

Learning disability and oligodendrocyte myelin glycoprotein (OMGP) gene in neurofibromatosis type 1

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SUMMARY: Terzi YK, Oğuzkan-Balcı S, Anlar B, Erdoğan-Bakar E, Ayter Ş. Learning disability and oligodendrocyte myelin glycoprotein (OMGP) gene in neurofibromatosis type 1. Turk J Pediatr 2011; 53: 75-78.

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease where phenotypic heterogeneity is explained by the effect of modifier genes. Thirty to 65% of patients have learning disability. The oligodendrocyte myelin glycoprotein (OMGP) gene located within the neurofibromatosis type 1 (NF1) gene might affect the phenotype of learning disability because it is expressed in the brain, and OMGP gene mutations have been associated with cognitive disturbances. We analyzed the OMGP gene in NF1 patients with and without learning disability (n=50 each) and healthy controls (n=100). The allele distribution of OMGP62 polymorphism was not significantly different between the groups (p=0.447). These results do not support a relationship between the OMGP gene and the learning disability phenotype observed in NF1. Other modifying genes, post-translational modifications or receptor interactions might be involved in the phenotypic variability of NF1.

Key words: neurofibromatosis type 1, oligodendrocyte myelin glycoprotein, OMGP, learning disability, cognitive.

Neurofibromatosis type 1 (NF1) (Online Mendelian Inheritance in Man Number 162200) is a common multisystem disorder of autosomal dominant inheritance affecting approximately 1 in 3500 individuals in all ethnic groups. Its frequent manifestations are café-au-lait spots, Lisch nodules and freckling of axillary and inguinal regions. Other features such as bone lesions, macrocephaly and malignancies are observed at various rates^{1,2}. Cognitive difficulties and learning disabilities, defined as academic achievement disproportionately lower than predicted from general intellectual function, have been reported in 30-65% of NF1 patients, significantly higher than the 7-10% rate in the general population³⁻⁵. Up to 40% of children with NF1 also have problems with attention or impulse control, meeting the diagnostic criteria of attention deficit-hyperactivity disorder. The pathogenesis of cognitive and behavioral impairment in NF1 is not well understood. Signal intensity changes or “unidentified bright objects” on

magnetic resonance imaging (MRI), probably representing spongiform changes in myelin, have been associated with cognitive problems. The volumes of gray matter and corpus callosum are significantly greater in NF1 subjects than control subjects. Macrocephaly and delayed developmental apoptosis, probably related to diminished Ras activity, were suggested to underlie these morphologic and behavioral changes⁴. However, these associations have remained controversial⁶.

The NF1 gene maps to chromosome 17q11.2 and encodes neurofibromin, a protein consisting of 2818 amino acids. One of the known functions of the gene is the tumor-suppressor activity of the GAP-related domain encoded from the central portion of the gene, encompassing exons 21-27a⁷. The NF1 gene also contains the oligodendrocyte myelin glycoprotein (OMGP) gene embedded within exon 27b⁸. OMGP is a membrane glycoprotein and a minor component of central nervous

system myelin. It is expressed mainly on large projection neurons such as Purkinje cells, pyramidal cells of the hippocampus, and motor neurons of the brainstem and anterior horn of the spinal cord, and also in proliferating neural stem cells, especially in the developing brain. Although OMGP has no defined role, subjects carrying a single copy of the OMGP gene due to heterozygote deletion in the NF1 locus, 5-20% of all NF1 patients, present severe cognitive impairment such as mental retardation and learning disabilities⁹. In order to examine the possible role of OMGP in the learning disability phenotype of NF1 patients, we analyzed the OMGP gene and its 5' untranslated region in this disorder.

Material and Methods

Patients

Neurofibromatosis type 1 (NF1) patients with (n=50) and without (n=50) learning disability were recruited from the Pediatric Neurology Clinic of Hacettepe University Children's Hospital. NF1 was diagnosed according to the National Institutes of Health (NIH) criteria². Learning disability was diagnosed by a pediatric neurologist and child psychologist, based on the operational definition of discrepancy: achievement below the class average in one or more school subjects (reading, writing, mathematics, etc.) despite an intelligence level within the normal range as assessed by Wechsler Intelligence Scale for Children-Revised (WISC-R)⁵. The healthy control group (n=100) consisted of volunteer children from schools and well-child clinics in the same districts. The study was approved by the institutional ethics committee, and informed consents were obtained from all subjects and parents.

Mutation Analysis

Genomic DNA was extracted from peripheral blood. We analyzed the entire OMGP gene and its 5' untranslated region by direct DNA sequencing. In addition, we tested a coding single nucleotide polymorphism (OMGP62). DNA sequencing was performed using the Big Dye Terminator 3.1 kit (PE Applied Biosystem, Foster City, CA, USA) and ABI-PRISM 3130 Genetic Analyzer (PE Applied Biosystem, Foster City, CA, USA), according to the manufacturer's instructions, with specific primers for the 5' untranslated region and the coding exon. Primer sequences are given in Table I. OMGP62 polymorphism genotyping was performed by polymerase chain reaction (PCR)-Restriction Fragment Length Polymorphisms (RFLP) methods¹⁰. PCR products were digested with MvaI restriction endonuclease enzyme, electrophoresed on 3% agarose gels containing ethidium bromide, and visualized under ultraviolet light. The PCR product contains two restriction sites for MvaI, and one of these sites involves the OMGP62 polymorphism. The MvaI restriction site is present on allele G and not on allele A.

