NICHD
NEONATAL RESEARCH NETWORK

Candidate Gene Study of Retinopathy of Prematurity in Extremely Low Birthweight Infants from the Neonatal Research Network

ME Hartnett, M Morrison, G Page, M Cotten, J Murray, M DeAngelis

Department of Ophthalmology, Moran Eye Center, University of Utah; RTI International, Department of Pediatrics Duke University, Department of Pediatrics University of Iowa, on behalf of he Eunice Kennedy Shriver NICHD Neonatal Research Network

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

SUPPORTED BY Research to Prevent Blindness

The University of Utah Moran Eye Center

Background

- Retinopathy of prematurity (ROP) risk is increased by extreme prematurity, oxygen exposure, and potentially inflammation.
- Analysis of mono- and di-zygotic twins found 70% variance in ROP risk from genetic factors.
- Candidate gene associations: *NORRIN/FZD4/LRP5* pathway, *EPAS1, VEGF, SOD*; however, studies have been small and results inconsistent perhaps due to heterogeneity in populations, phenotype, or diagnosis.

Objective

- To determine associations between candidate genes and ROP risk in a defined population within U.S. intensive care nurseries.

Methods

- Subjects:
  - Multiracial sample of 1,013 infants born 1998-2001;
  - birthweight <1000g;
  - blood spot samples in the NICHD Neonatal Research Network's anonymized DNA biorepository;
  - at least one ROP screening exam.
- ROP classified by zone and stage by ophthalmologists prior to death or discharge. Severe ROP = treated with laser or cryotherapy.
- Whole genome amplified DNA genotyped on Illumina GoldenGate platform for 1614 SNPs of 145 candidate genes.
- SNP data cleaned and analyzed using PLINK.
- SNPs removed: >10% genotypes missing; not in Hardy Weinberg Equilibrium.
- Analyses performed: **any ROP vs. no ROP**; **non-severe ROP vs. severe ROP**; **severe ROP vs. non-severe ROP and no ROP**.
- Epidemiologic variables tested for association with ROP using logistic regression (SAS).
- Stepwise logistic regression to determine significant epidemiologic factors.
- Minor allele for each SNP tested for association using logistic regression in PLINK.
- Correction for multiple testing by Bonferroni, Sidak, FDR.
- Bioinformatic analyses with Ingenuity Pathway Analysis and SNP and CNV Annotation Database.

Acknowledgements

R01EY015130 (PI: MEH)
HD52593 (PI: JM)
NRN 5U10 HD040492-12

References

Bizzarro et al.Pediatric 2006;118:1858-63
Drenser et al Arch Ophthalmol 2009;127:1649-54
Mohamed et al Pediatr Res 2009;65:193-197
Ells et al Ophthalmic Genetics 2010; 31:37-43
Giusti et al Free Radic Res 2012; 46:1130-1139
Kondo H et al Mol Vis 2013;19:476-485

Results

Genotyping

1494 SNPs were genotyped. After cleaning, the data set included 1324 SNPs and 964 infants.
49/1013 infants were removed because of low genotyping rates.
170 markers were excluded, 105 because of failure to be in Hardy Weinberg Equilibrium and 93 for low genotyping. 28 SNPs overlapped.

Epidemiologic Variables

42 epidemiologic variables tested included demographic, treatment, and outcome variables. After stepwise regression and controlling for multiple comparisons, variables retained were:

1. days of ventilation within 28 days for **ROP vs. no ROP**;
2. occurrences of seizures for **severe ROP vs. non-Severe ROP**;
3. both occurrences of seizures and days of ventilation for **Severe ROP vs. no ROP and non-Severe ROP** (see **Tables 2 a-c** at right).

