1.99)\* |
| Offspring |  | *n*=1100 | *n*=626 | *n*=277 | *n*=197 |
| rs1745464 (*FADS1*) | G | 0.94 (0.78, 1.14) | 0.77 (0.59, 1.01) | 0.96 (0.76, 1.22) | 0.66 (0.47, 0.93)\* | 0.94 (0.69, 1.29) | 1.04 (0.53, 2.06) | 1.05 (0.80, 1.39) |
| rs2727270 (*FADS2*) | A | 1.06 (0.87, 1.29) | 1.10 (0.85, 1.42) | 1.28 (1.00, 1.65) | 1.20 (0.85, 1.68) | 0.80 (0.56, 1.14) | 0.95 (0.63, 1.42) | 0.78 (0.50, 1.23) |
| rs174593 (*FADS2*) | G | 1.07 (0.89, 1.28) | 1.33 (0.91, 1.93) | 1.13 (0.88, 1.44) | 1.87 (0.92, 3.79) | 0.93 (0.68, 1.29) | 1.16 (0.73, 1.86) | 1.08 (0.85, 1.37) |
| rs498793 (*FADS2*) | A | 1.04 (0.85, 1.27) | 1.07 (0.68, 1.69) | 1.03 (0.77, 1.39) | 1.61 (0.60, 4.34) | 1.08 (0.77, 1.50) | 1.26 (0.61, 2.58) | 0.94 (0.76, 1.16) |
| rs17156506 (*FADS2*) | A | 1.10 (0.88, 1.37) | 2.41 (0.89, 6.48) | 1.18 (0.89, 1.58) | 3.89 (0.54, 27.77) | 0.94 (0.64, 1.38) | 1.85 (0.59, 5.81) | 1.11 (0.73, 1.68) |
| rs174450 (*FADS3*) | C | 1.22 (1.01, 1.48)\* | 1.51 (1.20, 1.89)\*\*\* | 1.27 (0.99, 1.61) | 1.80 (1.31, 2.48)\*\*\* | 1.29 (0.86, 1.94) | 1.45 (0.96, 2.18) | 0.94 (0.74, 1.20) |
| rs1000778 (*FADS3*) | A | 1.15 (0.97, 1.36) | 1.28 (0.98, 1.68) | 1.10 (0.88, 1.37) | 1.64 (1.10, 2.44)\* | 1.20 (0.87, 1.66) | 1.12 (0.70, 1.77) | 1.13 (0.89, 1.43) |
| rs174455 (*FADS3*) | G | 1.17 (0.97, 1.42) | 1.40 (1.11, 1.77)\*\* | 1.20 (0.95, 1.52) | 1.65 (1.19, 2.30)\*\* | 1.29 (0.87, 1.92) | 1.39 (0.93, 2.09) | 0.94 (0.74, 1.19) |
| 1 Values are cause-specific hazard ratios (95% CI) for the incidence of birth by spontaneous labor for heterozygotes Mm and minor allele homozygotes mm (as compared to major allele homozygotes MM). Estimates were assessed by cause-specific proportional hazards model. *p*-values: \*<0.05, \*\*<0.01, \*\*\*<0.001.2 Models in all ethnic subgroups were adjusted for ethnicity.3 In Indians, homozygotes for the minor allele were too few and were therefore combined with the heterozygote group.4 rs174546 is in negative linkage disequilibrium with the other SNPs. |

Table 4. Mean differences in birth length and weight according to *FADS* gene variants in mother-offspring pairs from the GUSTO cohort.1

