PERSPECTIVE

Genetic susceptibility to retinopathy of prematurity: the evidence from clinical and experimental animal studies

Gerd Holmström, Peter van Wijngaarden, Douglas J Coster, Keryn A Williams

Br J Ophthalmol 2007;**91**:1704–1708. doi: 10.1136/bjo.2007.117283

Despite advances in management and treatment, retinopathy of prematurity remains a major cause of childhood blindness. Evidence for a genetic basis for susceptibility to retinopathy of prematurity is examined, including the influences of sex, ethnicity, and ocular pigmentation. The role of polymorphisms is explored in the genes for vascular endothelial growth factor and insulin-like growth factor-1, and of mutations in the Norrie disease gene. Insights into the genetic basis of retinopathy of prematurity provided by the animal model of oxygen induced retinopathy are examined. Evidence for a genetic component for susceptibility to retinopathy of prematurity is strong, although the molecular identity of the gene or genes involved remains uncertain.

Retinopathy of prematurity (ROP) is a major cause of blindness in many parts of the world and recent consecutive, population based studies have revealed that, despite advances in neonatology, the disease remains an important cause of morbidity in prematurely born infants.[1–6] Although more mature babies born today have a reduced risk of developing ROP compared with past eras, a new population of extremely immature babies is at high risk of developing severe disease. Some infants will develop the recently described aggressive posterior ROP.[7] While guidelines for management of ROP have been refined over the past three years, difficulty remains in distinguishing those infants in whom disease will spontaneously regress from those in whom it is likely to progress.[8]

ROP, first described as a distinct entity by Terry in 1942,[9] is a multifactorial disease which affects the retinal vasculature. Of the numerous risk factors that have been investigated, gestational age and birth weight remain the most important.[3 6] Genetic influences on the pathogenesis of ROP were proposed by Flynn[10] and have been implicated in human as well as experimental animal studies.[11] Identification of such influences may be helpful in the design of screening programmes for ROP, and may also be a tool in discriminating infants at high risk of severe ROP, and therefore in need of treatment, from those at lesser risk. Our purpose in this article is to provide the ophthalmologist with an update on genetic susceptibility to ROP, based both on clinical and animal studies.

EVIDENCE FROM CLINICAL STUDIES OF GENETIC SUSCEPTIBILITY TO ROP

A genetic component to susceptibility to ROP was suggested by several early clinical studies, which are detailed below. Such influences were recently confirmed in a retrospective study of monozygotic and dizygotic twins.[12] Taking into account the effect of gestational age and the duration of supplemental oxygen use, the investigators showed that 70% of the variance in susceptibility to ROP was the result of genetic factors alone.

The role of sex

Most population based studies, in accordance with the early CRYO-ROP[13–15] and later ETROP[8] data, have been unable to demonstrate a relation between sex and the development of ROP.[2 3 5 16 17] However, a few investigators have reported an increased prevalence or disease severity in male infants.[18–22] For example, Darlow *et al*—in a prospective, essentially population based study—found a significant relation between male sex and severe ROP.[22] There is no obvious explanation for these findings. A different susceptibility to illness in females and males might be one possibility. Female sex has been associated with increased survival rates in premature infants.[23 24] However, Yang *et al* recently showed that any relation of male sex with severe ROP was independent of overall health status and concluded that other physiological or genetic differences might be involved.[20] Overall, the weight of evidence from large cohorts of infants in population based studies suggests no particular influence of sex on the incidence of ROP, but male sex may be associated with increased severity of disease in affected infants.

The role of ethnic background

Ethnicity was first identified as a significant influence on ROP in 1952, when Zacharias published her observation that in several cohorts of premature infants in the USA, black infants were less likely to develop retinopathy than white infants.[19] Other investigators,[13 16 20 26] including those involved in the CRYO-ROP cohort of 4099 premature infants and a British study of 505 such babies, have since confirmed a reduced risk of severe ROP in black infants compared with white infants, at least in North American and British populations. More severe disease was also reported in Asian infants than in black infants in the British

Abbreviations: CRYO-ROP, Cryotherapy for Retinopathy of Prematurity Cooperative Group; ETROP, Early Treatment for Retinopathy of Prematurity Cooperative Group; FEVR familial exudative vitreoretinopathy, ; IGF1, insulin-like growth factor1; OIR, oxygen induced retinopathy; ROP, retinopathy of prematurity; VEGF, vascular endothelial growth factor

