ORIGINAL ARTICLE

Cognitive outcome and cyclo-oxygenase-2 gene (−765 G/C) variation in the preterm infant

D R Harding, S E Humphries, A Whitelaw, N Marlow, H E Montgomery

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See end of article for authors' affiliations

Correspondence to: D Harding, St Michael's NICU, University of Bristol, D level, St Michael's Hospital, Bristol BS2 8EG, UK; david.harding@bristol.ac.uk

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Background: Cyclo-oxygenase (COX) inhibition by indomethacin does not result in an improvement in long-term neurocognitive outcome, despite reducing the incidence of both severe intraventricular haemorrhage and white matter injury visible on ultrasound. Diffuse brain injury after preterm birth may have inflammatory origins. These two points suggest that, in the preterm brain, COX inhibition may have a dominant proinflammatory or neuropathological role. The inducible form of the COX2 gene is polymorphic: the −765 C (rather than G) variant of the gene is associated with reduced COX2 activity.
Objective: To test the hypothesis that the C allele of COX2 is associated with worse neurodevelopmental outcomes after premature birth.
Outcomes: Cerebral palsy, disability, Griffith's developmental quotient at 2 years and British Ability Scales-11 general cognitive ability and motor performance (movement assessment battery for children) at 5½ years were compared with COX2 genotype.
Results: The C allele (GC 65 (31%), CC 3 (1%)) was independently associated with worse cognitive performance at 2 and 5½ years: C allele mean (SEM) developmental quotient 92.7 (1.7), *v* GG 97.6 (1.5), p = 0.039; C allele mean (SEM) general cognitive ability , 94.3 (2.2) *v* GG 100.9 (1.7), p = 0.028.
Conclusion: An antineuropathological role for COX2 in the preterm brain may help account for the lack of effect of indomethacin treatment in improving neurocognitive outcomes in children born preterm, despite reported reduction in apparent brain injury.

The developing brain of the preterm infant is especially sensitive to inflammation, a common consequence of preterm birth.[1] The cerebral inflammatory responses may contribute to the substantial prevalence of impairment identified in infants born prematurely. One half of all survivors born before 26 weeks gestation for instance are disabled as children, many from cognitive deficits.[2] In many cases there does not seem to be a clear correlation between structural abnormality and discrete abnormalities of higher function.[3]

The cyclo-oxygenase (COX) enzymes use arachidonic acid in the synthesis of prostaglandins. COX1 is constitutively expressed in many tissues, whereas COX2 is primarily thought of as the inducible form of the enzyme.[4] COX1 is mainly expressed in microglia and some neuronal cells throughout the brain, whereas COX2 is more widely expressed in neurones.[5] The prostaglandins generated by COX activity are vasoactive, but are also important regulators of the inflammatory response. As such they may, in theory, influence neurological outcome after preterm birth. However, whereas use of the non-selective COX inhibitor indomethacin to close the patent ductus arteriosus in the preterm infant has been associated with a reduction in severe intraventricular haemorrhage and periventricular leucomalacia (PVL) visible by cerebral ultrasound, a complementary improvement in neuro-cognitive outcome is not seen.[6] This observation suggests that COX inhibition may have detrimental effects on the brain of the preterm infant. Reduction in cerebral blood flow (associated with rapid administration) could contribute to this phenomenon,[7] but this seems unlikely given that PVL may be reduced by indomethacin treatment.

Increasing COX activity may result in both anti-inflammatory and proinflammatory activity depending on the tissues used.[8–10] Thus, although COX inhibition may be neuroprotective in animal and tissue culture models of certain brain injuries,[9] a principle COX2 product, prostaglandin E2 (PGE2), also seems to be neuroprotective.[10] Therefore, indomethacin, through the inhibition of formation of downstream products such as PGE2, could be having a direct and deleterious effect on the neurones of the preterm infant independent of cerebral perfusion.

The gene encoding COX2 is polymorphic, showing a G→C substitution in the promotor region, 765 base pairs before the start of the protein coding sequence.[11] The C allele is common in UK Caucasians (>25% of healthy people carry the C allele) and is associated with reduced gene expression and prostaglandin biosynthesis.[11 12] If COX2 has a predominantly anti-inflammatory role in the brain of human infants born preterm, the COX2 −765 C allele ought to be associated with worse cerebral outcomes after preterm birth. We have examined this hypothesis.