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Birth length | Birth weight |
| SNPs | Minor allele | Unadjusted2, cm | Gestational age-specific z-score | Unadjusted2, g | Gestational age-specific z-score |
| Mothers |  | *n*=502 | *n*=502 |
| rs1745463 (*FADS1*) | G | 0.33 (0.03, 0.62)\* | 0.10 (-0.04, 0.23) | 47 (-10, 104) | 0.06 (-0.08, 0.19) |
| rs2727270 (*FADS2*) | A | -0.34 (-0.63, -0.05)\* | -0.11 (-0.25, 0.02) | -42 (-98, 14) | -0.06 (-0.19, 0.07) |
| rs174593 (*FADS2*) | G | 0.02 (-0.34, 0.37) | 0.01 (-0.15, 0.17) | -3 (-71, 66) | -0.02 (-0.18, 0.14) |
| rs498793 (*FADS2*) | A | 0.24 (-0.14, 0.62) | 0.15 (-0.02, 0.33) | 73 (0, 147) | 0.24 (0.07, 0.41)\*\* |
| rs17156506 (*FADS2*) | A | -0.52 (-0.99, -0.05)\* | -0.30 (-0.52, -0.08)\*\* | -58 (-149, 33) | -0.16 (-0.38, 0.05) |
| rs174450 (*FADS3*) | C | -0.35 (-0.63, -0.07)\* | 0.00 (-0.13, 0.13) | -66 (-120, -12)\* | 0.00 (-0.13, 0.12) |
| rs1000778 (*FADS3*) | A | -0.17 (-0.45, 0.11) | 0.05 (-0.08, 0.18) | -45 (-99, 9) | -0.01 (-0.13, 0.12) |
| rs174455 (*FADS3*) | G | -0.37 (-0.63, -0.10)\*\* | -0.03 (-0.15, 0.10) | -75 (-127, -23)\*\* | -0.05 (-0.17, 0.07) |
| Offspring |  | *n*=601 | *n*=601 |
| rs1745463 (*FADS1*) | G | 0.52 (0.22, 0.81)\*\*\* | 0.15 (0.02, 0.28)\* | 66 (9, 122)\* | 0.05 (-0.08, 0.18) |
| rs2727270 (*FADS2*) | A | -0.28 (-0.57, 0.00) | -0.05 (-0.17, 0.08) | -32 (-86, 22) | 0.02 (-0.10, 0.15) |
| rs174593 (*FADS2*) | G | -0.31 (-0.62, 0.01) | -0.16 (-0.30, -0.02)\* | -34 (-94, 27) | -0.09 (-0.23, 0.05) |
| rs498793 (*FADS2*) | A | 0.13 (-0.23, 0.49) | 0.03 (-0.13, 0.19) | 63 (-5, 131) | 0.14 (-0.02, 0.29) |
| rs17156506 (*FADS2*) | A | -0.34 (-0.80, 0.12) | -0.20 (-0.41, 0.00) | -33 (-121, 55) | -0.09 (-0.30, 0.11) |
| rs174450 (*FADS3*) | C | -0.48 (-0.73, -0.23)\*\*\* | -0.13 (-0.25, -0.02)\* | -63 (-111, -15)\* | -0.05 (-0.16, 0.06) |
| rs1000778 (*FADS3*) | A | -0.33 (-0.60, -0.06)\* | -0.03 (-0.15, 0.09) | -57 (-109, -5)\* | -0.04 (-0.16, 0.08) |
| rs174455 (*FADS3*) | G | -0.43 (-0.68, -0.17)\*\* | -0.10 (-0.21, 0.02) | -77 (-125, -28)\*\* | -0.09 (-0.20, 0.02) |
| 1Values are mean differences (95% CI) in birth length and weight per copy of the minor allele among participants who experienced a spontaneous labor. Estimates were assessed by linear regression. *p-*values: \*<0.05, \*\*<0.01, \*\*\*<0.001.2Models were adjusted for ethnicity. 3rs174546 is in negative linkage disequilibrium with the other SNPs. |

**Figure legends**

Figure 1. Directed acyclic graph (DAG) representing the Mendelian randomization approach to estimate the causal effect of LC-PUFA status (E) on gestation duration (O) using *FADS* genetic variants (G) as instrumental variables. U, V and W denote potential unmeasured confounders, maternal dietary PUFA intake and ethnicity, respectively. Unmeasured factors (U) confound the relationship E🡪O, but not G🡪O. Dietary PUFA intake (V) is related to O only through E and therefore cannot confound the E🡪O or G🡪O relationships. Ethnicity (W) affects LC-PUFA status (E) through FADS variants (G) and dietary PUFA intake (V) and is therefore a confounder of both the E🡪O and G🡪O relationships.

Figure 2. Cumulative incidence of the event of delivery after spontaneous labor according to offspring *FADS3* rs174450 variant (major and minor alleles are A and C, respectively). Cumulative incidence function estimates were derived from cause-specific proportional hazards model adjusted for ethnicity (*n*=1100).



Figure 1.



Figure 2.