Mutations of the Norrie Gene in Korean ROP Infants

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The present study was conducted to evaluate if there is a Norrie disease gene (ND gene) mutation involved in the retinopathy of prematurity (ROP), and to identify the possibility of a genetic abnormality that may be linked to the presence of ROP. Nineteen premature Korean infants, with a low birth weight (1500g or less) or low gestational age (32 weeks or less), were included in the study. Eighteen infants had ROP, and the other did not. Genomic DNA was isolated from the peripheral blood leukocytes of these patients, and all three exons and their flanking areas, all known ND gene mutations regions, were evaluated following amplification by a polymerase chain reaction, but no ND gene mutations were detected. Any disagreement between the relationship of ROP to the ND gene mutation will need to be clarified by further investigation.

Key words: Korean, mutation, Norrie gene, retinopathy of prematurity

INTRODUCTION

Retinopathy of prematurity (ROP) is a well known cause of blindness in premature children, and various factors are known to be associated with its occurrence and progression. Of the controllable factors, oxygen is thought to be a major risk factor, and the careful control of its supplement brings a satisfactory proportional decrease in iatrogenic blindness in premature babies. However, an increase in the number of incidences, over those expected, has recently occurred. The risk factors associated with prematurity, including: a low gestational age and low birth weight, have emerged as being the most significant, but these factors are not totally predictive. The clinical courses for the presentation and progression are variable in patients with acute ROP even when at a similar stage, which can not be explained by a simple combination of risk factors.

Familial exudative vitreoretinopathy (FEVR) and Norrie disease have similar clinical manifestations to ROP. They both show vasoproliferative changes of the retinal vessels. It is known that X-linked FEVR and the Norrie disease gene are linked (ND gene), and the similarities in their clinical manifestations may be associated with their genetic linkage.

Conflicting studies concerning genetic linkage of ROP with ND gene have recently been reported, with some results showing a relationship between advanced ROP and the ND gene, but others
have not.\textsuperscript{18,19}

We have asked ourselves the question, which premature infants will develop advanced ROP, and we think the answer may lie in the underlying molecular or genetic abnormalities. Therefore, the present study was undertaken to identify any possible link between a genetic abnormality of the Norrie gene mutation and ROP.

\section*{MATERIALS AND METHODS}

\subsection*{Patients}

19 premature infants with a low birth weight (1500g or less), or a low gestational age (32 weeks or less), were included in this study. Retinal findings, including: time of onset and the tempo of disease progression, in 18 of the infants were consistent with ROP. One infant showed no suspected ROP related lesions, although vascular immaturity was evident. After acquiring the written informed consent for the procedure as to the purposes, risks, and benefits of this study were explained to their parents, as approved by the Institutional Review Board of the Seoul National University Hospital, a 2ml blood sample was collected from each infant. All patients were followed up for more than 2 years for evaluation of the retinal sequelae.

\subsection*{Genetic analysis}

Genomic DNA was isolated from peripheral blood leukocytes of the patients, and all three exons, and their flanking areas, amplified by a polymerase chain reaction (PCR) using previously reported primers.\textsuperscript{22} Three pairs of oligonucleotide primers were used: exon 1, 5'-TCCCGATAACGAGCGCCT-3', and 5'-CTTGGCAAGCCGGGACGCG-3'; exon 2: 5'-GTTCCATTAGTTGTTCTGG-3', and 5'-CTTGCTTGTCTCTGAGGGAA-3'; exon 3: 5'-CTTGCCATAGGGGTTGATATTA-3' and 5'-ACAGTTGTCCCATCCGAA-3'. PCR was performed on a final volume of 50 ml, containing: 200 ng of genomic DNA; 10mM Tris-HCl (pH 8.3); 1.5 mM MgCl\textsubscript{2}; 50 mM KCl; 200 \mu M of each dNTP; 20pmoles of each primer; and 1.25 U of Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN, USA), under the following conditions: the DNA was initially denatured at 95°C for 5 minutes, amplification was performed using 35 cycles of, 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C, which was followed by a final extension for 7 minutes at 72°C. The amplified products and the PCR primers were sequenced using a Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech, Little Chalfont, U.K.).

\section*{RESULTS}

Of the 19 infants, 7 were male and 12 were female. Their birth weights ranged from 650 to 1850g, with a mean \textpm SD of 1287 \pm 318 g, and their gestational ages ranged from 25 to 34 weeks, with a mean \textpm SD of 29.2 \pm 2.5 weeks.

