SNP Associations with Preterm Delivery: A Case-Control Replication Study and Meta-Analysis

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Running title

SNP Associations with Preterm Birth

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Conflicts of Interest

There is no conflict of interest to disclose.

Abstract

Background:

To replicate SNP associations with preterm birth (PTB, birth <37 completed weeks of gestation) and synthesise currently available evidence using meta-analysis.

Methods:

Spontaneous PTB cases and controls were selected from an existing cohort. Candidate SNPs were taken from an existing genotype panel. A systematic review was conducted for each SNP in the panel to determine suitability as a PTB candidate. Those with significant associations previously reported in Caucasians were selected for replication. Candidate SNPs were already genotyped in cases and controls and clinical data accessed from state perinatal and cerebral palsy databases. Association analysis was conducted between each SNP and PTB and meta-analysis conducted if there were ≥three studies in the literature. Maternal and fetal SNPs were considered as separate candidates.

Results:

A cohort of 170 cases and 583 controls was formed. Eight SNPs from the original panel of genotyped SNPs were selected as PTB candidates and replication based on systematic literature review results. In our cohort, fetal Factor V Leiden (FVL) was significantly associated with PTB (OR 2.6, 95% CI 1.31-5.17) and meta-analysis confirmed this association (OR 2.71, 95% CI 1.15-6.4).

Conclusion:

Replication and meta-analysis support an increased risk of PTB in Caucasians with the fetal FVL mutation.

Introduction

The prevalence of preterm birth (birth at less than 37 completed weeks of gestation) continues to rise around the world with current selected international estimates being 11.1% of all births [1]. Preterm birth is associated with poor outcomes for infants in both short and long terms and is accompanied with increased health care cost [2, 3, 4]. A global health priority is to reduce the incidence of preterm birth [5].

An extensive number of factors contributing to preterm birth have been investigated to date [6] including infection and inflammation related pathways [7], obstetric risks such as cervical insufficiency [8], multiple gestation [9], maternal history of preterm delivery [10], maternal BMI [11] and genetic predisposition [12].

Recurrence of preterm birth across generations and between siblings has lead to the investigation of specific genetic associations with preterm birth [12]. The growing number of SNP associations has been reviewed systematically in 2005 [13] and more recently curated to form an online resource incorporating a meta-analysis component [14, 15]. Many of the associations reported to date lack replication and there is substantial variation between reports including mixed ethnicities, variable cohort sizes, differing genetic models applied and different definitions of preterm birth between studies.

Commencing with an already recruited cohort and associated panel of cerebral palsy candidate SNPs already genotyped, our aim was to systematically review the literature examining each of these genotyped SNPs as a potential candidate for PTB. Where significant associations had been reported to date, these SNPs were considered PTB candidates. Replication of these PTB associations was then attempted in this new cohort and findings combined with those reported in the literature using meta-analysis.

Results

Cohort demographics

From within the Australian Cerebral Palsy Research Study, 170 cases and 583 controls were identified for spontaneous preterm birth analysis. Demographic details for these groups are shown in table 1. Cases of spontaneous preterm birth included 116 children diagnosed with cerebral palsy.

Systematic Review

When systematically searching Pubmed and Embase databases for evidence of SNP association with preterm birth in Caucasians, 1,521 articles were identified (see appendices for selection). 541 papers were removed as duplicates, leaving 980 papers for review of title and abstract. The full text of 78 papers were reviewed and eight SNPs from our panel were selected as replication candidates (see table 2).

SNP associations

Fetal Factor V Leiden (FVL) was significantly associated with spontaneous preterm birth (see table 3; odds ratio 2.6, 95% CI 1.31-5.17, p=0.008). In stratified analysis examining those children who had a diagnosis of cerebral palsy, significant associations were found for fetal FVL (odds ratio 2.65, 95% CI 1.24-5.56, p=0.02). Further stratification of the control group showed similar results (Tables S4 and S5).