See end of article for authors' affiliations

Correspondence to: Professor K A Williams, Department of Ophthalmology, Flinders Medical Centre, Bedford Park 5042 SA, Australia; keryn.williams@flinders.edu.au

Accepted 2 June 2007

study, although survival rates differed for the different ethnic groupings examined.[16] A recent prospective study of 873 infants weighing less than 1500 g at birth and admitted to Alaskan neonatal intensive care units between 1989 and 2003 is of some interest.[25] Threshold ROP was significantly more common in Alaskan native (24.9%) and Asian (15.9%) infants than in white (6.3%) or black (4.6%) infants. A study of ROP in Australia and New Zealand found that infants born to Asian mothers were at increased risk of severe ROP compared with infants of white, indigenous Australian, Maori, or Pacific Islander mothers, but the risk was not significant in multivariate analysis.[22] In a review of the data from the CRYO-ROP study, Saunders *et al* tested several hypotheses for the observed reduction in the risk for ROP in black infants.[26] Of those infants who never developed ROP, the rate of retinal vascularisation was similar for all ethnic groups, excluding race related differences in retinal vascular maturation as a likely cause. Furthermore, the effect of ethnicity was independent of gestational age, birth weight, general health, or socioeconomic factors.

Taking the above mentioned reports into account, it seems likely that there is an effect of ethnicity on susceptibility to ROP. A plausible basis for a difference among racial groups in susceptibility to ROP might be ocular pigmentation. One hypothesis is that the more heavily pigmented eyes of black infants might be less susceptible to phototoxic injury than those of white infants. However, Asian babies may be at higher risk of ROP than white babies[16 25] and furthermore, a multicentre randomised clinical trial—the light reduction in retinopathy of prematurity study—found no significant difference in the incidence or severity of ROP in infants exposed to either reduced light or standard lighting.[27] The nexus between ethnicity and ocular pigmentation in humans is thus not well established. The situation with respect to ethnicity is further complicated by a web of issues other than straightforward genetic influences, including different cultural and economic approaches to the provision of intensive neonatal care in different communities.

The role of polymorphisms and mutations in ROP

A great deal of attention has recently been paid to the association between particular genetic polymorphisms or mutations and specific disease,[28] including diseases involving aberrant angiogenesis.[29] Various investigators have examined possible influences of genetic polymorphisms or mutations on ocular disorders such as ROP. In considering the results of these studies, it is important to appreciate that the statistical association of a genetic polymorphism or mutation with a disease state does not prove causality.

The role of vascular endothelial growth factor gene polymorphisms

Vascular endothelial growth factor (VEGF) is a central mediator of angiogenesis[30] and is known to play a role in the pathogenesis of human ROP.[31] Thus increased expression of VEGF mRNA was found in the avascular retina just anterior to the ridge in the eye of a premature neonate with severe ROP, and levels of the factor in the fellow eye, which had undergone laser peripheral retinal ablation, were reduced.[32] An evaluation of VEGF protein levels in the subretinal fluid of eyes with stage 4 ROP showed significant elevation over levels in control eyes.[33] Similarly, VEGF was detected in 65% of fibrovascular membranes removed from eyes of infants with stage 5 ROP at vitrectomy.[34]

The VEGF gene is highly variant, with more than 70 known polymorphic loci.[35 36] Several studies have identified single nucleotide polymorphisms in the VEGF gene that are associated with alterations in protein expression and which appear to segregate with the risk of progression to threshold disease. In their study of 91 infants with threshold ROP and 97 comparison infants without disease, Cooke *et al* showed that carriage of the VEGF −634 G allele in the 5′-untranslated region of VEGF gene was an independent risk factor for threshold ROP.[36] Homozygotes for this G allele were twice as likely to progress to threshold ROP as were other genotypes. However, the opposite association was found by Vannay *et al*,[37] who showed that the VEGF −634 C allele was significantly more common in the group of infants with threshold disease than in those without threshold ROP. The C allele was identified as an independent risk factor for severe disease, with doubling of the risk for heterozygotes and more than trebling of the risk for homozygotes. Some investigators have speculated that the VEGF −634 G/C polymorphism may be differentially linked to other polymorphisms in the 5′ flanking sequence.[36 38] Certainly, the risk of threshold ROP in infants homozygous for the VEGF −634 C allele was increased in association with another polymorphism, VEGF −1498 T/C.[37] Interestingly, VEGF polymorphisms have recently been shown to be associated with development of macular oedema in patients with diabetic eye disease,[38] and also with the development of neovascular age related macular degeneration.[39] At present, our understanding of the contributions made by polymorphisms in the VEGF gene to the risk of ROP is incomplete. It is possible that the combined effects of these polymorphisms may differ in different racial groups, accounting for some of the observed differences in susceptibility.

