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# Clustering of mutations in the 5' tertile of the *NF1* gene in Slovakia patients with optic pathway glioma

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Optic pathway gliomas (OPG) occur in 15% of patients with neurofibromatosis type 1 (NF1; OMIM 162200). Genotype-phenotype correlations in patients with NF1 may help to determine the risk group for developing complications such as OPG in coincidence with other NF1.features.

We evaluated 52 patients with NF1 (25 with OPG and 27 without OPG). All subjects underwent a clinical examination focused on neurofibromatosis type 1 and molecular diagnostics of NF1 gene using protocol based on RNA analysis confirming the diagnosis of NF1.

In the group with OPG patients, there was a significantly higher incidence of freckling (P=0.017), neurofibromatosis bright objects (NBO) (P=0.0038), compared to the group without OPG. The differences between the groups with respect to Lisch nodules were on the borderline of statistical significance (P=0.088). The frequency of neurofibromas in the group with OPG was not significant (P=0.9). From all patients with the mutation localized in the first tertile of the NF1 gene majority (71%) had optic glioma compared to individuals who didn't have the OPG 29% (P=0.0049).

Our results present the clustering of mutations in the 5'tertile of *NF1* gene in patients with optic nerve glioma and suggest higher incidence of freckling and neurofibromatosis brain objects in these patients. Molecular analysis of *NF1* gene is important part in complex management of NF1 patients and contributes to a better understanding of clinical picture of NF1 patients.

*Key words: optic pathway glioma, NF1 mutation, genotype-phenotype correlation*

Neurofibromatosis type 1 (NF1; OMIM 162200) is one of the most common autosomal dominant disorders (incidence 1: 2500-3000) with a progressive disease course and variable clinical manifestations, including neurological complications such as cognitive impairment and central nervous system tumors. Optic pathway gliomas (OPG) account for 5% of low-grade gliomas in children [1-3], and the majority (70%) are present in children with NF1 [4]. Approximately 15-20% patients with NF1 develop OPG [5-8]. Fortunately, these gliomas are mostly asymptomatic, but 5% have complications, including visual impairment or protrusion of the bulbus [3,5, 9-11]. The optimal clinical assessment of OPG is still a challenge for a few reasons. One is the difficult diagnosis, which is based mostly on radiological findings rather than on histological proof. Other reasons are long clinically silent periods and periods of rapid growth or stagnation that might influence the beginning of treatment. Limited cooperation by the young patients and, in some cases, cognitive impairment represents additional challenges. The initial management of OPGs typically includes a close observation. When therapy is indicated, chemotherapy

is usually the treatment of choice because an exclusive surgical and radiologic treatment has a limited outcome [12-17]. Vincristine and carboplatin have been proven to be the most efficient treatments [4,12-18]. To estimate the severity of the clinical outcome of OPGs, a radiologic modified Dodge classification was proposed as a better indicator of localisation and severity of optic pathway gliomas [19, 20].

The *NF1* gene (17.q11.2, 280kb genomic DNA) is one of the largest genes in human genome, consists of 57 constitutive and approximately 3 alternatively spliced exons (9br, 23a, 48a) codes for 8454 nucleotides long transcript [21, 22, 23]. The broad spectrum of mutations includes various small *NF1* mutations, microdeletions or deletions of entire *NF1* gene (in some cases including neighbouring genes) [24, 25, 26] and intragenic copy number changes of one or more exons.

Recently, there have been attempts to use molecular data as a prognostic indicator. The current work of Sharif et al. suggests that a significant number of mutations in NF1 patients with optic nerve gliomas are located in the 5'tertile of the *NF1* gene [27]. Many other studies have tried to determine a genotype-phenotype correlation, but there are no data referring to the broad spectrum of the NF1 features.

Our aim was to assess the probability of a more severe form of NF1 in patients with OPG based on the molecular diagnostics. The disclosure of a genotype-phenotype correlation in OPG in NF1 patients may improve the prognosis prediction of these patients.

## Patients and methods

In our attempt to better understand the disease course and burden of optic gliomas, we summarised the follow-up documentation of 60 patients with neurofibromatosis and optic gliomas from two clinics: 2nd Department of Paediatrics in the Children's Faculty Hospital (45 patients) and the Department of Clinical Genetics in the National Cancer Institute in Bratislava (15 patients). This study was approved by the ethical boards of both institutions.

