DOI: 10.1111/j.1471-0528.2007.01653.x
www.blackwellpublishing.com/bjog

Fetal exposure to herpesviruses may be associated with pregnancy-induced hypertensive disorders and preterm birth in a Caucasian population*

CS Gibson,[a] PN Goldwater,[b] AH MacLennan,[a] EA Haan,[c] K Priest,[d] GA Dekker[a] for the South Australian Cerebral Palsy Research Group

[a] Discipline of Obstetrics and Gynaecology, The University of Adelaide, Adelaide, South Australia, Australia [b] Discipline of Microbiology and Infectious Diseases and [c] Discipline of Genetic Medicine, Children, Youth and Women's Health Service, Adelaide, South Australia, Australia [d] Epidemiology Branch, Department of Health, Adelaide, South Australia, Australia
Correspondence: Dr CS Gibson, Department of Obstetrics and Gynaecology, The University of Adelaide, Women's and Children's Hospital, 1st Floor Queen Victoria Building, 72 King William Road, North Adelaide 5006, Adelaide, South Australia. Email catherine.s.gibson@adelaide.edu.au

Accepted 6 December 2007.

Objective To investigate the role of fetal viral infection in the development of a range of adverse pregnancy outcomes (APOs), including pregnancy-induced hypertensive disorders (PIHD), antepartum haemorrhage (APH), birthweight <10th percentile (small for gestational age, SGA) and preterm birth (PTB).

Design Population-based case–control study.

Setting Laboratory-based study.

Population The newborn screening cards of 717 adverse pregnancy cases and 609 controls.

Methods Newborn screening cards were tested for RNA from enteroviruses and DNA from herpesviruses using polymerase chain reaction (PCR). The herpesviruses were detected using two PCRs, one detecting nucleic acids from herpes simplex virus (HSV)-1, HSV-2, Epstein–Barr virus (EBV), cytomegalovirus (CMV) and human herpesvirus (HHV)-8, hereafter designated Herpes PCR group A viruses, and the other detecting nucleic acids from varicella-zoster virus (VZV), HHV-6 and HHV-7, hereafter designated Herpes PCR group B viruses.

Main outcome measure Odds ratios and 95% CIs for specific APOs.

Results For both term and PTBs, the risk of developing PIHD was increased in the presence of DNA from Herpes PCR group B viruses (OR 3.57, 95% CI 1.10–11.70), CMV (OR 3.89, 95% CI 1.67–9.06), any herpesvirus (OR 5.70, 95% CI 1.85–17.57) and any virus (OR 5.17, 95% CI 1.68–15.94). The presence of CMV was associated with PTB (OR 1.61, 95% CI 1.14–2.27). No significant association was observed between SGA or APH and exposure to viral infection.

Conclusions Fetal exposure to herpesvirus infection was associated with PIHD for both term and PTBs in this exploratory study. Exposure to CMV may also be associated with PTB. These findings need confirmation in future studies.

Keywords Adverse pregnancy outcomes, fetal viral infection, pregnancy-induced hypertension, preterm birth.

Please cite this paper as: Gibson C, Goldwater P, MacLennan A, Haan E, Priest K, Dekker G for the South Australian Cerebral Palsy Research Group. Fetal exposure to herpesviruses may be associated with pregnancy-induced hypertensive disorders and preterm birth in a Caucasian population. BJOG 2008; 115:492–500.

Introduction

Clinical maternal viral infection is not uncommon in pregnancy and when it occurs, either as a primary or reactivational infection with viraemia, the fetus is placed at risk of infection through transplacental transmission.[1] Subclinical maternal viral infection must be common as there is nucleic acid evidence of viral exposure in 40–44% of healthy newborns.[2,3] It has been postulated that fetal viral infection *in utero* may increase the risk of adverse pregnancy outcomes (APOs), such as pregnancy-induced hypertensive disorders (PIHD),

* This research was conducted in Adelaide, South Australia, Australia.

birthweight <10th percentile (small for gestational age, SGA) and preterm birth (PTB).[4–6]

The placenta acts as a potential barrier to the transfer of viruses from mother to fetus during the viraemic phase of maternal infection.[7] The placental barrier may be less effective in early pregnancy and when the placenta is damaged, for example by infarction caused by vascular disease. Placental dysfunction has also been associated with acquired and genetic thrombophilia, systemic lupus erythematosus and pre-existing diabetes. Clinical syndromes associated with placental insufficiency and/or placental vasculopathy include pre-eclampsia, SGA, PTB and fetal demise. Very little is known about the passage of viruses across the placenta or the role of placental viral infection in adverse pregnancy and fetal outcomes. However, it is postulated that viral infection of extravillous trophoblast cells may alter the process of placental invasion and predispose the mother and fetus to adverse reproductive outcomes that result from placental dysfunction.[8]