The role of insulin-like growth factor 1

Insulin-like growth factor 1 (IGF1) is a somatic growth factor known to regulate physical growth and to influence birth weight. It is essential for the normal development of the retinal vasculature and appears to work synergistically with VEGF.[40 41] In prospective studies of premature infants, evidence has recently emerged to suggest that a low serum level of IGF1 is a risk factor for retinopathy.[42] If systemic IGF1 levels are sufficiently high after birth, normal vessel development occurs and retinopathy of prematurity does not develop. When IGF1 is persistently low, vessels cease to grow and the maturing avascular retina becomes hypoxic. Retinal hypoxia is then associated with a marked increase in VEGF expression. As IGF1 increases to a critical level, retinal neovascularisation is triggered. Delayed production of IGF1 in these infants has been shown to correlate with the proliferative phase of ROP.[42] It has been suggested that measurement of serum IGF1 in premature infants might thus help to predict which of them will develop ROP.[43] The same group has also presented an algorithm based on measurement of serum IGF1 and IGF binding protein-3 levels, together with postnatal weight gain, for prediction of ROP requiring treatment.[44] It has recently been speculated that genetic factors may affect IGF1 expression,[34] especially given that several polymorphisms of the IGF1/IGF1 receptor system have been identified.[45] However, Balogh *et al* have recently failed to show an association between one such IGF1 polymorphism and ROP.[46]

The role of mutations in the Norrie disease gene

Norrie disease, familial exudative vitreoretinopathy (FEVR), and ROP form a group of retinal diseases with similar phenotypic manifestations. Norrie disease is a rare X linked hereditary disorder characterised by bilateral blinding retinopathy, deafness, and mental retardation.[47] It is caused by mutation of the Norrie disease pseudoglioma gene, which results in deficiency of functional Norrin protein.[48] Deficiency of the protein causes retarded retinal vascularisation leading to retinal ischaemia and the accumulation of hypoxia induced angiogenic factors. FEVR is a related disorder, characterised by

deficient retinal vascularisation often associated with exudation, retinal traction, and ultimately, retinal detachment.[49] Mutations of the Norrie disease gene are known to cause the X linked form of FEVR, while other forms of the disease are associated with mutations in other genes.[50 51] Thus FEVR and Norrie disease appear to be caused by dysfunction of the same signalling axis.

There are conflicting reports on the importance of the Norrie disease gene in the pathogenesis of ROP. It has been suggested that mutations of the gene may account for a proportion of advanced cases of ROP.[52–54] However, a recent study found no significant increase in the prevalence of Norrie disease gene polymorphisms in infants with severe ROP and the investigators concluded that Norrie disease gene polymorphisms do not play a major pathogenic role in severe ROP.[55] Further, Haider *et al* were unable to find an association between Norrie disease mutations and ROP in premature Kuwaiti infants.[56 57] Recently, however, the same group identified a polymorphism in an untranslated region of the Norrie disease gene (C597A) that was associated with severe ROP.[58] The functional significance of this polymorphism and its prevalence in other populations remain to be determined. Similarly, Dickinson *et al* concluded that several novel Norrie disease gene mutations and deletions did not contribute to severe ROP in their population of Australian infants.[59] It may be that the conflicting results about the importance of Norrie disease gene mutations in the susceptibility to ROP could be explained by ethnic differences in the populations studied.[56 60] Further studies are needed to evaluate the relevance of mutations in the Norrie disease gene in the development of ROP.