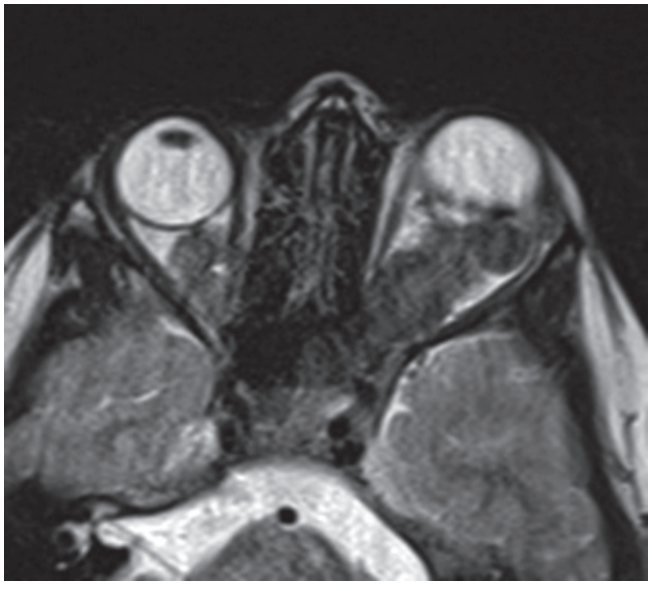

**Figure 1. Optic pathway glioma in patient 12 (P12) with NF1**

The patients were selected based on meeting at least two out of six major diagnostic criteria for neurofibromatosis type 1 (café-au-lait spots, freckling, Lisch nodules, neurofibromas, typical skeletal dysplasia and affected relative with NF1) [28]. All patients underwent genetic testing of *NF1* gene, which confirmed the diagnosis in 52 cases. Eight patients with a negative test result were excludes from the study. In remaining 52 subjects we evaluated the demographics, clinical status based on the NF1 diagnostic criteria [28], the therapy, treatment outcomes, the visual acuity and neurological status. The diagnosis and the progression of OPG were made using MRI scans and included the assessment of the size (thickness) of the optic nerve, tortuosity and changes in the intensity of post–gadolinium contrast enhancement (Figure 1). Both the classic Dodge classification [19] and newly proposed Modified Dodge Classification (MDC) [20], were used as indicators of localisation and extent of the OPG on MRI scans. These classifications were used in order to assess the potential severity of OPG and the impact on vision.

The majority of patients had their vision checked regularly. Ophthalmologic data included visual acuity, visual fields and fundoscopic examination. Visual impairment was assessed by the scale 0-2 (0: normal vision V5/5; 1: mild impairment from V5/7,5 to V5/25; 2: severe V5/25 to blindness). Methods of examination were adapted to the child's developmental age and cognitive status. In children older than 3 years of age, the Snellen linear chart method was used, and single optotypes were used in children younger than 3 years or in children who were not able to cooperate due to cognitive impairment.

**Genetic diagnostics.** All NF1 patients in the study underwent genetic testing with their informed consent. The implemented diagnostic method is based on the analysis of the entire coding region of *NF1* according to the modified protocol of Messiaen and Wimmer (2012) [29, 30]. The mutation detection is based on the cDNA sequencing, which allows the identification of splicing mutation variants [29-31]. All mutations identified at the cDNA level were confirmed in the genomic DNA. In those cases for which the cDNA screening did not uncover any mutations, the samples were analysed using MLPA (multiple ligation dependent probe amplification) with *NF1* microdeletion kits P122, P081 and P082 from MRC Holland 11, which enable the identification of whole *NF1* gene deletions and possible deletions of one or more individual *NF1* exons. For the exon numbering, the old nomenclature was used, with the new nomenclature indicated in parentheses (e.g., exon 12a (16)).

The pathogenicity of these mutations was evaluated according to the Human Gene Mutation Database (HGMD). If the mutations resulted in a truncated protein or nonsense, frameshift or splice-site changes, they were classified as

pathogenic. The effect of the novel missense mutations on the structure and function of *NF1* was evaluated using the SNAP and Polyphen2 prediction programmes [29]. In addition, several polymorphisms were observed in the cohort of patients with NF1 that were classified as non-pathogenic changes [29].

**Statistics.** In order to evaluate accumulation of mutations in the first tertile of the *NF1* gene in the group of patients with OPG, we used the Exact two-sided (mid-p) to correlate two independent proportions. The level of statistical significance was $P<0.05$. The logistic regression analysis was employed to assess the frequency of neurofibromatosis signs (e.i. freckling, Lisch nodules, neurofibromas and NBO) with respect to the occurrence of OPG. To confirm an association between the deterioration of visual acuity and the presence of OPG we used the Goodman Kruscal gamma coefficient. (The levels of agreement: -1 negative association, +1 positive association).

## Results

We identified causal mutations in the *NF1* gene in 52 patients, and based on the presence of OPG, we divided them into two groups. In the first group, there were 25 NF1 patients with OPG, diagnosed based on an MRI scan of the brain (Table 1, Figure 2). The second group contained 27 NF1 patients without OPG (Table 2, Figure 3). With respect to the male/female ratio, there was a slight predominance of males in both groups (14/11 in the group with OPG, 14/13 in the group without OPG). The median age in the group without OPG was higher (15.5 yrs, maximum 46 yrs, minimum 1 yr) compared with the group with OPG (10.75 yrs, maximum 24 yrs, minimum 3 yrs).

**Clinical manifestations.** All 52 patients had café-au-lait spots. The frequency of freckling in the OPG group was significantly higher (96.1%) then in the other group (44.4%) ($P=0.016$) as was that of neurofibromatosis bright objects (NBO) (87.7% vs. 26%) ($P=0.0038$). The differences between the groups with respect to Lisch nodules (48.1% vs. 18.5%, $P=0.08$) and neurofibromas (75.8% vs. 55.5%, $P=0.97$) were not significant (Table 1, 2, Figure 2,3). We also confirmed a strong association between the deterioration of visual acuity and the presence of OPG (Goodman Kruscal gamma coefficient = 0.83 ($P<0.0001$).

