

# Polymorphism in the Neuregulin Gene and Brain Damage in Preterm Newborns

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## Abstract

**Background:** Neuregulins (NRGs) are ligands of the erbB family of the receptor tyrosine kinases. NRG1 plays essential roles in the developing nervous system, heart, breast, and lung. In the nervous system, NRG regulates radial glial formation, neurotransmitter expression, oligodendrocyte development, and the onset of puberty. NRG1 is also neuroprotective in scenarios of experimental hypoxia-ischemia. An association has been identified between single nucleotide polymorphisms (SNPs) of NRG1 and schizophrenia. A possible role for NRG in adult neurodegenerative disorders has been reported.

**Objective:** To test the hypothesis that a variant in the NRG1 promoter region (SNP8NRG221533) modulates the risk for brain damage in preterm newborns.

**Design/Methods:** We collected whole blood from 54 singleton children < 32 weeks gestation from the Developmental Follow Up Program at Hannover Medical School (Germany). Children were at least two years of age. We established SNP8NRG221533 genotypes by PCR and RFLP analysis. Clinical data were abstracted from medical charts. Data were collected and analyzed in a case-control-fashion with periventricular echodensity (PVE), cystic periventricular leukomalacia (cPVL), cerebral palsy (CP) and developmental delay (DD) >= 12 months as per Denver Developmental Screening test as case definitions.

**Results:** Twenty-two children (41%) had PVE, 11 (19%) had cPVL, 5 (9%) had CP, and 9 (17%) had DD. Among 6 children homozygous for the C allele in the gene region of interest, 1 child had PVE, but none had cPVL, CP, or DD. Among 36 heterozygous children, these percentages were 50% (PVE), 19% (cPVL), 6% (CP), and 14% (DD). Among 12 children homozygous for the T allele, these risks were 25% for PVE, cPVL, and CP, and 33% for DD. The one-sided Cochran Armitage p-value for trend was 0.09 for CP even in this very small sample. The odds ratio for CC/CT genotypes vs TT predicting CP was 0.2 (0.02-1.0)

**Conclusions:** We conclude that NRG1 might be a candidate gene for further research into the function(s) of the SNP8NRG221533 C variant and the mechanism(s) of NRG1-mediated neuroprotection.

## Background

Antenatal infection is a major risk factor for prematurity. It is also an important predictor of neonatal disorders of the lung and brain among preterm infants (1). Apparently, a fetal inflammatory response to intrauterine infection contributes to such disorders (2), which are in turn associated with long-term developmental dysfunction.

The four variants of neuregulin (NRG), NRG-1, NRG-2, NRG-3, and NRG-4, and their transmembrane tyrosine kinase erbB receptors have various crucial functions in the developing brain (3), heart (4), and lung (5). In the nervous system, NRG regulates radial glial formation (6), neurotransmitter expression (7), oligodendrocyte development (8), and the onset of puberty (9).

NRG-1 the first polypeptide growth factor of the NRG family is also neuroprotective in scenarios of experimental hypoxia-ischemia (10) and appears to play a role in the etiology of several neurologic diseases in adults (11) (12) (13). There is also increasing evidence of an association between single nucleotide polymorphisms (SNPs) of NRG1 and schizophrenia (14, 15).

Some recent observations suggest that NRG-1 together with the erbB receptors might influence the development of myelinating cells (16, 17). Due to the fact that damage or aberrant maturation of immature oligodendrocytes (that characterize the cerebral white matter near the end of the second trimester) might be a pathogenetic factor in diffuse perinatal WMD (18), we wanted to explore a possible NRG-1-mediated neuroprotection in preterm newborns.

## Hypothesis

The goal of our study was to test the hypothesis that a variant in the NRG1 promoter region (SNP8NRG221533) modulates the risk for brain damage in preterm newborns.

## Methods

**Study population:** We evaluated 54 singleton children < 32 weeks gestation from the Developmental Follow Up Program at Hannover Medical School (Germany). Children were at least two years of age. A comprehensive physical examination and the Denver developmental screening (DDST) II test were administered by one pediatrician (FD). Clinical data were abstracted from medical charts.

