Single-nucleotide polymorphism associations with preterm delivery: a case–control replication study and meta-analysis

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BACKGROUND: The aim of this study was to replicate single-nucleotide polymorphism (SNP) associations with preterm birth (PTB; birth at <37 completed weeks of gestation) and synthesize 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 were accessed from state perinatal and cerebral palsy databases. Association analysis was conducted between each SNP and PTB, and meta-analysis was conducted if there were ≥3 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 for replication on the basis of systematic literature review results. In our cohort, fetal factor V Leiden (FVL) was significantly associated with PTB (odds ratio (OR): 2.6, 95% confidence interval (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.

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

An extensive number of factors contributing to PTB 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 PTB across generations and between siblings has led to the investigation of specific genetic associations with PTB (12). The growing number of single-nucleotide polymorphism (SNP) associations was reviewed systematically in 2005 (13) and more recently was 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 PTB 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 were 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 PTB analysis. Demographic details for these groups are shown in **Table 1**. Cases of spontaneous PTB included 116 children diagnosed with cerebral palsy.

Systematic Review

When systematically searching the PubMed and Embase databases for evidence of SNP association with PTB in Caucasians, 1,521 articles were identified (see appendixes for selection). A total of 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 (**Table 2**).

SNP Associations

Fetal factor V Leiden (FVL) was significantly associated with spontaneous PTB (**Table 3**; odds ratio (OR): 2.6, 95%

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Table 1. Demographics of cases and controls used for preterm birth analysis

	Cases	Controls
<i>n</i>	170	583
Mean gestational age (wk): mean (SD, range)	31 (4, 23–37)	39.8 (1.1, 37–43)
Mean birth weight (g): mean (SD, range)	1,783 (793, 595–4,000)	3,552 (441, 1,460–5,060)
Cerebral palsy: <i>n</i> (%)	123 (72)	101 (17)
Male: <i>n</i> (%)	92 (54)	289 (50)
Plurality: <i>n</i> (%)	33 (20)	4 (0.7)
Mean maternal age at birth in years: mean (SD, range)	32 (5, 22–45)	31 (4.9, 16–47)
Apgar score at 1 min <4: <i>n</i> (%)	13 (11)	20 (4)
Mean birth weight centile: mean (SD, range)	51 (32, 0–100)	58 (25, 10–100)
Maternal hypertension: <i>n</i> (%)	7 (4)	0 (0)

Table 2. SNPs identified from systematic review to have previously reported associations with preterm birth in Caucasians

SNP	Gene	Maternal/fetal	Previous association	References
rs1800629	<i>TNF-α 308</i>	Fetal	Yes	26–29 ^a
rs4986790	<i>TLR4 299</i>	Fetal	Yes	30–31
rs1800795	<i>IL-6–174</i>	Maternal	Yes	32–33
rs6103	<i>PAI-2_2</i>	Fetal	Yes	17 ^b
rs1800576	<i>THBD</i>	Fetal	Yes	17
rs6025	<i>FVL</i>	Fetal	Yes	16,17,33
rs1799963	<i>PGM</i>	Fetal	Yes	16,17,33
rs4961	<i>ADD1</i>	Fetal	Yes	17

ADD1, adducin 1; CI, confidence interval; FVL, factor V Leiden; IL-6, interleukin 6; OR, odds ratio; PAI-2, plasminogen activator inhibitor 2; PGM, prothrombin gene mutation; SNP, single-nucleotide polymorphism; THBD, thrombomodulin; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α.

^aSignificance reported using Fisher's exact test on genotype contingency table.

Analysis conducted using dominant genetic model to allow meta-analysis.

^bSignificance reported using Fisher's exact test on genotype contingency table.

Analysis conducted using recessive genetic model to allow meta-analysis.

confidence interval (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 (OR: 2.65, 95% CI: 1.24–5.56, $P = 0.02$). Further stratification of the control group showed similar results (**Supplementary Tables S1 and S2** online).

Meta-analysis

Meta-analysis was performed for five SNPs (**Figure 1**; fetal tumor necrosis factor (TNF)-α 308, fetal Toll-like receptor (TLR)4, maternal interleukin (IL)-6, fetal FVL, and fetal

prothrombin gene mutation). 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 (OR: 2.71, 95% CI: 1.15–6.40) with PTB.

DISCUSSION

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

Replication analysis of fetal FVL associations with PTB were significant in the overall cohort and also in subanalysis examining only cases with cerebral palsy (OR: 2.69, 95% CI: 1.22–5.92, $P = 0.02$). An 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 PTB 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 article). We detected statistical heterogeneity in these data, possibly indicating differences in case or control definitions between the papers included (e.g., Göpel *et al.* (16) define PTB as <35 wk, whereas Gibson *et al.* (17) and the present analysis use <37 wk). We were unable to further refine our definition of PTB for this and other potential subanalyses due to the small number of studies included.