The overall length of the clinical follow-up of patients in both groups was approximately the same (6/8 years). The mean age for the diagnosis of an OPG was 7.5 years, and in

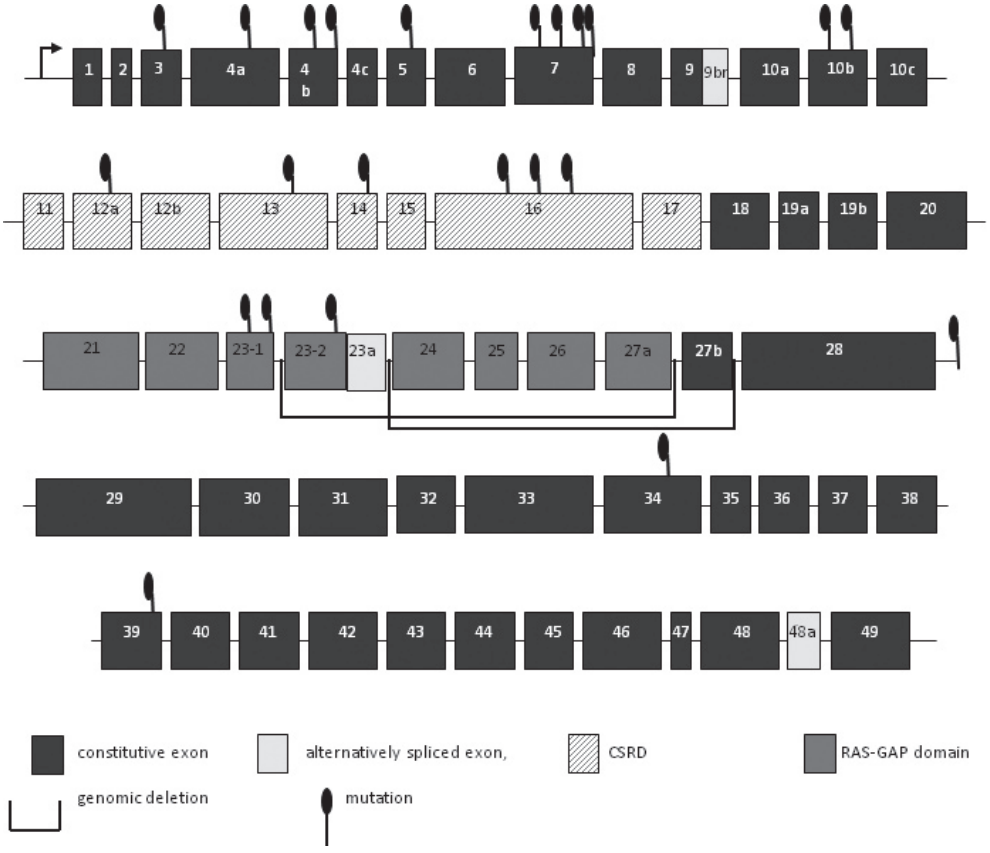


**Figure 2. Diagram of mutations in 24 NF1 patients with optic pathway glioma (OPG), 1 patient had the type 1 deletion**

**Table 1. Clinical manifestation and mutation screening in individuals with optic pathway glioma (OPG)**

| p.No. | age | OPG | | | VIS | FR | LN | FIB | NBO | IN | other | exon/ intron* | NF1 mutation* | mutation type | recurrent/ novel | therapy/therapy outcome | lenght of fol-low-up (years) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | age of dg. | DC | MDC | | | | | | | | | | | | | |
| P63 | 3 | 2 | A | 1aL | 1 | | | | | de novo | hypotonus | 3 (3) | p.Gln83ValfsX23, c.240_241delTC (also silent mutation in ex 31: c5895A>C, p.Lys1965Lys) | FS del | [30] | none/stac. | 2 |
| P46 | 14 | 7 | A | 1aR | 1 | | | | | 1(1) | FC, PP | 4a (4) | p.Gln97ValfsX13, c.291_479del (skipping of exon 4a) due to c.479+5G>A (ivs4a+5G>A) | splicing | [30] | none/stac. | 7 |
| P103 | 14 | 11 | A | 1aR | 1 | | | | | de novo | none | 4b (5) | p.Leu161Phe X4, c.483delA | FS del | [30] | none/stac. | 3 |
| P12 | 9 | 2 | C | 3Hb | 2 | | | | | de novo | none | 4b (5) | p.Cys167GlnfsX10, c.499_502delTGTT | FS del | [48] | VCR-CAP, temozol./ progress. | 7 |
| P58a | 14 | 11 | A | 1aR | 0 | | | | | 1(1) | HP, CP | 5 (7) | p.Met242ArgfsX39, c.725delT | Fs del | [49] | none/stac. | 11 |
| P68 | 7 | 5 | A | 1aL | 0 | | | | | de novo | none | 7 (9) | p.Ala330Val, c.989C>T, creates a cryptic donor splice site and thus deletion c.988_1062del75bp | untypical splicing (missense) | [30] | none/stac. | 2 |
| P18 | 6 | 4 | B | 2bR | 0 | | | | | 2(1) | none | 7 (9) | p.Tyr333XfsX1, c.998_999insA | FS ins | [49] | none/stac. | 4 |
| P18b | 4 | 2 | B | 2bR | 1 | | | | | 2(1) | none | 7 (9) | p.Tyr333XfsX1, c.998_999insA | FS ins | [50] | VCR-CAP/regress. | 3 |
| P122 | 19 | 8 | C | 3Hb | 2 | | | | | de novo | MBG, MR, | 7 (9) | p.Arg304X, c.910C>T | nonsense | [51] | refused to be treated | 11 |
| P37a | 21 | 19 | A | 1aL | 2 | | | PN | | 2(1) | WS | 10b (13) | p.Ile500AsnfsX11, c.1497_98insA | FS ins | [30] | none/stac. | 12 |
| P37b | 18 | 16 | A | 1aR | 0 | | | | | 2(1) | WS | 10b (13) | p.Ile500AsnfsX11, c.1497_98insA | FS ins | [30] | none/stac. | 12 |
| P118 | 13 | 10 | A | 1aR | 0 | | | | | de novo | none | 12a (16) | p.Thr586ValfsX18, c.1756_59delACTA | FS del | [52] | none/stac. | 3 |