Herpesviruses (including cytomegalovirus (CMV), herpes simplex viruses (HSV) 1 and 2, varicella-zoster virus (VZV), Epstein–Barr virus (EBV) and human herpesviruses (HHV) 6, 7 and 8) and enteroviruses are capable of crossing the placenta and causing *in utero* infection[9–16] and could potentially contribute directly or indirectly to APOs. It has been shown that CMV infection impairs critical aspects of cytotrophoblast function, which may explain some of the deleterious effects of this virus on pregnancy outcome.[17] The likelihood of maternal infection resulting in fetal infection varies according to the specific virus, whether the infection is primary or recurrent, and the gestational age of the fetus at the time of infection. Once the infection has crossed the placenta into the fetal circulation, there is the potential for adverse fetal outcomes. These can be caused by the infectious agent directly or indirectly through the fetal and/or placental inflammatory response to infection, where proinflammatory cytokines may adversely affect the developing brain and perhaps also placental function.

This study investigated the role of fetal exposure to viral infection (detected through the presence of viral nucleic acids in newborn screening cards) in APOs, including SGA, PIHD, antepartum haemorrhage (APH) and PTB. This is the largest case–control study to date investigating the role of maternal and fetal infection in APOs.

Methods

Patient selection

The cases and controls in this cohort were selected as part of a study investigating the role of genetic polymorphisms and viruses in the development of cerebral palsy (CP). The selection process for these cases and controls has been detailed previously.[18] For this analysis, we disregarded CP as an outcome and combined our cohort of 443 CP cases and 883 controls (total 1326) before separating them on the basis of APOs.[19]

A total of 717 of the 1326 babies (54.1%) met the following selection criteria for cases. Some cases had more than one condition:

1 PTB <37 weeks of gestation (451/717, 62.9%).
2 SGA <10th percentile calculated from Roberts and Lancaster[20] (241/717, 33.6%).
3 APH (any recorded bleeding at or after 20 weeks of gestation) (340/717, 47.4%). The classification of APH within the South Australian Perinatal Data Collection of births includes diagnosis of placenta praevia and placental abruption, as well as other and unknown causes of APH.
4 PIHD (blood pressure ≥140/90 mmHg or higher on two occasions at least 4 hours apart, or ≥170/110 mmHg or higher on one occasion, first noted after 20 weeks of gestation). The South Australian Pregnancy Outcome Database does not contain data on proteinuria; therefore, in this study, cases with PIHD include both gestational hypertension and pre-eclampsia (23/717, 3.2%).

The remaining 609 babies (45.9%) had none of the above selection criteria and were used as the comparison group for analysis.

Subanalysis was also performed, using the following selection criteria. These subgroups were identified *a priori*, before the data were analysed.

1 All PIHD plus SGA (9/717, 1.3%).
2 All APH plus SGA (108/717, 15.1%).
3 All PTB <37 weeks of gestation plus SGA (82/717, 11.4%).

Ethical considerations

This research was approved by the Research Ethics Committee of the Children, Youth and Women's Health Service in Adelaide, Australia, and followed the National Health and Medical Research Council of Australia Guidelines. The ethics committee deemed that cases and controls must be deidentified, and collection of clinical data was limited to that contained within the South Australian Supplementary Birth Record. No linkage was allowed with case notes or other neonatal outcomes. The supplementary birth record unfortunately does not collect data on perinatal infectious morbidity. The collection uses notifications of births in South Australia made by hospital and homebirth midwives and hospital neonatal nurses and includes comprehensive data on medical conditions present in pregnancy and obstetric complications. Validation of this perinatal data collection form has been undertaken, which showed that the data, such as major pregnancy events, are accurate and reliable in comparison with hospital medical records.[21]

Virus detection

The viruses of interest were categorised into DNA and RNA viruses. The DNA viruses included: HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7 and HHV-8. The RNA viruses included members of the enterovirus family. DNA viruses

were detected using two polymerase chain reactions (PCRs) using previously published primers,[22] and results were assigned to the respective PCR test groups: the first detected nucleic acids of HSV-1, HSV-2, EBV, CMV and HHV-8, hereafter designated Herpes PCR group A, and the second detected nucleic acids of VZV, HHV-6 and HHV-7, hereafter designated Herpes PCR group B. The separation of the herpesviruses into these groups by primer pairs corresponded to the G+C content of their DNA and not to the phylogenetic grouping based on the complete genome.[22] Within the Herpes PCR group A, differentiation between CMV and the remaining viruses (HSV-1, HSV-2, EBV and HHV-8) was possible because of differences in PCR product band size visually determined by agarose gel electrophoresis.

Punches of dried blood (1.2 mm) on newborn screening cards (collected by heel-prick at approximately 3–5 days of life) were extracted for DNA viruses using the NucleoSpin® Tissue Extraction Kit (Macherey-Nagel, Düren, Germany). The newborn screening cards were extracted for RNA using a phenolic wash method.[23]

All amplification conditions were optimised using reference RNA and DNA samples extracted from viral stocks. These reference samples were included as positive controls for all subsequent amplifications in addition to no template controls.