METHODS

Subjects

The subjects were Caucasian children who had survived beyond 2 years after birth at ⩽32 weeks gestation. They had participated in a randomised control trial designed to measure any effect of a developmental support programme initiated soon after birth for 2 years (The Avon Premature Infant Project).[13] To be included in this study, the blood samples stored routinely after use in the newborn metabolic screening programme had to be available for DNA extraction. One child of any identical twin birth was randomly excluded before data were analysed on the basis of COX2 and other genotypes[14] and sex (six infants in total).

Abbreviations: BAS, British Ability Scales-11; COX2, cyclo-oxygenase-2; GCA, general cognitive ability; IVH, intraventricular haemorrhage; PGE2, prostaglandin E_2; PVL, periventricular leucomalacia

Genotyping

DNA was extracted as described previously.[14] COX2 genotypes were determined by two scientists blinded to neonatal information using the original method[11] (http://atvb.ahajournals.org). Briefly, *Aci*I restriction endonuclease digestion (after CF8–CR7 primed polymerase chain reaction) generated two bands of 188 and 118 bp at −765G variant of the gene compared with the 306 bp band at −765C. Digested products were separated in an 8% Madge gel.[15]

Neurodevelopment

Neurodevelopmental functions were assessed at 2 and 5½ years. Griffith's mental developmental scales were used to assess motor and cognitive performance at 2 years corrected age.[16] Griffith's scales comprise five subscales, including personal and social, hearing and speech, locomotor, eye hand co-ordination and performance domains, from which an overall developmental quotient is derived. A lower developmental quotient reflects a worse neurodevelopmental performance. Cognitive developmental progress at 5½ years of age was assessed using the British Ability Scales (BAS).[17] The BAS-II computes general cognitive ability (GCA) together with visuospatial, verbal and non-verbal subscales. The GCA is a developmental quotient similar to an intelligence quotient estimate, normalised at 100 (SD 15) in which a lower score also indicates poorer conceptual ability. The movement assessment battery for children (ABC Movement)[18] was applied at 5½ years, and it measures manual dexterity, ball skills and balance over 10 tests, the scores of which are summed to produce a score ranging from 0 to 40. High scores indicate more impaired motor skills, and scores close to 0 indicate normal skills.

Cerebral palsy was defined as a disorder of movement or posture, including hypertonia, associated with disability, and was formally assessed at both ages (2 years corrected for prematurity and 5½ years for chronological age). Disability was defined as any disability, using published descriptions,[19] by outcome over neuromotor, vision, hearing and cognitive domains, and included those with disabling cerebral palsy only (not those with abnormality of tone without significant functional deficit). At 2 or 5½ years children who were non-ambulant, had developmental scores <70, were blind or had profound deafness were considered severely disabled.

A psychologist performed the Griffith's scales of mental development, a second psychologist performed the BAS–II and a research nurse carried out the ABC Movement tests. These assessments were blind to the child's neonatal course and progress. All other neurodevelopmental assessment was performed by NM, who, being a lead clinician, was not blinded to the children's early medical course.

Statistics

Data were stored and analysed using SPSS for Windows (V.9.0). Outcomes were examined for simple association with carriage of COX2 promoter C allele (GC+CC) compared with GG genotype.[11 12] Categorical comparisons were made by χ^2 or Fisher exact test where appropriate. Continuous data, if normally distributed (eg developmental quotient and GCA), were compared with genotypes using Student's t test. Mann–Whitney U test was used if the distribution of data was skewed. As for the original Avon Premature Infant Project, stepwise linear regression was used to determine whether any described relationship between genotype and outcomes persisted after controlling for factors previously identified as potential confounders[13] (perinatal factors (birthweight <1251 g), abnormal cranial ultrasound; severe IVH; white matter injury; disability; the uptake of support programmes (portage or parental advice): social and demographic factors (maternal age, mother educated beyond 16 years, manual parental occupation or unemployed, maternal car use and the presence of two parents at home)).

A probability of <0.05 was considered significant. Correction for multiple comparison was not performed, as is appropriate for an exploratory study of this nature.[20]

Consent

The study was approved by the ethics committees of Southmead Hospital and United Bristol Health Care Trust. Parental consent was obtained for participation in neurodevelopmental follow-up[13] (see below). Consent was not required for the genetic component of this study as all personal information was held separately from the genetic information, patients were identified by study codes, and the DNA collection was obtained and analysed before current legislation.