**Gene polymorphisms:** A whole blood sample was obtained for genetic analysis. We established SNP8NRG221533 genotypes by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

**Data analysis:** We report univariable and multivariable relationships between clinical variables, SNPs and outcomes in terms of odds ratios (ORs) and their 95% confidence intervals (CIs). In cases where we found an association with an OR whose CI excluded the null (i.e., 1.0) we still considered it worthwhile reporting these results. However, in constellations where the CI included the null, we do not believe that our study bears the potential of proving the absence of an association.

## Results

	All children			NRG			PVL			CP			DD => 12 mths		
	N=54	CC	CT	TT	OR	CI 95%	Yes	No	OR	Yes	No	OR	Yes	No	OR
		N=42	N=12				N=10	N=44	(CI 95%)	N=5	N=49	(CI 95%)	N=9	N=45	(CI 95%)
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
<b>NRG CC/CT</b>	-	-	-	-	-	-	70	80	0.6 (0.1-2.8)	40	82	0.2 (0.02-1.0)	56	82	0.3 (0.1-1.2)
<b>Gestation &lt; 30 wks</b>	63	62	67	0.8 (0.2-3.1)	90	57	6.8 (0.8-59)	80	61	2.5 (0.3-24)	89	58	5.9 (0.7-51)		
<b>Birth weight &lt; 1000g</b>	44	48	33	1.8 (0.5-7.0)	70	39	3.7 (0.8-16)	80	41	5.8 (0.6-56)	67	40	3.0 (0.7-14)		
<b>Small for gestational age</b>	22	24	17	1.6 (0.3-8.4)	20	23	0.9 (0.2-4.7)	40	20	2.6 (0.4-18)	33	20	2.0 (0.4-9.8)		
<b>Male</b>	50	45	67	2.4 (0.6-9.3)	60	48	1.6 (0.4-6.6)	60	49	1.6 (0.2-10)	67	47	2.3 (0.5-10)		
<b>Mother German</b>	72	69	83	0.5 (0.1-2.3)	60	75	0.5 (0.1-2.1)	100	69	-	78	71	1.4 (0.3-7.8)		
<b>Multiple pregnancy</b>	17	14	25	0.5 (0.1-2.4)	10	18	0.5 (0.1-1.4)	16	13	0.1 (0.1-13)	11	18	0.6 (0.1-5.3)		
<b>Perinatal glucocorticoid, complete</b>	81	76	100	-	70	84	0.4 (0.1-1.1)	80	82	0.9 (0.1-9.0)	78	82	0.8 (0.1-4.3)		
<b>Pregnancy induced hypertension</b>	19	19	17	1.2 (0.2-6.5)	20	18	1.1 (0.2-6.3)	20	18	1.1 (0.1-1.1)	11	20	0.5 (0.1-4.5)		
<b>Preterm labor</b>	65	60	83	0.3 (0.1-1.5)	80	61	2.5 (0.5-13)	80	63	2.3 (0.2-22)	89	60	5.3 (0.6-46)		
<b>Rupture of membranes &gt;12hrs</b>	22	24	17	1.6 (0.3-8.4)	30	20	1.7 (0.4-7.8)	20	22	0.9 (0.1-8.5)	22	22	1.0 (0.2-5.6)		
<b>Chorioamnionitis</b>	30	29	33	0.8 (0.2-3.2)	60	23	5.1 (1.2-22)	20	31	0.6 (0.1-5.5)	44	27	2.2 (0.5-9.6)		

**Table 1:** Antenatal characteristics of the study population. Data are given for all children (left), and in groups defined by NRG genotype CC/CT vs TT (middle) and the presence of absence of PVL, CP and DD => 12 months (right). If not indicated otherwise, numbers are column percents.