Sensitivity of virus detection

The minimum number of detectable viral nucleic acid copies was determined for each PCR and extrapolated back to a minimum number of detectable viral nucleic acid copies per millilitre of blood. The minimum number of detectable viral nucleic acid copies was 2.8/bloodspot (5.6×10^3/ml blood) for enterovirus, 1.6/bloodspot (3.2×10^3/ml blood) for herpes PCR group A viruses and 15/μl (3.2×10^3/ml blood) for herpes PCR group B viruses. Viral nucleic acids were detected from newborn screening samples that had been stored for up to 18 years. Not all samples had a valid test result for all viruses, and therefore the numbers in the tables may add up to less than the total number of cases and controls available for testing. The storage time of the newborn samples did not affect the ability to amplify genomic DNA, which was amplified from all samples tested.

Statistical analysis

As controls were not matched for important covariates, such as gestational age, analysis was undertaken using all controls without taking account of matching. Data analysis (GraphPad Instat version 3.06) then considered cases by gestational age range (<32 weeks, 32–36 weeks, <37 weeks, ≥37 weeks and all gestational ages). Results were expressed as odds ratios with 95% CIs comparing positive with negative virus detection. Tables S1–S8 detail all calculated odds ratios and confidence intervals; only significant results are presented in the main text. *P* values of <0.05 are highlighted in the tables. No adjustments were made for multiple testing in this largely exploratory study into the associations of *in utero* exposure to viral infection and APOs.

Results

All APOs versus non-CP APO

Significantly, more babies with a subsequent diagnosis of CP were in the case group of this study (OR 2.00, 95% CI 1.56–2.54). We therefore investigated whether the CP babies were overrepresented in the APOs studied and found that CP babies were overrepresented in the PTB group, irrespective of whether the PTB was spontaneous or iatrogenic (Table 1). There were no significant differences for APH, SGA, or PIHD. As a result of these findings, PTB was analysed only using non-CP babies in the case (n = 251) and control (n = 455) groups.

We also investigated whether the prevalence of viral infection differed between the APOs comparison group and the non-CP APOs comparison group. Table 2 illustrates the prevalences of viral exposure for each of these groups. No significant differences were observed between the two control populations.

SGA <10th percentile

Two hundred and forty-one babies in the case cohort (33.6%) were classified as SGA. There were no associations between any of the viruses tested and SGA.

Pregnancy-induced hypertensive disorders

The mothers of 42 babies in the case cohort (5.9%) suffered from hypertension, either pregnancy-induced (23) or pre-existing (20). One mother suffered from both. The low frequency of PIHD in this study is most likely explained by the high incidence of PTB (62.9% of the overall study population).

Detection of Herpes PCR group B viruses was associated with PIHD (OR 3.57, 95% CI 1.10–11.57) (Table 3). This significant association was also observed in preterm babies. The detection of CMV was also significantly associated with PIHD (OR 3.89, 95% CI 1.67–9.06) (Table 3). The detection of any herpesvirus or any virus was also associated with PIHD, with odds ratios of 5.70 (95% CI 1.85–17.57) and 5.17 (95% CI 1.68–15.94), respectively (Table 3).

PIHD and SGA <10th percentile

Of the 23 mothers of babies in the case cohort who developed PIHD, 9 (39.1%) also gave birth to an SGA baby. No significant associations were observed between exposure to viral infection and PIHD plus SGA.

Antepartum haemorrhage

The mothers of 340 of 717 babies in the case cohort (47.4% of cases) suffered from APH. No significant associations were observed between exposure to viral infection and APH.

Table 1. Odds ratios (95% CI) for CP versus non-CP babies in the APO case group for the APOs of interest

Adverse pregnancy outcome	APO cases (n = 717)		OR (95% CI)	P value
	CP babies (n = 289)	Non-CP babies (n = 428)		
APH (n = 340)	132	208	0.89 (0.65–1.21)	0.49
No APH (n = 377)	157	220		
SGA (n = 241)	91	150	0.85 (0.61–1.19)	0.36
No SGA (n = 476)	198	278		
PTB (n = 451)	200	251	1.58 (1.14–2.20)	0.005
No PTB (n = 266)	89	177		
Spontaneous PTB (n = 266)	118	148	1.00 (0.67–1.49)	0.93
Iatrogenic PTB (n = 185)	82	103		
PIHD (n = 23)	8	15	0.78 (0.30–2.00)	0.74
No PIHD (n = 694)	281	413		

APH and SGA <10th percentile

Of the 340 mothers of babies in the case cohort diagnosed with APH, 108 (31.8%) gave birth to an SGA baby. The majority of associations investigated were nonsignificant. Babies born at 32–36 weeks of gestation who tested positive for Herpes PCR group B viral DNA were at greater risk of APH and SGA (OR 2.79, 95% CI 1.08–7.25) (Table 3).

