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Assessment of the contribution of insulin-like growth factor I receptor 3174 G→A polymorphism to the progression of advanced retinopathy of prematurity

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PURPOSE. *Retinopathy of prematurity (ROP) is a leading cause of blindness in children with short gestational age and low birthweight. The condition can cause abnormal vessel development that can lead to retinal detachment and blindness. It has been recently reported that a low level of insulin-like growth factor I (IGF-I) is associated with ROP. However, the most prevalent polymorphism of IGF-I receptor (IGF-IR 3174 G→A) that was reported to be producing low level of IGF-I was not found to be associated with ROP in a certain population. In order to reproduce this data in a different cohort and to learn more about the contribution of IGF-IR polymorphism to ROP, the authors hypothesized that it is possible that such a polymorphism would occur more frequently in a different cohort of infants with advanced ROP than those children with mild or no disease.*

METHODS. *For genetic analysis, eligible patients were selected consecutively by experienced pediatric ophthalmologists and leukocyte DNA from affected (n=52) and normal patients (n=33) were amplified by polymerase chain reaction. The amplified products were subjected to restriction enzyme digestion with 10 units of MnlI enzyme. The digested products were analyzed by polyacrylamide gel electrophoresis followed by ethidium bromide staining to visualize the restriction fragment length polymorphism.*

RESULTS. *The analysis suggests that there is no statistically significant difference in allelic frequency of the most prevalent IGF-IR gene polymorphism between normal subjects and patients with ROP in this cohort. The G→A polymorphism did not occur more frequently in patients with ROP.*

CONCLUSIONS. *The results do not support the association of the most prevalent IGF-IR gene polymorphism and the risk of advanced ROP in a different cohort, confirming the earlier report. (Eur J Ophthalmol 2007; 17: 950-3)*

KEY WORDS. *Growth factor, Polymorphism, Prematurity, Receptor, Retinopathy*

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INTRODUCTION

Retinopathy of prematurity (ROP) is a retinal vascular disease affecting infants with low birthweight and short gestational age. In advanced cases, the condition is characterized by abnormal vessel growth in the vitreous that can lead to vitreoretinal traction, retinal detachment, and other complications resulting in blindness. The disorder clinically shares some common features with familial exudative vitreoretinopathy (FEVR), which occurs in full-term infants. The pathogenesis and the etiology of advanced ROP are not fully understood. In the past, many causative factors

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such as supplemental oxygen, excessive light exposure, and hypoxia have been suggested, but evidence for these as independent risk factors in recent years is not compelling (1). Although some degree of abnormal vascularization occurs in all low birthweight infants, only a small percentage of children develop a more severe ROP. It is not clear why ROP in a subset of infants with low birthweight progresses to a severe stage despite timely intervention while in other infants with similar clinical characteristics ROP regresses spontaneously. Recent research with the candidate gene approach (2-4) and twin studies (5) together with several indirect lines of evidence such as racial variation suggest a strong genetic predisposition to ROP besides environmental factors such as prematurity. However, at present there is no reliable method to predict which premature infant will develop retinal detachment and blindness. The ability to identify these high-risk infants would permit more aggressive monitoring and surgical intervention of these patients at the early stages of the disease process.

In light of this, it has been recently reported that a prolonged period of low levels of insulin-like growth factor I (IGF-I) may predict the development of ROP and other complications of premature birth (6). Infants with higher IGF-I do not develop ROP and exhibit better vascular development. IGF-I is an intrauterine growth factor and is expressed in several retinal cells. Several studies also suggest that it is essential for vascular development of the eye in the postnatal period. These results were supported by IGF-I knockout mice that developed abnormal retinal vascular growth. Since prematurity is one of the factors that contributes to ROP and the IGF-I level is determined by the IGF-I receptor (IGF-IR) and the most prevalent polymorphism IGF-IR 3174 G→A exhibited low levels of free plasma IGF-I, we hypothesized that such a single nucleotide polymorphism would occur more frequently in infants with advanced ROP in our cohort than in those with mild or no disease. In order to understand the relationship between advanced ROP and the most prevalent IGF-IR polymorphism, we carried out the following small-scale study in a cohort of patients.