FVL is a well-characterized functional SNP contributing to thrombosis (18). The association of fetal FVL with PTB 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 recommended only in exploratory analyses (21). However, chance statistical 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 PTB (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 is not typical of the wider PTB 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

Table 3. SNP associations with spontaneous preterm birth (controls with and without CP)

SNP	Cases without CP					Cases with CP					Cases with and without CP				
	Genotype	Count		OR (95% CI)	P value ^a	Genotype	Count		OR (95% CI)	P value ^a	Genotype	Count		OR (95% CI)	P value ^a
		Cases	Controls				Cases	Controls				Cases	Controls		
Fetal TNF- α 308 (rs1800629) ^b	GG	32	370			GG	78	370			GG	110	370		
	GA	14	196	0.76 (0.4–1.46)	0.41	GA	36	196	0.94 (0.62–1.42)	0.77	GA	50	196	0.89 (0.62–1.28)	0.52
	AA	0	16			AA	6	16			AA	6	16		
Fetal TLR4 299 (rs4986790)	AA	40	511			AA	111	511			AA	151	511		
	AG	7	69	1.25 (0.55–2.85)	0.53	AG	12	69	0.78 (0.41–1.48)	0.70	AG	19	69	0.91 (0.53–1.54)	0.92
	GG	0	1			GG	0	1			GG	0	1		
Maternal IL-6 174 (rs1800795)	GG	19	189			GG	51	189			GG	70	189		
	GC	16	286	1.0 (0.66–1.53)	1.0	GC	54	286	0.76 (0.57–1.01)	0.06	GC	70	286	0.83 (0.64–1.06)	0.13
	CC	12	103			CC	17	103			CC	29	103		
Fetal PAI2_2 (rs6103) ^c	CC	31	344			CC	76	344			CC	107	344		
	CG	13	199	0.95 (0.22–4.13)	0.94	CG	44	199	0.86 (0.61–1.22)	0.40	CG	57	199	0.51 (0.18–1.48)	0.18
	GG	2	26			GG	2	26			GG	4	26		
Fetal THBD (rs1800576)	GG	46	570			GG	123	570			GG	169	570		
	AG	0	7	0 (NA)	1	AG	0	7	0 (NA)	0.61	AG	0	7	0 (NA)	0.36
	GG	43	561			GG	111	561			GG	154	561		
Fetal FVL (rs6025)	GA	4	21	2.49 (0.82–7.57)	0.14	GA	11	21	2.65 (1.24–5.56)	0.02	GA	15	21	2.6 (1.31–5.17)	0.008
	GG	46	567			GG	117	567			GG	163	567		
	GA	1	15	0.82 (0.11–6.36)	0.85	GA	6	15	1.94 (0.74–5.1)	0.2	GA	7	15	1.62 (0.65–4.05)	0.31
Fetal PGM (rs1799963)	GG	32	372			GG	73	372			GG	105	372		
	GT	13	179	0.78 (0.44–1.37)	0.37	GT	45	179	1.06 (0.75–1.48)	0.75	GT	58	179	0.98 (0.72–1.32)	0.87
	TT	1	27			TT	3	27			TT	4	27		

ADD1, adducin 1; CI, confidence interval; CP, cerebral palsy; FVL, factor V Leiden; IL-6, interleukin 6; NA, not applicable; OR, odds ratio; PAI-2, plasminogen activator inhibitor 2; PGM, prothrombin gene mutation; SNP, single-nucleotide polymorphism; THBD, thrombomodulin; TLR4, Toll-like receptor 4; TNF- α , tumor necrosis factor- α .

^aLog additive model unless otherwise stated. ^bDominant genetic model. ^cRecessive genetic model.

Figure 1. Forest plots for meta-analysis of SNPs and preterm birth. (a) TNF- α 308 and PTB. (b) TLR4 and PTB. (c) FVL and PTB. (d) Maternal IL-6 and PTB. (e) PGM and PTB. Solid squares, effect estimate and weight of each study; solid diamonds, overall effect. CI, confidence interval; FVL, factor V Leiden; IL-6, interleukin 6; M-H, Mantel-Haenszel; OR, odds ratio; PGM, prothrombin gene mutation; PTB, preterm birth; SNP, single-nucleotide polymorphism; TLR4, Toll-like receptor 4; TNF- α , tumor necrosis factor- α .

in our meta-analysis did not report details of cerebral palsy diagnosis, preventing us from stratifying our meta-analysis in a similar way. In addition, 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 wk gestation.

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

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

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 PTB.

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 y, and were 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 defined as children without cerebral palsy. A total of 587 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

PTB cases used in this analysis were taken from the Australian Cerebral Palsy Research Study. PTB cases were selected as infants whose gestational age was less than 37 wk and who were 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 ≥ 37 wk 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 10th 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) (**Supplementary Table S3** online). 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 PTB. 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 database 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 Table S4** online 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 attempt to replicate only 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 (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 ORs, 95% CIs, 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 CIs, and genetic models used were extracted. Details for study quality assessment were also extracted for recommended checklists (**Supplementary Table S5** online) (25). Fixed effects analysis was used where there was no evidence for heterogeneity ($P > 0.05$, χ^2 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.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

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