PTB <37 weeks—non-CP

Four hundred and fifty one (62.9%) of the case cohort were born prematurely at a gestational age of less than 37 weeks. Of these premature babies, 251 (55.7%) were not diagnosed with CP. Because of the significant association observed between PTB and CP diagnosis within the PTB cohort (Table 1), all results for prematurity were calculated for only the non-CP population to avoid skewing of the results. Detection of CMV DNA was significantly associated with PTB (OR 1.61, 95% CI 1.14–2.27). The detection of Herpes PCR group A viruses and any herpesvirus was also associated with PTB (OR 1.51, 95% CI 1.08–2.10 and OR 1.43, 95% CI 1.02–2.01, respectively).

PTB and SGA <10th percentile

Eighty two (18.2%) of the 451 preterm babies were classified as SGA. Of these, 43 (52.4%) were not diagnosed with CP. Detection of any herpesvirus was associated with combined PTB at 32–36 weeks of gestation and SGA (OR 2.21, 95% CI 1.03–4.73) (Table 3). No other significant associations were observed between PTB with SGA and exposure to viral infection.

Discussion

This is the largest study to investigate the associations between perinatal exposure to viral infection, detected by the presence of viral nucleic acids in blood collected within the first 3–5 days of neonatal life, and APOs—SGA, PIHD,

Table 2. Prevalence of viral infections in the total APO control population and the non-CP APO control population, expressed as percentage positive of the total tested*

Virus	All APO comparison group		Non-CP APO comparison group	
	Positive/total	Prevalence % (95% CI)	Positive/total	Prevalence % (95% CI)
Herpes PCR group B	41/480	8.5 (6.2–11.4)	26/362	7.2 (4.7–10.3)
Herpes PCR group A	167/587	28.5 (24.8–32.3)	120/441	27.2 (23.1–31.6)
CMV	147/587	25.0 (21.6–28.8)	104/441	23.6 (19.7–27.8)
HSV	25/587	4.3 (2.8–6.2)	20/441	4.5 (2.8–6.9)
Enterovirus	19/585	3.4 (2.0–5.2)	14/442	3.2 (1.7–5.3)
Any herpesvirus	191/502	38.0 (33.8–42.5)	136/380	35.8 (31.0–40.8)
Any virus	203/503	40.4 (36.0–44.8)	145/382	38.0 (33.1–43.0)

*No significant differences were observed between the two control populations. Not all samples had a valid test result for all viruses, therefore the total number of samples included in analyses may be less than the total number of cases and controls available for study.

Table 3. Significant odds ratios (95% CI) for all APOs and virus exposures*

APO	Virus	Gestation (weeks)	Cases (positive/total)	OR (95% CI)	*P* value
PIHDs	Herpes PCR group B	All	4/16	3.57 (1.10–11.57)	<0.05
		<37	4/12	5.35 (1.55–18.55)	<0.05
		<32	4/8	10.71 (2.58–44.42)	<0.01
	CMV	All	13/23	3.89 (1.67–9.06)	<0.01
		≥37	5/6	14.97 (1.73–129.21)	<0.01
		<37	8/17	2.66 (1.00–7.02)	0.05
	Herpes PCR group A	All	13/23	3.27 (1.41–7.60)	<0.01
		≥37	5/6	12.58 (1.46–108.50)	<0.01
	Any herpesvirus	All	14/18	5.70 (1.85–17.57)	<0.01
		≥37	5/5	17.89 (0.98–325.63)	<0.01
		<37	9/13	3.66 (1.11–12.06)	<0.05
	Any virus	All	14/18	5.17 (1.68–15.94)	<0.01
		≥37	5/5	16.24 (0.89–295.57)	<0.05
		<37	9/13	3.33 (1.01–10.95)	<0.05
APH + SGA	Herpes PCR group B	32–36	6/29	2.79 (1.08–7.25)	<0.05
PTB (non-CP APO)	CMV	<37	82/247	1.61 (1.14–2.27)	<0.01
		32–36	48/145	1.60 (1.06–2.42)	<0.05
		<32	34/102	1.62 (1.02–2.58)	<0.05
	Herpes PCR group A	<37	89/247	1.51 (1.08–2.10)	<0.05
		32–36	54/145	1.59 (1.07–2.36)	<0.05
	Any herpesvirus	<37	98/221	1.43 (1.02–2.01)	<0.05
		32–36	62/134	1.55 (1.04–2.30)	<0.05
PTB + SGA (non-CP APO)	Any herpesvirus	32–36	16/29	2.21 (1.03–4.73)	<0.05

Positive, total number of samples testing positive for the viruses; total, total number of samples with a valid test result. Herpes PCR group B: VZV, HHV-6, HHV-7; Herpes PCR group A: HSV-1, HSV-2, EBV, CMV, HHV-8; Any Herpesvirus: HSV-1, HSV-2, EBV, CMV, VZV, HHV-6, HHV-7, HHV-8; Any Virus: any herpesvirus or enterovirus.