METHODS

Patients

In order to validate our hypothesis and to further understand the genetic contribution to advanced ROP, eligible patients (stages 4 and 5 involving partial and complete retinal detachment) were selected consecutively by experienced pediatric ophthalmologists. These patients have undergone a controlled oxygen treatment in a single hospital by an experienced pediatrician. After informed consent was obtained from one of each child's parents, blood samples were collected from a total of 52 children with advanced ROP including three pairs of dizygotic and two pairs of monozygotic twins. The birthweight and gestational age ranged from 600 to 1300 g (mean 882 g) and 23 to 30 weeks (mean 26 weeks). The control group consisted of 33 unrelated randomly selected full-term normal adults with no sign of ROP. In the patient set there were 63% males and 37% females and all patients had clinical findings typical of advanced ROP (stages 4 and 5). Exclusion criteria included family history of FEVR, Norrie disease, bleeding disorders, or ocular findings considered atypical for ROP. The study was approved by the Institutional Review Board of Oakland University.

Study design

For genetic analysis, leukocyte DNA was amplified by polymerase chain reaction (PCR) using pairs of primers described previously (7). Briefly, the primers were annealed at 55 °C for 1 min and extended at 72 °C for 2 min with 32 cycles using the FailSafe PCR buffer G system supplied by Epicenter (Madison, WI, USA). The amplified product was subjected to restriction enzyme digestion with 10 units of MnlI enzyme and the digested DNA fragments were analyzed by 12% polyacrylamide gel electrophoresis with 1 X tris-borate-EDTA buffer followed by ethidium bromide staining.

Statistical method

The significance of allele frequency differences was tested by using a chi-square test with Yates correction and confirmed by Fisher exact test. Because there is only a limited amount of information on frequency in the preterm population, study size estimations are difficult to make. However, the power of Fisher exact test shows that if the frequency of the A allele in the ROP group is increased by 25%, we would have 98% chance of detecting the difference with 52 infants.

TABLE I - ALLELE FREQUENCIES OF IGF-IR GENE POLYMORPHISM IN PATIENTS WITH RETINOPATHY OF PREMATURITY (ROP) AND NORMAL PATIENTS

Sequence polymorphism	ROP	Normal
G3174A, allelic frequency	G 0.51	0.62
	A 0.49	0.38
Birthweight, g	600–1300	2430–3960
Gestational age, wk	23–30	34–40

RESULTS

In order to understand the relationship between the most prevalent IGF-IR polymorphism (3174 G→A) and the progression of ROP, in this study we analyzed 52 infants with advanced ROP and 33 normal patients with no sign of ROP. The restriction digestion pattern indicates that all three genotypes (GG/GA/AA) are present in patients with advanced ROP as well as in control group. However, the allelic frequency (Tab. I) suggests that there is no significant statistical difference between patients with ROP and the normal controls. Chi-square test with Yates correction and Fisher exact test gave p values of 0.19 and 0.16, respectively. Since these are large values, we conclude that there is no association between ROP and the IGF-IR polymorphism. The 95% confidence intervals for the odds ratio are 0.32, 1.22, which supports our conclusion.

DISCUSSION

Despite advances in our understanding and management of ROP, the disorder is still a leading cause of blindness in children. It is unknown why ROP in some infants with low birthweight progresses to a severe stage despite timely intervention, while in other infants with similar clinical characteristics, ROP regresses spontaneously. Recent studies suggest that this unpredictability could be due to genetic factors in addition to environmental factors. However, there are no reliable methods of predicting which premature infants develop retinal detachment and blindness. The ability to identify these high-risk infants would permit more aggressive monitoring and surgical intervention at early stages of the disease process. Because IGF-IR 3174 G→A polymorphism exhibited low levels of free plasma IGF-I and low levels of serum IGF-I are reported to predict the development of ROP, we hypothesized that this single nucleotide polymorphism in the IGF-IR gene may be more prevalent in ROP in our cohort than in controls. However, our results do not support our hypothesis. The allelic frequency suggests that there is no significant statistical difference between patients with ROP and normal controls. These results are consistent with another recent report (7) in a different population. However, we cannot eliminate IGF-IR gene involvement in ROP because there are other polymorphisms in the coding and noncoding regions that may contribute to the low levels of IGF-I. Additionally, we cannot directly compare our results with other studies because we have not used preterm babies without ROP as controls. However, the information obtained by using term babies is valuable because the distribution of an unrelated polymorphism must be the same between preterm and term babies. Although we cannot draw a strong conclusion from this small-scale study, and we are addressing a specific situation and not a general population, the power calculation supports our conclusion. In the future, a large-scale study and complete analysis of the IGF-IR gene genotype in ROP will be required to understand the contribution of IGF-IR gene to ROP.

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