Statistics

The differences in genotype and allele frequencies for OMGP62 polymorphism in patients and controls were analyzed using the chi-square (χ^2) test. Hardy-Weinberg equilibrium for patients and controls was calculated using the de Finetti program (<http://ihg.gsf.de/cgi-bin/hw/hwa2.pl>).

Results

The distribution of OMGP genotypes and allele frequencies were similar in the NF1 patients

Table I. Primer Sequences for Polymerase Chain Reaction (PCR)

Primer	Forward (5'-3')	Reverse (5'-3')
OMGP UTR-1	GAA AAC TAT CCA TGA GGA AGG	ATA ACA TCT TAC CGT GGT GTC
OMGP UTR-2	AGA TGC TGA TGT TGA AGA CGA	AAA TAA ATA GTT GCA GTT GTA GGC
OMGP UTR-3	CTC ATC TGA GTA AGA AAG CAT ATC	GTG CAT ATA CAT TGG AGA GGA
OMGP-1	CCC ATG CAG ATG CCT AAA CT	TTC CAC AGA GAC CGA GGT AA
OMGP-2	TAT ACC AAT CTG AGG ACC CT	TCT GGA ATG AAT GTG AAC TT
OMGP-3	TGC CCT CCA AAC TAC ATA TC	TTA ATG GTG TCC ACT GTC TG
OMGP-4	ACC TTC TGG ATT TAC CTC AA	AGT GAT ACT TAG GGT CAT GG
OMGP-5	ATG GTC ACA AAC ACA AGC CT	GAC AGT AAA ATA GCA GCA AG

Table II. Distribution of OMGP62 Polymorphism in NF1 Cases With and Without Learning Disability (LD) and Control Groups (HWE: Hardy-Weinberg Equilibrium)

Groups	Genotypes*			n	Alleles**	
	G/G	G/A	A/A		G	A
NF1 with LD ^a	40 (80 %)	10 (20 %)	0	50	90 (90 %)	10 (10 %)
NF1 without LD ^b	43 (86 %)	7 (14 %)	0	50	93 (93 %)	7 (7 %)
Control ^c	86 (86 %)	13 (13 %)	1 (1 %)	100	185 (92.5 %)	15 (7.5 %)

*genotype distribution comparison for three groups ($\chi^2=0.682$, $df=4$, $p= 2.293$)

**allele distribution comparison for three groups ($\chi^2=0.747$, $df=2$, $p=0.688$)

^aHWE $p=0.595$, ^bHWE $p=0.432$, ^cHWE $p=0.528$ ($df=1$)

with ($n=50$) and without ($n=50$) learning disability and the control group ($n=100$). The observed genotype counts were not significantly deviant from those expected according to the Hardy-Weinberg equilibrium (Table II). When groups were compared by pairs, the distribution of OMGP62 alleles did not show any difference: NF1 with and without learning disability ($\chi^2=0.579$, $df=1$, $p=0.447$), NF1 with learning disability vs. control ($\chi^2=0.550$, $df=1$, $p=0.460$), and NF1 without learning disability vs. control ($\chi^2=0.025$, $df=1$, $p=0.875$). No sequence alteration was observed in coding exons and the 5' untranslated region of the *OMGP* gene.

The WISC-R test results in NF1 patients and control subjects were significantly different in all tests: verbal IQ (78.7 ± 17.7 in NF1 vs. 110.4 ± 8.97 in control), performance IQ (78.0 ± 17.4 vs. 118.22 ± 11.67), full scale IQ (76.9 ± 17.7 vs. 115.65 ± 10.92), Bender-Gestalt test (6.1 ± 4.8 vs. 2.72 ± 1.62), and Judgment of Line Orientation test (10.5 ± 3.4 vs. 18.17 ± 3.36). Results of cognitive tests and their interpretation are published in more detail elsewhere¹¹.

Discussion

Neurofibromatosis type 1 (NF1) presents a heterogeneous phenotype: the distribution and severity of symptoms and signs differ among patients and even within the same family. The only well-known function of the gene product, its tumor-suppressing action, does not explain all the clinical features of the disorder. It is assumed that modifier genes might play a role in the phenotypic variability. The *OMGP* gene may be a candidate modifier for several reasons. It is located within the *NF1* gene, its protein product is expressed in the central nervous system, especially during development, and it

contains binding sites for transcription factors involved in neural development. NF1 and OMGP are regulated by common mechanisms and both may synergistically inhibit Ras activity. Therefore OMGP might contribute to the downregulating role of neurofibromin on Ras, which is impaired in NF1. Patients with large deletions in the *NF1* locus have severe cognitive impairment⁹. Another observation of our group, the tendency of unaffected siblings of NF1 patients to obtain mildly but consistently lower scores in certain cognitive tests compared to healthy controls, also suggests shared genetic characteristics different from, but in proximity of, *NF1* mutation¹¹. OMGP62 polymorphisms have also been associated with autism and non-syndromic mental retardation^{10,12}. Despite these findings suggesting a role of *OMGP* in cognition, we have not observed any relationship between *OMGP* gene mutations and learning disability in NF1. This is the first report in the literature analyzing this possible relationship. Learning disability in NF1 can be related to other functional domains of neurofibromin, brain parenchymal lesions associated with the disease, or post-translational modifications and structure of receptors involved in the proper function of *OMGP*^{4,11}.

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