*Not all samples had a valid test result for all viruses, therefore the total number of samples included in analyses may be less than the total number of cases and controls available for study.

APH and PTB. The link between prematurity and infection is well established.[24–26] The role of infection in other APOs is not so well studied, although over the past decade, the potential involvement of infection and inflammatory responses in the placenta and mother in the pathogenesis of pre-eclampsia has received attention.[27–31]

Using this large cohort of pathology-enriched cases and term controls, specific viral DNA and RNA nucleic acid sequences have been identified, and an association has been shown between exposure to viral infection and APOs, particularly PIHD.

Caveats

While this is the largest study of its kind, there are a number of caveats. Our cases and controls were derived from another study hypothesis and were not matched to each other. Furthermore, 217 separate analyses were performed on the individual viruses, with 10.6% (23) yielding significant associations. Such multiple analyses increase the likelihood of identifying chance statistical associations (type 1 error) and because of small numbers in some of the subanalyses, associations cannot be confidently excluded (type 2 error). Limitations imposed by our ethics committee meant that we were unable to access case notes or other relevant clinical information about our cases and controls. Information such as Doppler studies on umbilical and uterine arteries, if available, would have enhanced this study. Our chosen outcomes may causally interact to result in an APO, and there are inherent difficulties in determining the roles played by the individual outcomes. Finally, although the viruses detected in the neonatal blood spots probably reflected exposure *in utero*, neonatal exposure is also possible. Findings must be reported but interpreted with caution, and further large-scale prospective studies are necessary to confirm these associations.

Despite these caveats, this study has demonstrated that the presence of viral nucleic acids, in particular Herpes PCR group B and CMV, in newborn screening blood samples may be associated with PIHD over a wide range of gestational ages. One mechanism by which such associations could be explained is the inflammatory response caused by these viruses. Vessel inflammation and artery wall thickening, as a result of viral infection, can contribute to the increased

resistance of blood vessels,[4] thus promoting a hypertensive state. HSV-1, HSV-2 and CMV, members of the herpesvirus family, are capable of causing thrombogenic changes to host cells and initiating the clotting cascade through the generation of thrombin,[32] thus promoting vascular disease. A large cross-sectional study by Sun *et al.*[4] found that HSV-2 infection was associated with essential hypertension.

Our results demonstrated associations between CMV exposure and PIHD in all infants and term-born infants born to mothers who had PIHD. A marginally significant trend was observed ($P = 0.05$) between CMV exposure and PIHD in preterm infants born to mothers who had PIHD. We did not observe significant findings for the small subgroup PIHD with SGA. This may be because of the very small numbers in this subgroup, and large prospective studies would be needed to rule out such associations. von Dadelszen *et al.*[33] showed that women with early-onset pre-eclampsia had higher levels of anti-CMV antibodies than those with late-onset pre-eclampsia, those with normotensive intrauterine growth restriction (IUGR) and those with normal pregnancies. Exposure to CMV and subsequent production of anti-CMV antibodies may generate pathogenic antiphospholipid antibodies, which are capable of binding and activating endothelial cells.[34] This in turn may enhance thrombus formation and increase inflammation, resulting in hypertension. A similar connection has been identified for enteroviral infection and atherosclerosis,[35] suggesting that several viral infections are capable of increasing blood pressure and causing hypertension. A study reported by Belfort *et al.*[36] demonstrated a significant association between evidence of CMV infection and pre-eclampsia ($P < 0.01$), thus supporting an association between pre-eclampsia and viral infection during pregnancy. An alternative hypothesis to explain the link between CMV and APOs is that CMV may affect the biology of cytotrophoblasts and thus critical cytotrophoblast function.[17]

Our study demonstrated associations between PTB and exposure to viral infection, particularly CMV. This provides further evidence that the presence of infectious agents *in utero* is associated with subsequent PTB.[25,37–39]

Our study did not show any associations between exposure to viral nucleic acids and SGA, despite other research suggesting possible links.[5,6] Fetal viral infection, in particular CMV, can cause SGA, however, no association was found in the present study. The results do, however, agree with van Dongen *et al.*,[40] who found no associations between IUGR and adenoviruses or enteroviruses. Discrepancies may be explained by study design differences. Our study used non-quantitative PCR methodology on archived newborn screening cards, and there remains the possibility of the results reflecting differing viral loads. Low viral loads may not invoke significant inflammatory/cytokine responses and therefore may not create susceptibility to APOs, such as PTB or SGA; alternatively, high viral loads, possibly associated with major inflammation and/or damaging cytokine production, could be necessary before such adverse outcomes are observed. Furthermore, these results may reflect different gestational ages at which exposure to the virus first occurred. Infections that occur earlier in intrauterine life tend to be associated with more severe clinical sequelae compared with those occurring later.[41] Positive amniotic fluid viral DNA PCR results have been associated with an increased rate of fetal structural malformations, IUGR, hydrops and other fetal abnormalities.[6] These tests were performed between 19–20 weeks of gestation compared with 3–5 days after birth in the current study, which may explain the differing results. Prospective studies designed to quantify viral loads at various gestational ages are planned to investigate this hypothesis further. Such prospective studies will also determine whether these infections are primary infections or reactivation of latent viral infection. This study was unable to test every potential virus worthy of investigation (e.g. adenoviruses, rotaviruses, human coronaviruses, parvovirus, paramyxoviruses and lymphocytic choriomeningitis virus), and these should be investigated in future studies.