	PVL (n=10)		CP (n=5)		DD => 12 (n=9)	
	OR	CI 95 %	OR	CI 95 %	OR	CI 95 %
<b>NRG any C</b>	0.6	(0.1-3.1)	0.2	(0.02-1.1)	0.3	(0.1-1.3)
<b>&lt; 30 wks</b>	6.8	(0.8-58)	2.5	(0.2-26)	6.0	(0.7-54)
<b>NRG any C</b>	0.5	(0.1-2.4)	0.1	(0.01-0.9)	0.2	(0.04-1.0)
<b>Birth weight &lt; 1000g</b>	4.1	(0.9-19)	11	(0.9-140)	4.1	(0.8-22)
<b>NRG any C</b>	0.7	(0.1-3.4)	0.2	(0.02-1.1)	0.3	(0.1-1.4)
<b>Male</b>	1.5	(0.4-6.4)	1.1	(0.2-8.1)	1.9	(0.4-9.0)
<b>NRG any C</b>	0.7	(0.2-3.5)	0.2	(0.02-1.2)	0.4	(0.1-1.6)
<b>Preterm labor</b>	2.4	(0.4-13)	1.5	(0.1-17)	4.4	(0.5-39)
<b>NRG any C</b>	0.6	(0.1-3.2)	0.1	(0.02-1.0)	0.3	(0.1-1.3)
<b>Chorioamnionitis</b>	5.1	(1.2-22)	0.5	(0.1-5.2)	2.2	(0.5-10)

**Table 2:** Five multivariable models predicting morbidities, using NRG any C and one confounding factor at a time.

➤ Twenty-two children (41%) had PVE (data not shown), 10 (19%) had cPVL, 5 (9%) had CP, and 9 (17%) had DD.

➤ Among 6 children homozygous for the C allele in the gene region of interest, 1 child had PVE (data not shown), but none had cPVL, CP, or DD.

➤ Among 36 heterozygous children, these percentages were 50% (PVE) (data not shown), 19% (cPVL), 6% (CP), and 14% (DD)

➤ Among 12 children homozygous for the T allele, these risks were 25% for PVE (data not shown), cPVL, and CP, and 33% for DD

➤ The one-sided Cochran Armitage p-value for trend was 0.09 for CP even in this very small sample (data not shown).

➤ The odds ratio for CC/CT genotypes vs TT predicting CP was 0.2 (0.02-1.0)

## Discussion

The major finding of our retrospective study is that even in a small cohort of preterm infants (n = 54) those who carry at least one C allele of the NRG-1 SNP seem to be at reduced risk for PVL, and even more prominent for CP and DD. Perinatal brain damage, especially in the preterm newborn, is usually attributed to one or more of multiple causes, i.e. inflammation, hypoxia-ischemia, excitotoxicity (19). In all these scenarios NRG seems to modulate pathways leading to perinatal brain injury.

Our own findings that NRG might be "protective" in the perinatal brain damage scenario is supported by the related finding of our group that human umbilical venous endothelial cells (HUVECs) express NRG-1 mRNA and protein (Hoffmann et al., 2007 PAS, Session 6298 – Developmental Biology, Presentation Number 258). This expression seems to be affected by the gestational age as well as by an exposure to lipopolysaccharide (LPS). The NRG-1 SNP appears to exert a quantitative influence on these LPS stimulated expression patterns, with C alleles bearing at least one C-allele exhibiting relatively higher NRG-1 production at 24 hours. Together with the known enhanced late fetal lung surfactant synthesis by NRG-1 (5), this might also support our suggestion of a protective function of NRG in the perinatal setting.

One disadvantage of our study is its small sample size. Although this makes it difficult to generate definitive statements due to the lack of formal statistical significance, it allows for the identification of point estimate (OR) trends. On the basis of the described consistent trend showing a risk reduction for PVL, CP, and DD without being affected by potential confounders, we conclude that NRG1 might be a candidate gene for further research aiming at the elucidation of the function(s) of the SNP8NRG221533 C variant and the mechanism(s) of NRG1-mediated neuroprotection.

## Conclusion

Our findings support the hypothesis that NRG-1 is an endogenous protector in perinatal brain damage pathogenesis among preterm newborns (Dammann et al, submitted).

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