Meta-analysis

Meta-analysis was performed for five SNPs (see figure 1; fetal TNF-α 308, fetal TLR4, maternal IL-6, fetal FVL and fetal PGM). With the exception of fetal TNF-α 308, all SNP associations

showed statistical heterogeneity and random effects methods were used for meta-analysis. Fetal FVL was positively associated (odds ratio 2.71, 95 % CI 1.15-6.40) with PTB.

Discussion

Our replication analysis supports the association of fetal FVL with preterm birth and meta-analysis supports a positive association of fetal FVL.

Replication analysis of fetal FVL associations with preterm birth were significant in the overall cohort and also in sub-analysis examining only cases with cerebral palsy (OR 2.69, 95% CI 1.22-5.92, p=0.02). Association was detected for cases specifically without cerebral palsy (OR 2.71, 95% CI 0.87-8.45) however this was not significant (p=0.1), possibly due to the low number of individuals in this strata (n=42).

Our meta-analysis association of fetal FVL with spontaneous preterm birth is based on data from 1,690 cases and 3,324 controls. These data are taken from four published studies (in addition to the analysis we report in this paper). We detected statistical heterogeneity in these data, possibly indicating differences in case or control definitions between the papers included (e.g. Gopel *et al*. [16] define preterm birth as <35 weeks, Gibson *et al.* [17] and the present analysis use <37 weeks). We were unable to further refine our definition of preterm birth for this and other potential sub-analyses due to the small number of studies included.

FVL is a well characterised functional SNP contributing to thrombosis [18]. The association of fetal FVL with preterm birth that we report may indicate a causal relationship between thrombosis and preterm delivery. Our association analysis did not include mechanistic investigations so we can only speculate that FVL may increase the likelihood of placental thrombus formation [19], a risk factor for preterm delivery [20].

The strengths of the present study include a well refined group of candidate SNPs for replication. A larger number of SNPs could have been examined, but we were unable to justify the selection of further candidate SNPs based on the evidence available in the published literature. The analyses we report are strictly replication, and as such do not require statistical correction for multiple testing which is only recommended in exploratory analyses [21], however chance significance may still explain our results. Our analysis also integrates a meta-analysis component with our replication, providing further evidence from a much larger sample size.

The weaknesses of this report include the limited number of SNPs genotyped in the original study. The literature provides evidence supporting other SNPs as candidates for preterm birth (e.g. IL1RN) but we were unable to assess them in this study (resource limitations) [14, 15]. It is possible that publication bias limited the number of SNPs we selected for replication. We also highlight that the proportion of cases diagnosed with cerebral palsy in a not typical of the wider preterm birth population. We acknowledge this limitation and the inclusion of stratification by cerebral palsy diagnosis provides at least partial control for this potential confounder. The majority of reports included in our meta-analysis did not report details cerebral palsy diagnosis preventing us from stratifying our meta-analysis in a similar way. Also, our controls had exclusion criteria of hypertension during pregnancy and growth restriction which were present in a few cases and could confound the data. Gestational age data were obtained from state perinatal databases which typically collect the "best clinical estimate" of gestational age. This estimate includes data from ultrasound in nearly all cases but occasionally uses last menstrual period dating methods only and may possibly lead to inaccuracies in case or control allocation where birth was close to 37 weeks gestation.

This study is limited to the Caucasian population and we acknowledge there are robust candidate SNPs for preterm birth that warrant replication in other ethnic groups. We are unable to generalise the findings we report beyond Caucasians.

Due to the low number of studies reported, meta-analysis was conducted for only 5/8 selected candidate SNPs, highlighting the need for more work in the area of SNP associations with preterm birth.

Despite these limitations, our replication analysis and meta-analysis provide support for the positive association of fetal FVL with preterm delivery in Caucasians. As in many meta-analyses of gene associations, few candidate genes remain associated with the pathology investigated. More sophisticated genetic technologies such as massively parallel sequencing may uncover genetic alterations that have a greater effect on preterm birth.