No associations with APH were identified, an outcome not previously investigated for associations with viral infection. Within our pathology-enriched case cohort of 717 babies, the mothers of 340 (47.4%) were diagnosed with some form of APH. The classification of APH within the South Australian Perinatal Data Collection of births includes diagnosis of placenta praevia and placental abruption, as well as other and unknown causes of APH. However, no associations were evident in this large group.

A high prevalence of viral DNA was observed in the control group, with 203/503 (40%) controls with a valid PCR result for all viruses testing positive for at least one virus. A similar control group prevalence of 44% was reported in 2005 by other authors,[42] suggesting that antenatal viral exposure is common but not clinically relevant unless other factors are present to initiate infection and/or an inflammatory response. It is important to recognise that the presence of viral DNA does not necessarily indicate active congenital or neonatal infection. Newborn screening cards were tested for the presence of viral DNA and RNA. While detection of viral nucleic acids in the blood of neonates indicates exposure to and replication of the respective virus or viruses, this study was not designed to detect evidence of an accompanying inflammatory response. Given the small sample volume (1.2 mm diameter) of dried blood and the limit of detection of 1–10 viral nucleic acid copies, it could not be confirmed that true viraemic infection was occurring. It would seem unlikely that the detection of viral nucleic acid in such small samples merely reflected maternal and fetal cell trafficking or maternal blood contamination. The possibility of nosocomial infection needs to be considered, although it is thought exceedingly unlikely that this infection would have had time to incubate

and cause infection in the newborn infant within the first days of life without clinical symptoms. Clinically, a nosocomial infection rate of 40% in our control population would have been identified as a major epidemic, which was not evident. Prospective investigations are required to follow women through pregnancy, quantitatively testing antenatal blood samples for viral nucleic acids and determining if there is active infection in the fetus/neonate by examining leukocytes and sera for the presence of viral antigens associated with active viral replication. PCR contamination was excluded by working in two designated separate laboratories for preparation of PCR samples (to eliminate the possibility of PCR product contamination) and by using appropriate controls in all assays. Furthermore, no other viral PCR work was being conducted by other users of the PCR laboratories.

In summary, we have demonstrated that exposure to viral infection (as demonstrated by the presence of viral nucleic acids in blood on newborn screening cards) may be associated with PIHD and PTB. These exploratory findings require confirmation in other studies. These findings support the previously published indirect findings (such as antibody responses) of others that pointed to a relationship between CMV and hypertension of pregnancy. Our findings suggest that some APOs could potentially be prevented by immunisation or passive immunity through virus-specific immunoglobulin. It is likely that APOs are multifactorial and that other factors, such as genetic susceptibility to infection, genetically regulated proinflammatory cytokine responses and inherited thrombophilia[19] are needed for the adverse phenotypes to be expressed.

Funding

This research was supported by the Australian National Health and Medical Research Council, The Channel 7 Children's Research Foundation, The University of Adelaide and The South Australian Government Captive Insurance Corporation. The supporting sources had no influence on the analysis, writing or submission of the manuscript.

Details of ethics approval

The procedures of the study received ethics approval from the Women's and Children's Hospital Human Research Ethics Committee, South Australia. The date of approval was 6 June 2002, reference number REC1323/5/2008.

Contribution to authorship

All authors listed on this paper fulfil the uniform requirements for authorship. No one who fulfils these criteria has been omitted from authorship. C.S.G. contributed to the design of the study, collection, analysis and interpretation of data, writing of the manuscript and gave final approval of the version to be published. P.N.G. contributed to the study design and interpretation of the data, writing of the manuscript and gave final approval of the version to be published. A.H.M. contributed to the study design and interpretation of the data, writing of the manuscript and gave final approval of the version to be published. E.A.H. contributed to the study design, collection and interpretation of the data, writing of the manuscript and gave final approval of the version to be published. K.P. contributed to the collection and analysis of data, writing of the manuscript and gave final approval of the version to be published. G.A.D. contributed to the study design, analysis and interpretation of data, writing of the manuscript and gave final approval of the version to be published. Other members of the South Australian Cerebral Palsy Research Group were involved in the design of the study and are listed as follows: A/Prof Annabelle Chan, Dr William Hague, Dr Zbigniew Rudzki, Ms Phillipa van Essen, A/Prof T Yee Khong, Dr Mark R Morton, Mr Enzo Ranieri, Ms Heather Scott, Dr Heather Tapp, Mr Graeme Casey.