RESULTS

A total of 207 Caucasian children underwent cranial ultrasound assessment, neurodevelopmental follow-up for 2 years and determination of COX2 genotype; 122 (59%) were male and 85 (41%) female. The median (range) birth weight of the study group was 1470 g (645–2480) and the gestational age 30 weeks (22–32). In all, 156 had neurodevelopmental follow-up to 5½ years. Genotypes were GG 139 (67%), GC 65 (31%) and CC 3 (1%), similar to another group of Caucasians described by this group,[9] and were consistent with Hardy–Weinberg's equilibrium.

The characteristics of the patients and their families (table 1) were independent of genotype. There was no difference in the rates of developmental or family support provided to any genotype or allele group (table 1). There was no association between genotype and presence of patent ductus arteriosus (C allele 10 (15%) *v* GG genotype 24 (17%), p = 0.64), or patent ductus requiring treatment with indomethacin (C allele 7 (10%) *v* GG genotype 17 (12%), p = 0.77).

The COX2 C allele was not associated with cranial ultrasound abnormalities, cerebral palsy or disability (table 2).

Despite this, the C allele was associated with worse developmental quotient and GCA compared with the GG genotype (table 3).

The associations between COX2 C allele and worse developmental quotient and GCA were essentially unchanged among those with normal cranial ultrasound scans: mean (SEM) developmental quotient for C allele 93.7 (2.0), mean (SEM) developmental quotient for GG genotype 100.6 (1.3), 95% confidence interval (CI) −11.3 to −2.4, p = 0.003 (n = 127); mean (SEM) GCA for C allele 94.7 (2.8), mean (SEM) developmental quotient for GG genotype 102.1 (1.9), 95% CI −14.1 to −0.8, p = 0.029 (n = 98). Among the non-disabled, the COX2 C allele was also associated with a lower mean (SEM) developmental quotient, 95.4 (1.1), compared with the GG genotype, 100.8 (1.1) (95% CI −8.6 to −2.3, p = 0.001 (n = 153)) and a lower mean (SEM) GCA for the C allele, 94.0 (2.3), compared with the G allele, 100.3 (1.6) (95% CI −8.6 to −2.3, p = 0.001 (n = 122)).

Linear regression to model for potentially confounding variables showed similar findings, with the COX2 C allele being independently associated with a worse performance in the developmental scales at 2 and 5½years (table 4).

In contrast, the COX2 −765 C allele was not associated with motor development as assessed by performance in the ABC movement scales (median summative score, C allele 8.0, interquartile range 2.1–11.9) and median summative score for GG genotype 7.6 (IQR 2.0–11.5), p = 0.27. In addition, it was noted that there was no association between the C allele and

Table 1 Patient and family characteristics and genotype

Characteristic	Genotype GG (n = 139)	Genotype GC (n = 65)	Genotype CC (n = 3)
Gestation (week; median (IQR))	31 (28–32)	30 (29–32)	29 (25–31)
Birth weight (g; median (IQR))	1490 (1140–1720)	1390 (1145–1705)	1190 (830–1560)
Male	82 (59%)	65 (60%)	1 (33%)
Twin pregnancy	18 (22%)	18 (16%)	0
Preterm rupture of membranes	34 (25%)	14 (22%)	1 (33%)
Antenatal corticosteroids	28 (20%)	17 (26%)	1 (33%)
5-min Apgar score <6	12 (9%)	2 (3%)	1 (33%)
Drugs used during resuscitation	0	2 (3%)	0
All septicaemia	43 (31%)	25 (39%)	2 (67%)
Chronic lung disease (O_2 36 weeks)	5 (4%)	2 (3%)	0
Dexamethasone for CLD	9 (7%)	5 (8%)	1 (33%)
Duration of hospitalisation (days; median (IQR))	45 (29–69)	42 (31–64)	51 (27–126)
Developmental/parental support (parental adviser/portage)	95 (68%)	38 (59%)	2 (67%)
Maternal age (years)	27 (23–30)	26 (23–29)	26 (25–27)
Mother educated beyond 16 years	49 (35%)	29 (44%)	1 (33%)
Manual parental occupation or unemployed	82 (59%)	42 (64%)	2 (67%)
Maternal car use	78 (56%)	39 (60%)	1 (33%)

CLD, chronic lung disease; IQR, interquartile range.

the locomotor component of the Griffith's scales: mean (SEM) locomotor subscale perfomance for C allele versus GG genotype, 92.9 (1.4) *v* 91.4 (1.9), 95% CI −6.1 to 3.2, p = 0.53 (table 3).