Table 1. Characteristics of Sample Population

	No ROP	Any ROP	Severe ROP	non-Severe ROP	No ROP + non-Severe ROP	Whole Cohort
Gestational Age	27.1 (1.9)	25.4 (1.7)	24.5 (1.2)	25.7 (1.7)	26.2 (1.8)	25.9 (1.9)
Birth Weight	823.6 (126)	745.4 (134)	697 (125)	758 (133)	782 (135)	763 (141)
Small for Gestational Age (%)	64 (24.33)	61 (10.5)	6 (4.8)	55 (12.1)	119 (16.6)	125 (14.8)
Male (%)	113 (43%)	284 (49)	62 (50)	222 (48.8)	335 (46.7)	397 (47.1)
Days in Ventilation	8.2 (9.2)	19.2 (9.9)	25.4 (5.7)	17.5 (10.2)	14.1 (10.8)	15.4 (10.7)
Race/ Ethnicity Black (%)	133 (50.6%)	271 (46.8)	60 (48.4)	211 (46.4)	344 (47.9)	404 (48)
White (%)	123 (46.7%)	299 (51.6)	63 (50.9)	236 (51.8)	359 (50)	422 (50.1)
Hispanic (%)	29 (11%)	125 (21.6)	24 (19.5)	101 (22.2)	130 (18.1)	154 (18.3)
Occurrences of Seizures	13 (4.9%)	68 (11.7%)	25 (20.2%)	43 (9.4%)	56 (78%)	81 (9.6%)
Antenatal Steroids	218 (82.9%)	441 (76.3)	90 (73.2)	351 (77.1)	569 (79.3)	659 (78.4)

Pathway Analysis

e-QTL showed BDNF alltered expression of 24 genes. Pathways involved carbohydrate metabolism, behavior, cancer, nervous system development, cell movement, cell-cell signaling.

Results

Table 2a Any vs. no ROP

CHR	SNP	Gene	Allele	NMISS	p-value	Odds Ratio	FDR_BH p
10	rs297046	LOC645269 /NEUROG3	G	542	2.35E-06	0.2532	0.0031
10	rs12360522	PCDH15	A	534	0.000883	1.901	0.5842
2	rs4251956	IL1RN	T	543	0.006521	0.1987	0.9795
2	rs2280234	STAT1	G	543	0.006561	0.6365	0.9795
11	rs7929344	BNDF	A	543	0.006922	0.5406	0.9795

23 variables significant. After stepwise regression, only days of ventilation within 28 days was significant. After adjusting for days of ventilation, eigen values and multiple testing, only an intergenic SNP on chromosome 10 (rs297046) between *NEUROG3* and *LOC645269* was significantly protective.

Table 2b Severe ROP vs. non-severe ROP

CHR	SNP	Gene	Allele	NMISS	p-value	Odds Ratio	FDR_BH p
11	rs7934165	BNDF	C	376	0.000109	1.983	0.0885
11	rs2049046	BNDF	A	377	0.000134	1.986	0.0885
16	rs2057768	NSMCE1/IL 4R	T	377	0.000698	0.4873	0.2435
16	rs7204874	NSMCE1/IL 4R	A	377	0.000736	2.452	0.2435
12	rs2193154	GRIN2B	T	348	0.001406	2.679	0.3724

19 variables significant, but after stepwise regression, only occurrences of seizures remained significant. After adjusting for occurrence of seizures, eigen values and multiple testing, no SNPs were significant.

Table 2c Severe ROP vs. no ROP and non-severe ROP

CHR	SNP	Gene	Allele	NMISS	p-value	Odds Ratio	FDR_BH p
11	rs7934165	BDNF	C	542	1.18E-05	2.317	0.0098
11	rs2049046	BDNF	A	543	1.48E-05	2.325	0.0098
2	rs13419896	EPAS1	A	542	0.0012	2.366	0.5312
16	rs7204874	NSMCE1/IL 4R	A	543	0.00292	2.265	0.5503
23	rs45501198	NDP	A	517	0.00296	6.503	0.5503

21 variables significant, but after stepwise regression, both occurrences of seizures and days of ventilation within 28 days remained significant. After adjusting for occurrence of seizures, days of ventilation, and GWAS identified eigen values and correction for multiple testing, two intronic SNPs in *BNDF* gene on chromosome 11 were significant.

Conclusions

- In a US population of ELBW infants, a SNP on chromosome 10 was protective in ROP.
- SNPs involving *BDNF* on chromosome 11 were associated with increased risk of severe ROP.
- The findings, which require replication and physiologic and epidemiologic validation, suggest links between neural and retinal vascular development and pathology.

Acknowledgements: The National Institutes of Health and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development provided grant support for the Neonatal Research Network. We are indebted to the infants and their parents who agreed to take part in this study and to our medical and nursing colleagues at: Brown University; Case Western Reserve University; Cincinnati Children's Hospital Medical Center; Duke University; Emory University; Indiana University; RTI International; Stanford University; University of Alabama at Birmingham; University of California – San Diego; University of Miami; University of New Mexico; University of Tennessee; University of Texas Southwestern Medical Center; University of Texas Health Science Center at Houston; University of Utah; Wake Forest University; Wayne State University; Yale University.