Acknowledgements

We thank the staff of the Neonatal Screening Laboratory (Women's and Children's Hospital) for their technical assistance. We also thank Barry Slobedman (Westmead Millenium Institute, NSW, Australia) for providing us with positive control viral material.

Supplementary material

The following supplementary materials are available for this article:

Table S1. Odds Ratios (95% CI) for Herpes PCR Group B Results for specified adverse pregnancy outcomes.
Table S2. Odds Ratios (95% CI) for Herpes PCR Group A Results for specified adverse pregnancy outcomes.
Table S3. Odds Ratios (95% CI) for CMV results for specified adverse pregnancy outcomes.
Table S4. Odds Ratios (95% CI) for HSV results for specified adverse pregnancy outcomes.
Table S5. Odds Ratios (95% CI) for Enterovirus results for specified adverse pregnancy outcomes.
Table S6. Odds Ratios (95% CI) for any herpesvirus result for specified adverse pregnancy outcomes.
Table S7. Odds Ratios (95% CI) for any virus result for specified adverse pregnancy outcomes.
Table S8. Odds Ratios (95% CI) for all preterm birth and non-CP preterm birth for the specified virus groups.

These materials are available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1471-0528.2007.01653.x.

(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article. ■

References

1 Anteby E, Yagel S. Immune responses to viral infection. In: *Viral Diseases in Pregnancy*. New York, NY: Springer Verlag, 1994.
2 Gibson CS, MacLennan AH, Goldwater PN, Haan EA, Priest K, Dekker GA. Neurotropic viruses and cerebral palsy: population based case-control study. *BMJ* 2006;332:76–80.
3 Srinivas SK, Ma Y, Sammel MD, Chou D, McGrath C, Parry S, *et al*. Placental inflammation and viral infection are implicated in second trimester pregnancy loss. *Am J Obstet Gynecol* 2006;195:797–802.
4 Sun Y, Pei W, Wu Y, Jing Z, Zhang J, Wang G. Herpes simplex virus type 2 infection is a risk factor for hypertension. *Hypertens Res* 2004;27:541–4.
5 Pereira L, Maidji E, McDonagh S, Genbacev O, Fisher S. Human cytomegalovirus transmission from the uterus to the placenta correlates with the presence of pathogenic bacteria and maternal immunity. *J Virol* 2003;77:13301–14.
6 Reddy UM, Baschat AA, Zlatnik MG, Towbin JA, Harman CR, Weiner CP. Detection of viral deoxyribonucleic acid in amniotic fluid: association with fetal malformation and pregnancy abnormalities. *Fetal Diagn Ther* 2005;20:203–7.
7 Newton ER. Diagnosis of perinatal TORCH infections. *Clin Obstet Gynecol* 1999;42:59–70;quiz 174–5.
8 Koi H, Zhang J, Parry S. The mechanisms of placental viral infection. *Ann N Y Acad Sci* 2001;943:148–56.
9 Remington JS, Klein JO. *Infectious Diseases of the Fetus and Newborn Infant*, 4th edn. Philadelphia, PA: W.B. Saunders Company, 1995.
10 McLean LK, Chehab FF, Goldberg JD. Detection of viral deoxyribonucleic acid in the amniotic fluid of low-risk pregnancies by polymerase chain reaction. *Am J Obstet Gynecol* 1995;173:1282–6.
11 Zdravkovic M, Knudsen HJ, Liu X, Hager H, Zachar V, Aboagye-Mathiesen G, *et al*. High interferon alpha levels in placenta, maternal, and cord blood suggest a protective effect against intrauterine herpes simplex virus infection. *J Med Virol* 1997;51:210–3.
12 Mouly F, Mirlesse V, Meritet JF, Rozenberg F, Poissonier MH, Lebon P, *et al*. Prenatal diagnosis of fetal varicella-zoster virus infection with polymerase chain reaction of amniotic fluid in 107 cases. *Am J Obstet Gynecol* 1997;177:894–8.
13 Palmer AL, Rotbart HA, Tyson RW, Abzug MJ. Adverse effects of maternal enterovirus infection on the fetus and placenta. *J Infect Dis* 1997;176:1437–44.
14 Brown HL, Abernathy MP. Cytomegalovirus infection. *Semin Perinatol* 1998;22:260–6.
15 Wenstrom KD, Andrews WW, Bowles NE, Towbin JA, Hauth JC, Goldenberg RL. Intrauterine viral infection at the time of second trimester genetic amniocentesis. *Obstet Gynecol* 1998;92:420–4.
16 Takami T, Sonodat S, Houjyo H, Kawashima H, Takei Y, Miyajima T, *et al*. Diagnosis of horizontal enterovirus infections in neonates by nested PCR and direct sequence analysis. *J Hosp Infect* 2000;45:283–7.