EVIDENCE FROM ANIMAL MODELS OF GENETIC SUSCEPTIBILITY TO ROP

Oxygen induced retinopathy as a model of ROP

Studies involving human infants are necessarily limited in scope, but additional evidence can be gained from animal models of disease. The experimental animal equivalent of ROP is known as oxygen induced retinopathy (OIR).[61] OIR involves the exposure of neonatal animals (usually mice, rats, cats, or dogs) to inspired hyperoxia (which induces attenuation of the normal postnatal retinal vascular development) followed by subsequent exposure to the relative hypoxia of room air (which induces retinal vascular proliferation). The two stages of experimental OIR recapitulate the biphasic nature of human ROP. Different species differ in their susceptibility to oxygen induced retinopathy, and also in the extent to which the retinopathy resembles human ROP.[61 62] The neonatal animals used in experimental models of ROP are not premature and therefore lack many of the comorbidities of premature human infants such as problems resulting from cerebral, pulmonary, and gastrointestinal immaturity. Many of these comorbid ailments may modulate the risk of ROP and its rate of progression. Despite these shortcomings, animal models of oxygen induced retinopathy have proven to be of value in the study of human ROP.[62]

The role of strain in OIR

An advantage of small rodents (rats and mice) is that they can be inbred to genetic homogeneity. Different inbred strains of laboratory rat have been shown to have differential susceptibility to OIR. Thus the Brown Norway strain was found to be significantly more susceptible to OIR than the Sprague Dawley strain.[63] Differences in retinal vascular permeability between these rat strains were also observed.[64] To further investigate heritable influences, we examined susceptibility to OIR in neonatal rats of five different inbred strains.[65] The Fischer 344 (F344), Wistar-Furth, and Lewis rat strains were relatively resistant to OIR, whereas the Sprague Dawley and the Dark Agouti (DA) strains were very sensitive. These strain related differences were independent of litter size, body mass, and major histocompatibility complex haplotype. A marked and consistent differential susceptibility of the retinal vasculature to cyclic hyperoxia that was strongly suggestive of a heritable component to OIR in this species was thus identified. Formal backcross analysis—in which an OIR resistant rat strain was mated with an OIR sensitive strain and the progeny were crossed back to each of the parental strains—was then used to investigate the genetics of the susceptibility trait.[66] Segregation of the susceptibility trait for OIR in the offspring of these various matings could be accounted for by an autosomal dominant pattern of inheritance.

The role of ocular pigmentation in OIR

It is clear that in experimental animals, as in humans, the association between ocular pigmentation and susceptibility to retinopathy of prematurity is far from straightforward. Although the pigmented rat strains thus far examined are susceptible to oxygen induced retinopathy, the albino Sprague Dawley—long used in such studies—is also susceptible. Other work has shown greater susceptibility to ischaemic retinal injury[67] and to retinal gliosis following trauma and inflammation[68] in albino rats, compared with pigmented rats.

In a recent study, differences in retinal vascularisation were examined in five strains of mouse exposed to hyperoxia.[69] Strains differed by up to twofold in retinal vascular volume and almost threefold in intravitreal neovascularisation. Two pigmented strains were differentially sensitive to hyperoxia, lending support to the contention that factors in addition to ocular pigmentation are important in regulating susceptibility to oxygen induced retinopathy.[65 66]

The role of gene polymorphisms and mutation in OIR

VEGF was first shown to act as an essential survival factor for the developing retinal circulation in a murine model of OIR.[70] In general, the proliferative phase of OIR appears to be associated with a higher level of VEGF expression than is observed in control animals raised in room air. Strain related differences in retinal gene expression of VEGF at both mRNA and protein levels during OIR in rodents have already been reported.[63 64 69 71] It seems likely that polymorphisms or mutations in other genes important in regulating the development of the retinal microvasculature may also govern susceptibility to OIR. Erythropoietin is an archetypal hypoxia induced protein[72] that is a key factor in the development of retinal neovascularisation in OIR in mice.[73] The expression of erythropoietin in the retinas of inbred strains of rats and their backcross progeny following hyperoxic exposure closely paralleled the microvascular phenotypic findings: significantly higher levels of erythropoietin mRNA were demonstrable in neonates sensitive to the attenuating effects of hyperoxia.[66]

Genetic influences on corneal neovascularisation

Strain related heterogeneity of ocular angiogenesis in rodents is not limited to the retina. Murine strain dependent variations in the corneal response to angiogenic factors have recently been identified.[29] Corneal stromal implantation of basic fibroblast growth factor impregnated micropellets was associated with angiogenesis that differed by up to 10-fold among different inbred strains.[74] Similar heterogeneity was seen in the response to VEGF. No clear association between susceptibility to corneal neovascularisation and ocular pigmentation was apparent in these studies. Further, the extent of the resting limbal vasculature has been shown to differ considerably among mouse strains, and is predictive of the response to basic fibroblast growth factor in the corneal neovascularisation

model.[75] Together, these studies suggest that genetic factors play important roles in regulating angiogenesis in the eye.