There were 4 with stage 2 ROP in both eyes, 8 with stage 3 (plus: 6, no plus: 2), 1 with stage 4 and 3 with stage 5, 2 had the rush type ROP and 1 had a normal retina. No infant had asymmetric ROP. We performed diode laser photocoagulation, or cryotherapy, on 11 eyes that developed the threshold, or the rush type, ROP, an encircling procedure on 2 eyes at stage 4 ROP, and delamination on 6 eyes at stage 5 ROP. At the follow-up fundus examination at 2 years of age, retinal dragging was found in 5 eyes, a falciform retinal fold in 1, total detachment of the retina in 6, with all others having flat retinas.

For the genetic analyses, we thoroughly examined all regions of known ND gene mutations, but no genetic abnormalities were detected.

\section*{DISCUSSION}

Some conflicting studies concerning the genetic linkage of ROP with the ND gene have recently been reported. Some authors implied the possibility of genetic abnormalities being associated with the ND gene in ROP.\textsuperscript{15-17} They found several patients with advanced ROP and the ND gene mutations - missense mutations (R121W and L108P) in the 3\textsuperscript{rd} exon of the ND gene, an insertion of additional CT repeats (12bp) in the 1\textsuperscript{st} exon and a deletion of CT repeats (14bp) in the 1\textsuperscript{st} exon. Strong evidence has been provided supporting the idea that in some premature infants there was a genetic influence on the development of their ROP.
Conversely, there have been reports of no association of the ND gene mutation with advanced ROP. According to these results, the homozygous genotypes, [R121W], [L108P] and [A105T], in the missense mutations of the ND gene are commonly found in Kuwaiti premature infants, with the [V60G] mutation having no association with advanced ROP.

Although the number of infants in this study was small, we evaluated all known sites of the ND gene mutations at all stages of ROP. We found no missense mutations of the ND gene, which were significantly associated with ROP, and the homozygous genotypes, [R121W], [L108P] and [A105T], were not commonly found in our study, because we could not find any genetic abnormalities associated with the ND gene in ROP infants.

The results from our study were very different from those found previously, although ours do have the limitation of a small study group. We found no abnormal mutations in ROP infants, or common genetic characteristics, as found with the Kuwaiti infants, so this difference may be ethnic. Some epidemiological studies have shown these kinds of difference in the development of ROP, such as: low incidences of debilitating vision, in Jewish & Kuwaiti, and high incidences in Danish & Alaskan natives.

Our hypothesis was based on many epidemiological studies where ethnic differences were shown, and experimental studies showing a failure to reproduce cicatricial ROP in animal models and also in genetic studies showing ND gene mutations in advanced ROP. From these findings, there would appear to be no genetic abnormalities that are associated with the ND gene in Korean ROP infants. This may be due to ethnic differences, and suggests the hypothesis that: “the development of ROP is associated with genetic abnormalities”, is true, and may include the ND gene mutations.

There may be a genetic background in the development of ROP, and there is evidence that supports this hypothesis. For example; the required time, to arrive at the threshold is consistent, is about 34 to 42 weeks following conception, regardless of gestational age or birth weight variations at the time of diagnosis. The course to this threshold is usually consistent, regardless of the stage at the time of diagnosis, although the rush type ROP progresses via another pathway. The timing, and course, of the retinal vascular events implies that something other than postnatal environmental factors influences retinal vascular events. This ‘something’ may be the genetic background.

We could find no genetic abnormality associated with the ND gene mutation at stages 2 to 5 ROP in Korean infants. We had expected to identify that the severe ROP progression might be associated with genetic abnormalities, especially the ND gene mutation, and thereby contribute to the development of more accurate predictive tools for the prognosis, and more effective treatment modalities, which eventually would lead to genetic counseling of the parents of ROP infants. We could find no abnormalities that supported our hypothesis of a genetic association between ROP and the ND gene mutation. However, we can not conclude there is no evidence for a genetic association between ROP and the ND gene mutation in Korean infants, due to our limited sample size.

We are confront with confusing problems: why does an infant of 2500 g unusually has more severe ROP than an infant of 850 g, why does an infant have a very immature vascular network in his retina for their gestational age, or why a given patient has a progression to advanced ROP regardless of treatment modality. These problems can not be solved by knowledge of previously known risk factors. The answers may lie in an extrauterine environmental, or genetic, mechanism. Further genetic studies will give us a better understanding to this perplexing question.

REFERENCES
4. Hammer ME, Mullen PW, Ferguson JG, Pai S,