| P74 | 10 | 8 | B | 2bL | 0 | | | | | de novo | none | 13 (18) | p.Arg711ProfsX5, c.2129dupT | FS ins | [30] | none/stac. | 2 |
| P49 | 18 | 12 | C | 3Hb | 1 | | | | | de novo | MS | 14 (19) | p.Arg752LeufsX17, c.2252_2325del (skipping of exon 14) due to c.2325+1G>A (ivs14+1G>A) | splicing | [30] | none/stac. | 10 |
| P10 | 7 | 4 | A | 1aL | 0 | | | PN | | de novo | none | 16 (21) | p.Gly849GlufsX29, c.2546delG | FS del | [30] | none/stac. | 5 |
| P44 | 16 | 8 | C | 3Hb | 2 | | | | | de novo | MBG, HC | 16 (21) | p.Lys874X, c.2619insT | FS ins-stop | [30] | RAT/residuum stac. | 8 |
| P2 | 24 | 16 | B | 2bR | 1 | | | | | 1(1) | MPNST | 16 (21) | p.Leu847Pro, c.2540T>C | missense | [53] | VCR-CAP/death | 8 |
| P3 | 11,5 | 5 | B | 2bL | 2 | | | PN | | 2(2) | Pseudoart. of tibia | 23.1 (29) | p.Gln1298X, c.3892C>T | stop | [54] | VCR-Cyclophos/ progress. | 5 |
| P16 | 10 | 1 | B | 2bL | 2 | | | | | 1(1) | WS, SD | 23.1 (29) | p.Leu1305GlufsX5, c.3913delTinsGAA | FS del ins | [30] | surgery/regress. | 9 |
| P23 | 7 | 3 | A | 1aL | 0 | | | | | de novo | PA | ex 23.2-27a (30-35) | p.Leu1326_Arg1554del, c.3975_4661del675nt due to genomic deletion of exons 23.2,24,25,26,27a (30-35) | genomic del of 5 exons | [30] | none/stac. | 7 |
| P67 | 12 | 10 | A | 1aR | 0 | | | | | de novo | none | ex 24-27b (32-36) | p.Val1372GlnfsX8, c.4111_4772del650nt due to genomic deletion of ex 24,25,26, 27a,27b (32-36) | genomic del of 5 exons | [55] | none/stac. | 2 |

**Table 1. Continued**

| p.No. | age | OPG | | | VIS | FR | LN | FIB | NBO | IN | other | exon/ intron* | NF1 mutation* | mutation type | recurrent/ novel | therapy/therapy outcome | lenght of follow-up (years) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | age of dg. | DC | MDC | | | | | | | | | | | | | |
| P90 | 5 | 3 | A | 1b | 0 | | | | | de novo | none | in 28 (in37) | c.5206-5_5220delTCCAGGTTGGTTCTACTG CT causes skipping of ex29 (p.Gly1737SerfsX4, c.5206_5546del329nt) or of ex29 and 30 (p.Gly1737LeufsX3, c.5206_5749del532nt) | small del/ splicing/FS | [30] | none/stac. | 2 |
| P89 | 7 | 3 | B | 2bR | 1 | | | | | 1(1) | none | ex 34 (43) | p.Phe2176CysX44, c.6525_6528delTT | FS del | [30] | VCR-CAP/stac. | 4 |
| P57 | 8 | 8 | B | 2bR | 0 | | | | | 1(1) | none | ex 39 (48) | p.2366_67del AsnPhe, c.7096_ 7101delAACTTT | small in frame deletion | [56] | none/stac. | 6 |
| P11 | 13 | 10 | A | 1aL | 1 | | | | | de novo | MC | all | type I deletion of entire NF1 gene | genomic del of entire NF1 gene | [30] | none/stac. | 3 |