CONCLUSIONS

Despite decades of research on the pathogenesis of ROP, we still lack sufficient knowledge to predict in which infants ROP will regress spontaneously or progress in spite of adequate treatment. This overview of human and animal studies indicates that genetic influences in the pathogenesis of ROP may account for much of the heterogeneity in risk of progression of the disease. The evidence for genetic diversity affecting angiogenesis dependent processes in a variety of disparate diseases has recently been reviewed.[76] Given the complexity of the process of angiogenesis, alterations in the regulation or expression of any one of a large number of genes might contribute to a genetically determined susceptibility to ROP.

Further identification of new polymorphisms and mutations that may influence susceptibility to ROP is an area of active research. In the future, screening for candidate genes may be possible in the individual infant, to identify those at high risk ROP who are in need of frequent examinations and prompt treatment. Such screening should improve the visual outcome in high risk infants, and reduce the need for stressful examinations in low risk infants as well as the morbidity associated with unnecessary peripheral retinal ablation.

ACKNOWLEDGEMENTS

We acknowledge very helpful discussions with Associate Professor Jamie Craig. This work was supported by the NHMRC of Australia, the Ophthalmic Research Institute of Australia, the Flinders Medical Centre Research Foundation, and the Swedish Association of the Visually Impaired.

Authors' affiliations

Gerd Holmström, Department of Ophthalmology, Uppsala University, Uppsala, Sweden
Gerd Holmström, Peter van Wijngaarden, Douglas J Coster, Keryn A Williams, Department of Ophthalmology, Flinders University of South Australia, Adelaide, Australia

Competing interests: None declared.