DISCUSSION

The COX2 C allele is associated with reduced COX expression.[11] In keeping with a predominantly anti-inflammatory effect of COX expression, it is also associated with a reduction in circulating C reactive protein[11 12] and a reduced incidence of myocardial infarction and stroke.[12] In children born at ⩽32 weeks gestation, the COX2 –765 C allele (encoding downregulation of the COX2 gene) is, however, associated with a worse performance in tests of cognitive developmental progress, at both 2 and 5½ years of age. There was no association between genotype and cranial ultrasound abnormalities or measurements of motor skills.

We used stepwise linear regression to model for previously identified confounders of neurodevelopmental outcome to determine whether the effect of the COX2 C allele (if causal) was an independent risk factor for worse neurodevelopment. The models included adjustment for social factors and developmental interventions (family advice and portage). We found that the COX2 C allele was independently associated with a worse developmental quotient and GCA. Although relatively small, the size of these effects described here (a typical reduction in developmental quotient at 2 years of 5 points and GCA at 5½ years of 9 points; table 4) are of a magnitude similar to the gains of prolonged (2–5 year) developmental support programmes for preterm infants.[13 21]

These data suggest that COX2 inhibition could perhaps be deleterious to the areas of the brain of a child born prematurely responsible for cognitive development. These data are compatible with the products of COX2 activity in the newborn brain preferentially using the PGE2 receptor to exert downstream prostanoid effects:[10 12 22] genetic downregulation of COX2 production leading to reduction in synthesis of PGE and reduction in activation of the neuroprotective, cyclic adenosine monophosphate-coupled EP2 receptor. Given (a) that the EP2 receptor is abundant in the cerebral cortex, striatum and hippocampus,[10] (b) that many of the deficits suffered by children born prematurely are cognitive, and (c) the lack of association between C allele and worse motor development, receptors such as the EP2 receptor may perhaps offer a selective target for therapeutic interventions to enhance cognitive outcome after preterm birth. The effects of the C allele shown here (if causal) are presumably not due to any effect (of the C allele) on cerebral perfusion, as it seems unlikely that a polymorphic variant commonly found in the population would lead to a pathological constriction of cerebral blood flow rendering the brain ischaemic.

Table 2 Neurological abnormality and genotype

Neurological abnormality	Genotype GG (n = 139) (%)	C allele (n = 68) (%)	Probability
Severe IVH	10 (7)	3 (5)	0.55
White matter injury	16 (10)	7 (11)	0.79
Cerebral palsy	13 (9)	4 (6)	0.59
Any disability at 2 years	22 (16)	9 (14)	0.62

IVH, intraventricular haemorrhage.

The finding described here (a possible influence on cognition but not motor outcome) is consistent with the observation that relates different aetiological factors to different types of disabling injury in the extreme preterm infant.[23] Marlow *et al*[23] reported that illness severity, focal cranial ultrasound abnormality and postnatal corticosteroids were associated with poor motor outcome in these infants, whereas impaired cognitive development was associated with other factors such as chronic lung disease, socioeconomic status and a low level of maternal education (for which we have adjusted). That we did not show a link between global developmental performance and genotype is consistent with recent findings on different polymorphisms of the interleukin-6 genotype, where the interleukin-6 −174 CC genotype was associated with disabling brain injury but not cognitive outcome,[14] and the interleukin-6 −572 C allele with cognitive deficit but not disability.[24]

There was no association, or even a trend, between the C allele and a reduced incidence of clinically significant patent ductus arteriosus. The lack of association (or even trend) between COX genotype and cranial ultrasound abnormalities was interesting, and we believe that this is a genuine finding (rather than a lack of power) as we have been able to show association with the interleukin-6 –174 CC genotype and cranial ultrasound abnormality in this patient group.[14] It is inherent within the hypothesis proposed here that the postulated damage due to COX downregulation or inhibition is diffuse and beyond the resolution of standard imaging