Methods

Initial cohort

The Australian Cerebral Palsy Research Study cohort was used for this analysis. The original study design is described in detail elsewhere [22]. In summary, mother-child case and control pairs (one child per family) were recruited between July 2008 and March 2010 from around Australia with the following inclusion criteria: children were aged between 5 and 18 years, born in Australia and of Caucasian background. Cases in the initial cohort were defined as children with a diagnosis of cerebral palsy and controls were children without cerebral palsy. Five hundred and eighty-seven case families and 1,154 control families were recruited during the same time period and included in the analysis. Informed consent was obtained from all participants.

Case selection

Preterm birth cases used in this analysis were taken from the Australian Cerebral Palsy Research Study. Preterm birth cases were selected as infants whose gestational age was less than 37 weeks and who delivered spontaneously. Cases included children with cerebral palsy and those without, with separate analysis conducted for each group. Multiple births were included.

Control selection

Controls were also selected from the Australian Cerebral Palsy Research Study. Controls were required to be delivered spontaneously at greater than or equal to 37 weeks gestational age. For the primary analysis, controls included individuals with and without cerebral palsy. Individuals with maternal hypertension during pregnancy or growth restriction less than the 10^{th} centile were excluded.

Clinical data

Clinical data including gestational age, maternal hypertension during pregnancy, birth weight and mode of delivery were provided by state perinatal databases. Cerebral palsy diagnoses were provided by state cerebral palsy registers.

Candidate SNP selection – Systematic Review

Candidate SNPs were selected from those previously screened for association with cerebral palsy [23] (see Table S1). SNPs in the original cerebral palsy candidate panel were predominantly involved in regulation of coagulation and inflammation. All SNPs in the cerebral palsy candidate panel were used in this study with fetal and maternal SNPs considered as separate candidates. For each SNP, we conducted a systematic literature review, assessing the evidence for their association with preterm birth. The search strategy was developed by one author with

assistance from a university research librarian and considered only full papers published in English. Papers up to December 2008 were retrieved from an already published online data base of associations [15]. Relevant references within these papers were also selected. Papers from 2008 until June 2012 were searched using the same strategy (see supplementary material for details of search terms). Relevant references within these papers were also selected. For inclusion in our candidate panel, positive SNP associations must have been reported with PTB in at least one previous case-control study, and the reported study must have examined a Caucasian population. DNA samples were taken from a Caucasian population and we could therefore only attempt to replicate SNP associations that had previously been reported in Caucasians. Guidelines for replication were taken from a published international consensus statement [21].

Genotyping

DNA samples were collected using buccal swabs. SNP genotyping was performed by the Australian Genome Research Facility (AGRF, Brisbane Node, Brisbane, Australia) on a MassARRAY iPLEX Gold System (Sequenom, San Diego, CA).

Analysis

SNP association testing

Analysis was conducted using the package 'SNPassoc' in the R environment (R Foundation for Statistical Computing, Vienna, Austria) [24]. All association tests conducted are reported with odds ratio, 95% confidence intervals and p values. Association tests were conducted using the genetic model originally reported for each candidate SNP. SNPs were assessed for Hardy-Weinberg equilibrium and genotype failure rate. Genotype counts for cases and controls are

reported. Replication analyses followed international guidelines and did not include statistical correction for multiple testing [21].

Meta-analysis

Meta analysis was conducted where three or more associations had been reported (including this report) and was performed using Review Manager Software version 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). For each study included in the meta-analysis the number of cases and controls, minor and major allele counts, effect measures with confidence intervals and genetic models used was extracted. Details for study quality assessment were also extracted for recommended checklists [25]. Fixed effects analysis was used where there was no evidence for heterogeneity ($p>0.05$, Chi squared test) and a random effects analysis used when heterogeneity was significant. Forest plots were used to assess publication bias for each meta-analysis.

Ethics

This study was approved by the Women's and Children's Hospital Human Research Ethics Committee.

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Figure Legends

Figure 1. Forest plots for meta-analysis of SNPs and preterm birth.

M-H - Mantel-Haenszel

a. TNF-α 308 and PTB

b. TLR4 and PTB

c. FVL and PTB

d. Maternal IL-6 and PTB

e. PGM and PTB

black square – effect estimate and weight of each study

black diamond – overall effect