REFERENCES

1 **Gilbert C**, Awan H. Blindness in children. *BM J* 2003;**327**:760–1.
2 **Holmström G**, el Azazi M, Jacobson L, *et al.* A population-based, prospective study of the development of ROP in prematurely born children in the Stockholm area of Sweden. *Br J Ophthalmol* 1993;**77**:417–23.
3 **Larsson E**, Carle-Petrelius B, Cernerud G, *et al.* Incidence of ROP in two consecutive population-based studies. *Br J Ophthalmology* 2002;**86**:1122–6.
4 **Fledelius HC**, Kjer B. Surveillance for retinopathy of prematurity in a Danish country. Epidemiological experience over 20 years. *Acta Ophthalmol Scand* 2004;**82**:38–41.
5 **Darlow BA**. Incidence of retinopathy of prematurity in New Zealand. *Arch Dis Child* 1988;**63**:1083–6.
6 **Darlow BA**, Hutchinson JL, Simpson JM, *et al.* Variation in rates of severe retinopathy of prematurity among neonatal intensive care units in the Australian and New Zealand Neonatal Network. *Br J Ophthalmol* 2005;**89**:1592–6.
7 **International Committee**. An International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. *Arch Ophthalmol* 2005;**123**:991–9.
8 **ETROP**. Early Treatment for Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol* 2003;**121**:1684–94.
9 **Terry TL**. Extreme prematurity and fibroblastic overgrowth of persistent vascular sheath behind each crystalline lens. *Am J Ophthalmol* 1942;**25**:203–4.
10 **Flynn J**. The premature retina: a model for the in vivo study of molecular genetics? *Eye* 1992;**6**:161–5.
11 **Wheatley CM**, Dickinson JL, Mackey DA, *et al.* Retinopathy of prematurity: recent advances in our understanding. *Br J Ophthalmol* 2002;**86**:696–700.
12 **Bizzaro MJ**, Hussain N, Jonsson B, *et al.* Genetic susceptibility to retinopathy of prematurity. *Pediatrics* 2006;**118**:1858–63.
13 **Schaffer DB**, Palmer EA, Plotsky DF, *et al.* Prognostic factors in the natural course of retinopathy of prematurity. The Cryotherapy for Retinopathy of Prematurity Cooperative Group. *Ophthalmology* 1993;**100**:30–7.
14 **Palmer EA**, Flynn JT, Hardy RJ, *et al.* Incidence and early course of retinopathy of prematurity. The Cryotherapy for Retinopathy of Prematurity Cooperative Group. *Ophthalmology* 1991;**98**:1628–40.
15 **CRYO-ROP**. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Multicenter trial of cryotherapy for retinopathy of prematurity. Three-month outcome. *Arch Ophthalmol* 1990;**108**:195–204.
16 **Ng YK**, Fielder AR, Shaw DE, *et al.* Epidemiology of retinopathy of prematurity. *Lancet*, 1988;ii, 1235–8.
17 **Holmström G**, Broberger U, Thomassen P. Neonatal risk factors for retinopathy of prematurity – a population-based study. *Acta Ophthalmol Scand* 1998;**76**:204–7.
18 **Kinsey VE**, Zacharias L. Retrolental fibroplasia: incidence in different localities in recent years and correlation of the incidence with treatment given the infants. *JAMA* 1949;**139**:572–8.
19 **Zacharias L**. Retrolental fibroplasia: a survey. *Am J Ophthalmol* 1952;**35**:1426–54.
20 **Yang MB**, Donovan EF, Wagge JR. Race, gender and clinical risk index for babies (CRIB) score as predictors of severe retinopathy of prematurity. *J AAPOS* 2006;**10**:253–61.
21 **Noodgard H**, Andreasson H, Hansen H, *et al.* Risk factors with retinopathy of prematurity (ROP) in northern Jutland, Denmark. *Acta Ophthalmol Scand* 1996;**74**:306–10.
22 **Darlow BA**, Hutchinson JL, Henderson-Smart DJ, *et al.* Prenatal risk factors for severe retinopathy of prematurity among very preterm infants of the Australian and New Zealand Neonatal Network. *Pediatrics* 2005;**115**:990–6.
23 **Phelps DL**, Brown DR, Tung B, *et al.* 28-day survival rates of 6676 neonates with birth weights of 1250 grams or less. *Pediatrics* 1991;**87**:7–17.
24 **Ingemarsson I**. Gender aspects of preterm birth. *Br J Obstet Gynaecol*, 2003;**110**(suppl 20), 34–8.