17 Fisher S, Genbacev O, Maidji E, Pereira L. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. *J Virol* 2000;74:6808–20.
18 Gibson CS, Maclennan AH, Hague WM, Haan EA, Priest K, Chan A, *et al*. Associations between inherited thrombophilias, gestational age, and cerebral palsy. *Am J Obstet Gynecol* 2005;193:1437.
19 Gibson CS, MacLennan AH, Janssen NG, Kist WJ, Hague WM, Haan EA, *et al*. Associations between fetal inherited thrombophilia and adverse pregnancy outcomes. *Am J Obstet Gynecol* 2006;194:e1–10.
20 Roberts CL, Lancaster PA. Australian national birthweight percentiles by gestational age. *Med J Aust* 1999;170:114–18.
21 McLean A, Scott J, Keane RJ, Sage L, Chan A. *Validation of the 1994 South Australian Perinatal Data Collection Form*. Adelaide, Australia: Pregnancy Outcome Unit, Department of Human Services, 2001.
22 Johnson G, Nelson S, Petric M, Tellier R. Comprehensive PCR-based assay for detection and species identification of human herpesviruses. *J Clin Microbiol* 2000;38:3274–9.
23 Rogers CD, Burgoyne LA. Reverse transcription of an RNA genome from databasing paper (FTA(R)). *Biotechnol Appl Biochem* 2000;31:219–24.
24 Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. *Ment Retard Dev Disabil Res Rev* 2002;8:3–13.
25 Romero R, Chaiworapongsa T, Espinoza J. Micronutrients and intrauterine infection, preterm birth and the fetal inflammatory response syndrome. *J Nutr* 2003;133:1668S–73S.
26 Gibson CS, MacLennan AH, Goldwater PN, Dekker GA. Antenatal causes of cerebral palsy: associations between inherited thrombophilias, viral and bacterial infection, and inherited susceptibility to infection. *Obstet Gynecol Surv* 2003;58:209–20.
27 von Dadelszen P, Magee LA. Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? *Acta Obstet Gynecol Scand* 2002;81:642–8.
28 Borzychowski AM, Sargent IL, Redman CW. Inflammation and pre-eclampsia. *Semin Fetal Neonatal Med* 2006;11:309–16.
29 Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998;179:80–6.
30 Zaki M, Greenwood C, Redman CW. The spontaneous reversal of pre-eclampsia associated with parvovirus-induced hydrops and the placental theory of pre-eclampsia: a case report. *BJOG* 2003;110:1125–6.
31 Redman CW, Sargent IL. Placental debris, oxidative stress and pre-eclampsia. *Placenta* 2000;21:597–602.
32 Sutherland MR, Raynor CM, Leenknegt H, Wright JF, Pryzdial EL. Coagulation initiated on herpesviruses. *Proc Natl Acad Sci U S A* 1997;94:13510–4.
33 von Dadelszen P, Magee LA, Krajden M, Alasaly K, Popovska V, Devarakonda RM, *et al*. Levels of antibodies against cytomegalovirus and *Chlamydophila pneumoniae* are increased in early onset pre-eclampsia. *BJOG* 2003;110:725–30.
34 Gharavi AE, Vega-Ostertag M, Espinola RG, Liu X, Cole L, Cox NT, *et al*. Intrauterine fetal death in mice caused by cytomegalovirus-derived peptide induced aPL antibodies. *Lupus* 2004;13:17–23.
35 Kwon TW, Kim do K, Ye JS, Lee WJ, Moon MS, Joo CH, *et al*. Detection of enterovirus, cytomegalovirus, and *Chlamydia pneumoniae* in atheromas. *J Microbiol* 2004;42:299–304.
36 Belfort MA, Cortes J, Bowles N, Saade GR, Dorman K, Abadajos P, *et al*. Placental cytomegalovirus infection and preeclampsia: are they related? *J Soc Gynecol Investig* 1998;5:T403.
37 Friese K. The role of infection in preterm labour. *BJOG* 2003;110 (Suppl. 20):52–4.
38 Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. *BJOG* 2003;110 (Suppl. 20):71–5.

39 Jacobsson B. Infectious and inflammatory mechanisms in preterm birth and cerebral palsy. *Eur J Obstet Gynecol Reprod Biol* 2004;115: 159–60.

40 van Dongen AJ, Verboon-Maciolek MA, Weersink AJ, Schuurman R, Stoutenbeek P. Fetal growth restriction and viral infection. *Prenat Diagn* 2004;24:576–7.

41 Bittencourt AL, Garcia AG. Pathogenesis and pathology of hematogenous infections of the fetus and newborn. *Pediatr Pathol Mol Med* 2002;21:353–99.

42 Srinivas SK, Sammel M, Chou D, McGrath C, Parry S, Elovitz M. Are viral infections the cause of second trimester pregnancy loss? *Am J Obstet Gynecol* 2005;193:S33.