Legend: p.No.: patient number; grey color field: sign present; OPG: optic pathway glioma; DC: Dodge classification; A: single nerve, B: optic chiasma, C: optic tracts; MDC Modified Dodge Classification; 1aL/R: single nerve left/right; 2bL/R: asymmetric chiasmatic L>R (R>L), 3Hb: asymetric tracts; VIS: visual aquity; (0: normal vision, 1: mild deterioration; 2: severe deterioration; FR: freckling; LN:Lisch nodues; FIB:neurofibromas; PN: plexiform neurofibromas; IN: inheritance 1(1): one first degree relative; 2(2): two second degree relatives; NBO: neurofibromatosis bright objects; FC: femoral cyst; PP: precocious puberty; CP: cerebral palsy; HP: hepatopathy; WS: wrong stature; MBG: multiple brain gliomas; SD: speach delay; MR: mental retardation, MC: microcephaly; VCR: vincristin; CAP: carboplatin; cyclophos: cyclophosphamid, HC: hydrocephalus; stac.: stationary; progress.: progression; regress.: regression; * new exon/intron numbering is indicated in the brackets, FS: frameshift, del.: deletion; ins.: insertion

most patients, the diagnosis was based on an MRI, which also allowed staging of the disease. We evaluated the severity of the OPG using both classic Dodge [19] radiological classification and novel – Modified Dodge classification – MDC [20]. The mild form of OPG (grade 1 in MDC) was seen in 13/25 (52%) patients, the medium form with chiasma affected in 7/25 (32%) and a potentially severe form with hypotalamic involvement in 4/25 (16%) (Table 1). The absence of a glioma in hypothalamic region is generally difficult to verify in the mild form of OPG.

The management of optic nerve glioma varied, depending on the age at diagnosis and the clinical presentation. In most cases, it was characterised by close observation. In the case of disease progression or complicated early onset, 7/25 (26.6%) patients were treated with chemotherapy, surgery or stereotactic radiotherapy. Patient P16 (Table 1) underwent a successful surgical treatment with regression of OPG. Stereotactic radiotherapy was used as the treatment modality in patient P44 (Table 1) showing no progression seven years after the treatment. One patient (Table 1, P122) refused the treatment. Chemotherapy was given to 5 (20%) patients. The treatment of choice included vincristine and carboplatin (Table 1, patients P2, P12, P18b, P89 were treated for 12 months) or vincristine and cyclophosphamide in the case of an allergic reaction to carboplatin (Table 1, P3 treated for 12 months). Patient P12 (Table 1, Figure 1) was initially treated with vincristine and carboplatin for 12 months, after a significant progression in the size of the OPG and other brain gliomas, was then treated with temozolomide for another 12 months. The combination of treatments was sporadic. Two patients (Table 1, P3 and P12) had an inborn protrusion of the bulbus caused by the OPG. Patient P12 had an operation at first, followed by treatment with chemotherapy, Patient P3 underwent a total resection of the optic nerve with preservation of the bulbus. Both patients (Table 1, P3, P12) showed signs of progression on MRI scans after treatment. In general, two patients showed regression (Table 1, P18b, P16), one died at the age of 24 due to progression of malignant peripheral nerve sheath tumor (MPNST) on the neck (Table 1, P2).

**Patients with OPG – mutation spectrum and clinical findings.** The clinical and molecular data of NF1 patients with optic nerve glioma in our study are summarised in Table 1 and Figure 2.

In our study we found that 17/25 (58%) mutations in the OPG group were located in the range of the first 5'tertile: exons 1-16 (1-21, new classification) (P=0.01). This finding was confirmed by the assessment of mutation distribution in all patients in the study (52 patients) In this analysis all patients with the whole *NF1* gene deletion (type 1) were excluded. The causal mutations were distributed throughout the entire *NF1* gene (Figure 2,3). However, we observed that from 24 patients with the mutation localized in the first tertile of the *NF1* gene, 17 (70.9%) had OPG compared to 7 (29.1%) who didn't have the OPG (P=0.0049). Statistical analysis – the exact two-sided (mid-p) – was used to correlate two independent proportions