25 **Lang DM**, Blackledge J, Arnold RW. Is Pacific race a retinopathy of prematurity risk factor? *Arch Pediatr Adolesc Med* 2005;**159**:771–3.
26 **Saunders RA**, Donahue ML, Christmann LM, *et al.* Racial variation in retinopathy of prematurity. The Cryotherapy for Retinopathy of Prematurity Cooperative Group. *Arch Ophthalmol* 1997;**115**:604–8.
27 **Reynolds JD**, Hardy RJ, Kennedy KA, *et al.* Lack of efficacy of light reduction in preventing retinopathy of prematurity. Light Reduction in Retinopathy of Prematurity (LIGHT-ROP) Cooperative Group. *N Engl J Med* 1998;**338**:1572–6.
28 **Balasubramanian SP**, Brown NJ, Reed MWR. Role of genetic polymorphisms in tumour angiogenesis. *Br J Cancer* 2002;**87**:1057–65.
29 **Rogers MS**, D'Amato RJ. The effect of genetic diversity on angiogenesis. *Exp Cell Res* 2006;**312**:561–74.
30 **Aiello LP**. Vascular endothelial growth factor. 20th-century mechanisms, 21st-century therapies. *Invest Ophthalmol Vis Sci* 1997;**38**:1647–52.
31 **Smith LEH**. Pathogenesis of retinopathy of prematurity. *Acta Paediatr* 2002;**437**(suppl):26–28.
32 **Young TL**, Anthony DC, Pierce E, *et al.* Histopathology and vascular endothelial growth factor in untreated and diode laser-treated retinopathy of prematurity. *J AAPOS* 1997;**1**:105–10.
33 **Lashkari K**, Hirose T, Yazdany J, *et al.* Vascular endothelial growth factor and hepatocyte growth factor levels are differentially elevated in patients with advanced retinopathy of prematurity. *Am J Pathol* 2000;**156**:1337–44.
34 **Umeda N**, Ozaki H, Hayashi H, *et al.* Colocalization of Tie2 angiopoietin 2 and vascular endothelial growth factor in fibrovascular membrane from patients with retinopathy of prematurity. *Ophthalmic Res* 2003;**35**:217–23.
35 **Watson CJ**, Webb NJ, Bottomley MJ, *et al.* Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine* 2000;**12**:1232–5.
36 **Cooke RW**, Drury JA, Mountford R, *et al.* Genetic polymorphisms and retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2004;**5**:1712–15.
37 **Vannay A**, Dunai G, Banyasz I, *et al.* Association of genetic polymorphisms of vascular endothelial growth factor and risk for proliferative retinopathy of prematurity. *Pediatr Res* 2005;**57**:396–8.
38 **Awata T**, Kurihara S, Takata N, *et al.* Functional VEGF C-634G polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes. *Biochem Biophys Res Commun* 2005;**333**:679–85.
39 **Churchill AJ**, Carter JG, Lovell HC, *et al.* VEGF polymorphisms are associated with neovascular age-related macular degeneration. *Hum Mol Genet* 2006;**15**:2955–61.
40 **Smith LE**, Shen W, Perruzzi C, *et al.* Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. *Nat Med* 1999;**5**:1390–5.
41 **Hellstrom A**, Carlsson B, Niklasson A, *et al.* IGF-1 is critical for normal vascularization of the human retina. *J Clin Endocrinol Metab* 2002;**87**:3413–16.
42 **Hellstrom A**, Perruzzi C, Ju M, *et al.* Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc Natl Acad Sci USA* 2001;**98**:5804–8.
43 **Hellstrom A**, Engstrom E, Hard AL, *et al.* Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. *Pediatrics* 2003;**112**:1016–20.
44 **Lofqvist C**, Andersson E, Sigurdsson J, *et al.* Longitudinal postnatal weight and insulin-like growth factor I measurements in the prediction of retinopathy of prematurity. *Arch Ophthalmol* 2006;**124**:1711–18.
45 **Rasmussen SK**, Lautier C, Hansen L, *et al.* Studies of the variability of genes encoding the Insulin-like Growth Factor 1 receptor and its ligand in relation to type diabetes mellitus. *J Clin Endocrinol Metab* 2000;**85**:1606–10.
46 **Balogh A**, Derzbach L, Vannay A, *et al.* Lack of association between insulin-like growth factor 1 receptor +3174 A polymorphism and retinopathy of prematurity. *Graefes Arch Clin Exp Ophthalmol* 2006;**244**:1035–8.