Table 3 Developmental performance and genotype

Developmental and subscale quotients	GG genotype	C allele	Mean difference (95% CI)	p Value
Griffith's DQ	97.6 (1.5)	92.7 (1.7)	4.9 (−9.7 to −0.25)	0.039
Locomotor	92.9 (1.4)	91.4 (1.9)	1.5	
Personal-social	103.6 (1.7)	96.2 (1.7)	7.4	
Hearing and speech	94.9 (2.4)	88.8 (2.4)	6.1	
Hand eye development	93.8 (1.4)	89.3 (1.9)	4.5	
Performance	102.9 (1.8)	97.6 (2.5)	5.3	
GCA	100.9 (1.7)	94.3 (2.2)	6.6 (−12.4 to −0.7)	0.028
Verbal subscale	103.7 (1.5)	94.0 (3.3)	9.7	
Non-verbal subscale	100.0 (1,7)	95.3 (2.4)	4.7	
Spatial subscale	98.9 (1.8)	93.3 (2.2)	5.6	

DQ, developmental quotient; GCA, general cognitive ability.
207 had follow up to 2 years and 156 had follow-up to 5½ years.
Values are mean (SEM).

(ultrasound). However, given the poor resolution of cranial ultrasound (relative to magnetic resonance imaging) and that correlation between structural abnormality and higher function is weak,[3] it would be interesting to relate COX2 genotype and grey matter volume determined by cranial magnetic resonance imaging to cognitive outcome in children without IVH or PVL.

This is a small study in which we have used a group of preterm infants with defined neurodevelopmental outcomes to test a hypothesis. We have shown an association between the COX2 –765 C allele and worse cognitive attainment at 2 and 5½ years of age in children born prematurely ($\leqslant$32 weeks). Although these observed differences are relatively small in clinical terms, these data suggest that COX inhibition may have direct neuropathological effects on the preterm brain.

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Authors' affiliations

D R Harding, Peter Dunn Neonatal Intensive Care Unit, St Michael's Hospital, Bristol, UK
S E Humphries, H E Montgomery, Cardiovascular Genetics, University College London, London, UK
A Whitelaw, Department of Clinical Science, University of Bristol, Southmead Hospital, Bristol, UK
N Marlow, School of Human Development, University of Nottingham, Nottingham, UK

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What is already known on this topic

- Indomethacin, a non-selective cyclo-oxygenase (COX) antagonist, reduces severe intraventricular haemorrhage (and perhaps cystic periventricular leucomalacia) but not cognitive outcome in infants born preterm. It is also known that some products of COX action (eg, prostaglandin E2) are neuroprotective in animal models and tissue culture. These findings suggest that COX inhibition may be deleterious to the development of cognition after preterm birth
- Mechanistic in vivo human data are naturally sparse

What this study adds

- This study uses a gene–environment association study to derive the mechanism by which indomethacin could be deleterious to cognitive development in those born preterm, and shows that preterm infants who are genetically programmed to produce lower amounts of COX2 via carriage of the COX2 −765 C allele may have a worse cognitive outcome at 2 and 5½ years than those who are not so programmed
- These data suggest that COX2 activity is neuroprotective overall to the developing areas of the preterm human brain responsible for cognitive function

Table 4 Independent associations with developmental performance

Variable	Effect size (B)	SE (B) (95% CI)	Probability
DQ			
COX2 C allele	−4.9	2.2 (−9.2 to −0.58)	0.026
White matter injury	−9.1	3.3 (−15.6 to −2.5)	0.007
Disability	−24.9	3.3 (−31.3 to −18.5)	<0.001
Manual occupations or unemployed	−5.7	2.2 (−10.1 to −1.4)	0.010
Maternal car use	+5.3	2.4 (0.70 to 10.0)	0.025
GCA			
COX2 C allele	−8.9	2.8 (−14.5 to −3.3)	0.002
Manual occupations or unemployed	−8.1	2.7 (−13.4 to −2.8)	0.003
Maternal education beyond 16 years	+9.4	2.7 (4.1 to 14.7)	0.001

CI, confidence interval; COX2, cyclo-oxygenase-2; DQ, developmental quotient; GCA, general cognitive ability.

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