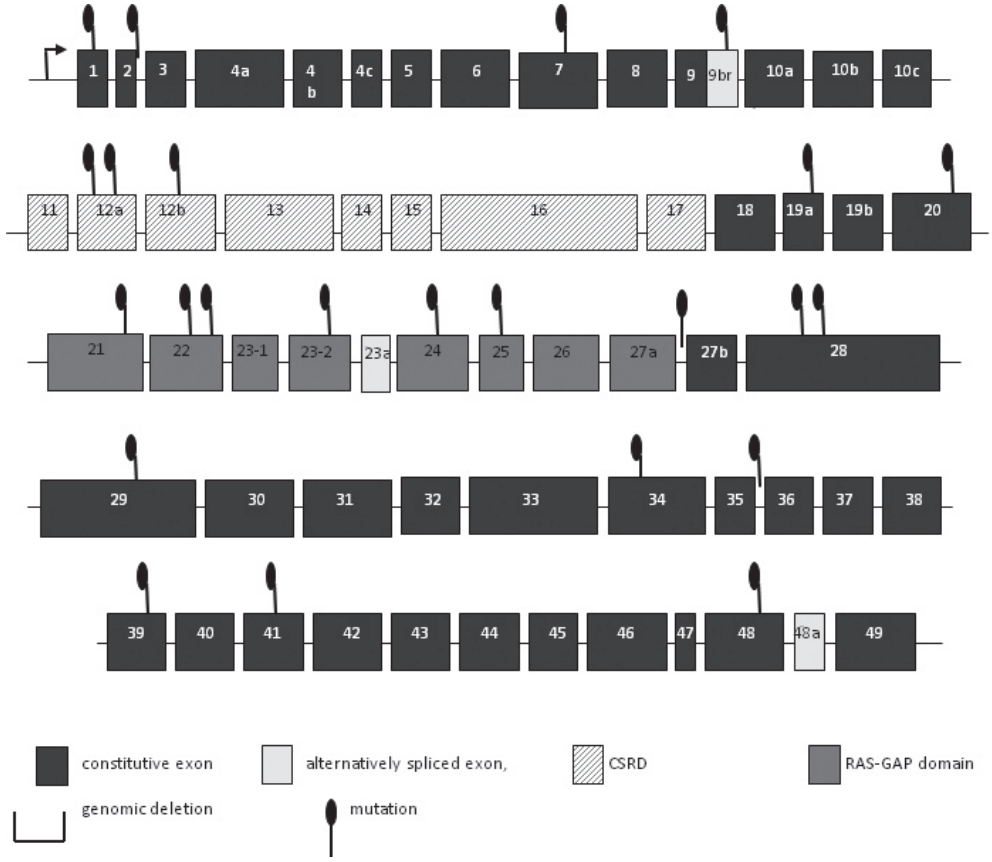


**Figure 3. Diagram of mutations in 24 NF1 patients without optic pathway glioma, 3 patients had the type 1 deletion**

(mutation location and OPG), and confirmed that clustering of mutations in the first 16 exons of the *NF1* gene positively correlates with the frequency of the OPG sign.

In the group of patients with OPG, one patient (table 1, P 11) had a whole gene deletion, and two patients (Table 1, P23, P67) had large genomic deletions including several exons, all of which were revealed by the MLPA analysis. Frameshift mutations were found in 14 patients, two of which were sib-pairs (7 deletions, 6 insertions and 1 deletion and subsequent insertion). Direct cDNA sequencing revealed also two simple splicing mutations, one missense mutation, one mutation causing a premature stop codon, and one small in-frame deletion in this group. Six mutations were located in the range of exon 11 to 17 – in the CSRD (cysteine/serine rich domain) (Table 1), and four were affecting the RAS-GAP related domain spanning from exon 21 to 27a. The rest of the mutations were located in other constitutive exons.

Despite a known association between a large deletion and a more severe disease progression [25-27], in our study, there was one patient with a type 1 deletion (Table 1, P11) with a mild form of OPG diagnosed at the age of 10 years, without progression until now (age 13). At the age of 13 years he presented with a mild form of NF1 (hyperpigmentations and a few cutaneous neurofibromas). We identified two NF1 patients with novel intragenic deletions and OPG. Patient P23 (Table 1) had a deletion spanning from 23.2 to 27a (30-35) (including an alternatively spliced exon, 23a), resulting in the inactivation of the RAS-GAP related domain. Phenotypically, he had a 9 mm wide intraorbital glioma and early manifestation Lisch nodules and neurofibromas. The second patient, P67 (Table 1), who had unilateral renal dysgenesis and cutaneous features of NF1, had an intragenic deletion of exons 24-27b (32-36) that also included the RAS-GAP related domain.

Among the OPG-positive cohort, there were also two sibling pairs, both having OPG. Interestingly, our OPG-positive cohort contained individuals from 9 families with a parent carrying *NF1* mutation. It would be helpful to know if the parents of five children (Table 1, P46, P58a, P18, P37a, P2) with the mutation located in the 5'tertile of the NF1 gene, showed also OPG. Unfortunately all of these parents refused to undergo MRI scanning and provide the data.

As expected, visual impairment proved more significant in patients with OPG compared to the group without OPG. This finding was confirmed by Goodman Kruscal gamma coefficient with the level of agreement 0.833. The correlation