47 **Berger W**. Molecular dissection of Norrie disease. *Acta Anat (Basel)* 1998;**162**:95–100.
48 **Xu Q**, Wang Y, Dabdoub A, *et al*. Vascular development in the retina and inner ear: control by Norrin and Frizzled-4 a high-affinity ligand-receptor pair. *Cell* 2004;**116**:883–95.
49 **Shastry BS**, Hejtmancik JF, Plager DA, *et al*. Linkage and candidate gene analysis of X-linked familial exudative vitreoretinopathy. *Genomics* 1995;**27**:341–4.
50 **Robitaille J**, MacDonald ML, Kaykas A, *et al*. Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat Genet* 2002;**32**:326–30.
51 **Toomes C**, Bottomley HM, Jackson RM, *et al*. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am J Hum Genet* 2004;**74**:721–30.
52 **Hiraoka M**, Berinstein DM, Trese MT, *et al*. Insertion and deletion mutations in the dinucleotide repeat region of the Norrie disease gene in patients with advanced retinopathy of prematurity. *J Hum Genet* 2001;**46**:178–81.
53 **Shastry B**, Pendergast S, Hartzer M, *et al*. Identification of missense mutations in the Norrie disease gene associated with retinopathy of prematurity. *Arch Ophthalmol* 1997;**115**:651–5.
54 **Talks SJ**, Ebenezer N, Hykin P, *et al*. De novo mutations in the 5′ regulatory region of the Norrie disease gene in retinopathy of prematurity. *J Med Gen* 2001;**38**:E46.
55 **Hutcheson KA**, Paluru PC, Bernstein SL, *et al*. Norrie disease gene sequence variants in an ethnically diverse population with retinopathy of prematurity. *Mol Vis* 2005;**11**:501–8.
56 **Haider MZ**, Devarajan LV, Al-Essa M, *et al*. Missense mutations in Norrie disease gene are not associated with advanced stages of retinopathy of prematurity in Kuwaiti Arabs. *Biol Neonate* 2000;**77**:88–91.
57 **Haider MZ**, Devarajan LV, Al-Essa M, *et al*. Retinopathy of prematurity: mutations in the Norrie disease gene and the risk of progression to advanced stages. *Pediatrics Int* 2001;**43**:120–3.
58 **Haider MZ**, Devarajan LV, Al-Essa M, *et al*. A C597→A polymorphism in the Norrie disease gene is associated with advanced retinopathy of prematurity in premature Kuwaiti infants. *J Biomed Sci* 2002;**9**:365–70.
59 **Dickinson JL**, Sale MM, Passmore A, *et al*. Mutations in the NDP gene: contribution to Norrie disease, familial exudative vitreoretinopathy and retinopathy of prematurity. *Clin Exp Ophthalmol* 2006;**34**:682–8.
60 **Shastry BS**. Ophthalmic genetics: complexity in paediatric vitreoretinopathy. *Clin Exp Ophthalmol* 2006;**34**:633–4.
61 **Madan A**, Penn JS. Animal models of oxygen-induced retinopathy. *Front Biosci* 2003;**8**:1030–43.
62 **Gole G**. Animal models of retinopathy of prematurity. In: Silverman WA, Flynn JT, eds. *Retinopathy of prematurity*. London: Blackwell Scientific Publishers, 1985:53–9.
63 **Gao G**, Li Y, Fant J, *et al*. Difference in ischemic regulation of vascular endothelial growth factor and pigment epithelium-derived factor in brown Norway and Sprague-Dawley rats contributing to different susceptibilities to retinal neovascularization. *Diabetes* 2002;**51**:1218–25.
64 **Zhang SX**, Ma JX, Sima J, *et al*. Genetic difference in susceptibility to the blood–retina barrier breakdown in diabetes and oxygen-induced retinopathy. *Am J Pathol* 2005;**166**:313–21.
65 **van Wijngaarden P**, Coster DJ, Brereton HM, *et al*. Strain-dependent differences in oxygen-induced retinopathy in the inbred rat. *Invest Ophthalmol Vis Sci* 2005;**46**:1445–52.
66 **van Wijngaarden P**, Brereton HM, Coster DJ, *et al*. Genetic influences on susceptibility to oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci* 2007;**48**:1761–6.
67 **Safa R**, Osborne NN. Retinas from albino rats are more susceptible to ischaemic damage than age-matched pigmented animals. *Brain Res* 2000;**862**:6–42.
68 **Engelmann R**, Dieterich DC, Bien A, *et al*. A different retinal glia response to optic nerve injury/lipopolysaccharide administration in hooded and albino rats. *Brain Res* 2001;**889**:251–5.
69 **Chan CK**, Pham LN, Zhou J, *et al*. Differential expression of pro- and antiangiogenic factors in mouse strain-dependent hypoxia-induced retinal neovascularization. *Lab Invest* 2005;**85**:721–33.
70 **Alon T**, Hemo I, Itin A, *et al*. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995;**1**:1024–8.
71 **Floyd BN**, Leske DA, Wren SM, at al. Differences between rat strains in models of retinopathy of prematurity. *Mol Vis* 2005;**11**:524–30.
72 **Wang GL**, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci USA* 1993;**90**:4304–8.
73 **Morita M**, Ohneda O, Yamashita T, *et al*. HLF/HIF-2alpha is a key factor in retinopathy of prematurity in association with erythropoietin. *EMBO J* 2003;**22**:1134–46.
74 **Rohan RM**, Fernandez A, Udagawa T, *et al*. Genetic heterogeneity of angiogenesis in mice. *FASEB J* 2000;**14**:871–6.
75 **Chan CK**, Pham LN, Chinn C, *et al*. Mouse strain-dependent heterogeneity of resting limbal vasculature. *Invest Ophthalmol Vis Sci* 2004;**45**:441–7.
76 **Cole CH**, Wright KW, Tarnow-Mordi W, *et al*. Pulse Oximetry Saturation Trial for Prevention of Retinopathy of Prematurity Planning Study Group. Resolving our uncertainty about oxygen therapy. *Pediatrics* 2003;**112**:1415–19.