**Table 2. Clinical manifestation and mutation screening in 27 individuals without optic pathway glioma (OPG)**

| p.No. | age | VIS | FRE | LN | FIB | NBO | other | exon | mutation | type of mutation | recurrent/novel |
|---|---|---|---|---|---|---|---|---|---|---|---|
| P56 | 17 | 1 | | | | | | 1 (1) | mut ex1, c.47G>C (CGC>CCC), p.Arg16Pro, | missense | [30] |
| P64 | 13 | x | | | | | | in2 (2) | c.204+2T>G,skipping of exon 2: c.61_204del, p.Gln20del48aa, in frame | splicing | [53] |
| P69 | 21 | x | | | | | | 7 (9) | p.Leu307ProfsX5, c.919_926delCTTGCTGG | FS del | [30] |
| P83 | 1 | x | | | | | | in9 (11) | p.Ala422LeufsX57, c.1261-19G>A | splicing | [30] |
| P60 | 7 | | | | | | WS | 12a (16) | p.Leu604AspfsX5, c.1808_09delTA | Fs del | [30] |
| P87 | 36 | | | | | | | 12a (16) | p.Thr586ValfsX18, c.1756_59delACTA | Fs del | [52] |
| P82 | 2 | x | | | | | | 12b (17) | p.Gln616Glu, c.1846C>G | missense | [30] |
| P29 | 19 | | | | | | | 19a | p.Trp1048Arg, c.3142T>C | missense | [30] |
| P47 | 35 | | | | | | | 20 (26) | p.Leu1153MetfsX4, c.3457_60delCTCA | Fs del | [57] |
| P85 | 2 | 1 | | | | | | 21 (27) | p.Gln1189Arg, c.3566A>G | missense | [30] |
| P61 | 3 | | | | | | | 22 (28) | p.Arg1276X, c.3826C>T (CGA>TGA) | stop | [58] |
| P88 | 31 | | | | | | | 22 (28) | p.Arg1276Gln, c.2827G>A | missense | [30] |
| P4 | 32 | 1 | | | | | | 23.2 | p.Gln1360SerfsX20, c.4076_4077insC | FS ins | [30] |
| P76 | 22 | | | | | | hemang. | 24 (32) | p.Lys1409IlefsX10, c.4226_4227delAGinsT | FS del ins | [30] |
| P51 | 21 | | | | | | HP | 25 (33) | p.Pro1442GlnfsX2, c.4325_29delCTTTC | FS del | [30] |
| P38 | 14 | | | | | | | in27a (35) | p.His1555TyrfsX10, c.4662_68delGCATCAG due to splicing mutation c.4662-2A>G | splicing | [53] |
| P45 | 8 | | | | | | | 28 (37) | p.Leu1610X, c.4827_29delT | FS del-stop | [30] |
| P41 | 21 | | | | | | | 28 (37) | | missense | [30] |
| P91 | 6 | 1 | | | | | | 29 (38) | p.Ser1755X, c.5264C>A | stop | [30] |
| P6 | 46 | 1 | | | | | | 34 (43) | p.Arg2162ThrfsX18, c.6483_6484insAC | FS ins | [30] |
| P17 | 5 | | | | | | | in35 (44) | p.Ala2174IlefsX6, skipping of ex.35 (c.6580_6641del) due to c.6641+1G>T (ivs35+1G>T) | splicing | [52] |
| P21 | 22 | | | | | | | 39 (48) | p.Asn2362ThrfsX13, c.7084_85delA | FS del | [30] |
| P31 | 6 | | | | | | WS, HP | 41 (50) | p.Thr2423AsnfsX4, c.7267dupA | FS ins | [59] |
| P1 | 6 | 1 | | | | | deafness | 48 (57) | p.Ala2716XfsX1, c.8146_8153delGCCTTGAT | FS del-stop | [30] |
| P32 | 20 | | | | | | PP | all | type I deletion | type I | [30] |
| P39 | 5 | 2 | | | | | GIST | all | type I deletion | type I | [30] |
| P52 | 5 | 1 | | | | | | all | type I deletion | type I | [30] |

Legend: p.No.: patient number; grey color field: sign present; VIS: visual aquity; (0: normal vision, 1: mild deterioration; 2: severe deterioration; X: insufficient data); FRE: freckling; LN:Lisch nodues, FIB: neurofibromas; NBO: neurofibromatosis bright objects; WS: wrong stature; hemang.: hemangiomas; HP: hepatopathy; MS: missence; PP: precocious puberty; GIST: Gastrointestinal stromal tumor; FS: frameshift, del.: deletion; ins.: insertion

of subgroups 0-2 were insignificant due to small numbers. The other limitation of the analysis is the insufficiency of data in the non-OPG group of NF1 patients.

**Patient without OPG – mutation spectrum.** The mutational spectrum and disease features of the group of NF1 patients without OPG was different (Figure 3, Table 2).

We identified a causal *NF1* mutation in all 27 patients. In the group without OPG, 7/27 (27%) mutations were distributed in the first 5'tertile of the *NF1* gene. (Figure 3, Table 2) A type 1 whole *NF1* gene deletion was found in three patients. Twelve patients had a frameshift mutation leading to a premature stop codon, with eight being caused by a nucleotide deletion, three by a nucleotide insertion, and one patient by both a deletion and an insertion. In this group, there were also four simple splicing mutations, six missense mutations and two stop mutations. In the CSRD, there were three mutations identified, and six other mutations affected the RAS-GAP domain. The rest of the mutations were positioned in other constitutive exons. Clinical signs were already mentioned. Patients without OPG had not developed any malignancy, thus they required no treatment.

## Discussion

This report is the first on the genotype-phenotype correlation in NF1 patients that combines genetic and various disease feature data focusing on the presence of optic pathway glioma.

The results show the clustering of mutations in the 5'tertile of the *NF1* gene in patients with an OPG and refer to a higher incidence of NF1-disease features and thus to a NF1 phenotype with more frequent NF1 features. These data represent new knowledge to supplement previously published genotype-phenotype correlations [32-35].

To date, only two genotype-phenotype correlations in patients with neurofibromatosis type 1 have been reported. The first clinical correlation relates to NF1 patients with a whole *NF1* gene deletion (type 1 deletion) [32]. Such patients present with a more severe disease status, having more cutaneous neurofibromas than expected for their age, dysmorphic features, learning disabilities and a higher risk of MPNSTs [33, 34]. The second study relates to a correlation between the presence of a 3 bp (AAT) deletion in exon 17 of the *NF1* gene and a milder NF1 phenotype and the complete absence of neurofibroma development [35]. Sharif and colleagues studied the mutational status in 29 NF1 patients with optic nerve glioma and focused on mutation distribution without the assessment of other clinical features of NF1. Their work suggested the clustering of mutations in the 5' tertile of the *NF1* gene in these patients [27]. Our data strongly support his findings and address the predictive value of mutation distribution in this region for optic glioma development.

The 5'tertile of the *NF1* gene is characterised by the presence of the CSRD spanning from the exon 11 to exon 17 (15-22). The role of this domain in the pathogenesis of OPG has not been completely elucidated, although there is emerging evidence that the CSRD could be an equally important functional domain in neurofibromin as the RAS-GAP domain (RGD). Using human and mouse nervous system cells and cell lines, in response to epidermal growth factor, neurofibromin has been shown to be phosphorylated on serine residues by protein kinase C (PKC), with this phosphorylation being prominent in CSRD [36]. The CSRD has also a region for ATP binding and three cAMP-dependent PK recognition sites phosphorylated by PKA thus, it is also known as the cAMP/PKA domain [37]. Due to the clustering of mutations in this region in individuals with OPG, we assumed that this NF1 domain may play an important role in the development of OPG. To confirm our finding, further extensive molecular studies with a larger group of patients are necessary. Mutation clustering in this domain was also described in a group of NF1 patients with pheochromocytomas, suggesting that the CSRD could be a mutation target in NF1-associated pheochromocytoma [38]. However, in our cohort, there were no pheochromocytomas observed.

In our study, the patients with optic glioma were diagnosed at an average age of 7 years old, which widely corresponds with the results from larger population studies [3-6]. The later onset of OPG has been described in a study by Lewis et al. that included 33 NF1 patients with a mean age of 19, 8 years [39] and also in the work of Listernick et al. [40]. In the group without OPG, there were 7/27 (27%) individuals with a mutation in the first tertile of the gene, 3 of whom were younger than 8 years old. These children are still at risk of developing an OPG later in life and might be in need of closer follow-up. The average length of follow-up was 7-8 years, and we realise a longer follow-up is required for a better understanding of the development of OPG into adulthood and of the association of pathogenic mutations and clinical course (prognosis) in NF1 patients with OPG. So far, gender has not been described as a significant determinant factor for OPG development, which agrees with our observation.

Further investigation is required regarding the observation of NF1 parents in 5 families with children carrying the mutation in the 5'tertile of *NF1* gene. Given that the parents refused MRI scanning as an objective method of OPG evaluation, it was not possible to verify the presence of OPG. Evaluating the disease history of each parent to verify whether an OPG was present at all or whether it was present but regressed spontaneously would be important [41-42]. We also observed the tendency of an increased incidence of the mutations affecting the RAS/GAP domain among the patients without OPG (30% compared with 19% in the group with OPG).

The modified Dodge classification proved to be a helpful tool in the assessment of the severity of the optic pathway glioma given that biopsy is not a recommended diagnostic tool in the management of OPG [20]. The involvement of the chiasma and hypothalamus in four patients with mutations located in the 5'tertile of the *NF1* gene is particularly interesting. This finding might indicate a higher risk of a more severe form of OPG in individuals with mutations located in this region. However, the significance of this finding would have to be confirmed in a study with a larger number of patients.

The decision about the severity and potential treatment was based on a combination of both radiological data and visual acuity. As we found in our study, studying visual outcomes in children with OPG in a retrospective manner before and after the treatment is difficult due to reporting in a non-standard manner. An objective method, such as visual-evoked potentials, has not met with universal agreement as a screening modality [43]. The decision regarding the treatment of most patients was made when there was radiographic progression or visual deterioration, which is advocated by several authors [44, 45]. Others reserve treatment only for patients with documented visual deterioration. In addition, in our study, the factors influencing the decision to treat were tumor size/extent, tumor enhancement, tumor location, progressive proptosis and visual loss [43-47]. It is possible that more detailed studies comparing the tumor location on MRI with visual defects may allow for a better prediction of visual problems. Our preliminary molecular and clinical data seem to be promising for prognosis of NF1 patients however for the final genotype-phenotype correlations regarding OPG much larger studies are necessary.

## Conclusion

Our results may contribute to a better prediction of the development of optic glioma in NF1 patients based on the molecular diagnostics. Additionally molecular analysis in early childhood may help to disclose early stages of optic glioma and improve their clinical management.

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