

Is Neurofibromatosis Type 1–Noonan Syndrome a Phenotypic Result of Combined Genetic and Epigenetic Factors?

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Abstract. *Background/Aim: Neurofibromatosis 1–Noonan syndrome (NFNS) presents combined characteristics of both autosomal dominant disorders: NF1 and Noonan syndrome (NS). The genes causing NF1 and NS are located on different chromosomes, making it uncertain whether NFNS is a separate entity as previously suggested, or rather a clinical variation. Patients and Methods: We present a four-membered Greek family. The father was diagnosed with familial NF1 and the mother with generalized epilepsy, being under hydantoin treatment since the age of 18 years. Their two male children exhibited NFNS characteristics. Results: The father and his sons shared R1947X mutation in the NF1 gene. The two children with NFNS phenotype presented with NF1 signs inherited from their father and fetal hydantoin syndrome-like phenotype due to exposure to that anticonvulsant during fetal development. Conclusion: The NFNS phenotype may be the result of both a genetic factor (mutation in the NF1 gene) and an epigenetic/environmental factor (e.g. hydantoin).*

Neurofibromatosis–Noonan syndrome (NFNS, MIM 601321) is considered by some clinicians a distinct clinical entity (1, 2), which presents combined characteristics of both autosomal dominant disorders: neurofibromatosis type 1 (NF1, MIM 162200) and Noonan syndrome (NS1, MIM 163950). NF1 is characterized by neurofibromas, café-au-lait spots, osseous lesions, and brain tumors such as gliomas (3). NS presents characteristic facial appearance, short stature, hypertelorism, strabismus, broad nasal bridge, low-set ears and low posterior hairline (4).

NS is characterized by large genetic heterogeneity. NS1 is the most common type, since more than 50% of patients with NS have been diagnosed with mutations in the protein

tyrosine phosphatase non-receptor type 11 (*PTPN11*) gene on chromosome 12q24 (5, 6). There are at least ten NS types, with most of them exhibiting autosomal dominant inheritance, while for one of them, namely NS2, autosomal recessive inheritance has been confirmed (7-15).

The genes causing NF1 and NS are neither allelic nor contiguous. NF1 is caused by mutations in the tumor-suppressor gene *NF1*, encoding neurofibromin, on chromosome 17p11.2 (16, 17). Neurofibromin is a large cytoplasmic protein, which functions as a rat sarcoma oncogene homolog (RAS) GTPase-activating protein regulating the initial stage of the RAS/mitogen activated protein kinase (MAPK) cascade (18). Interestingly, all known genes of NS types encode proteins that participate in the same RAS/MAPK signal-transduction pathway (19, 20).

The RAS/MAPK cascade is activated by extracellular signals, such as growth factors, that bind to a cell membrane-bound receptor tyrosine kinase (RTK), resulting in its dimerization and activation (19, 20). Activated RTK consequently triggers intracellular RAS GTPases, which in turn activate a series of protein kinases, transmitting a phosphorylation cascade signal to downstream targets, with each successive kinase serving as a substrate for the upstream one. Finally, the RAS/MAPK signal transduction cascade leads to activation of extracellular-signal-regulated kinases that regulate expression of genes involved in meiosis, mitosis, and post-mitotic functions of differentiated cells (21).

The RAS/MAPK cascade is involved in the pathophysiology of both NF1 and NS types. As previously mentioned, NF1 regulates RAS in the initial stage of the RAS/MAPK cascade (18). Another factor that also controls the initial activation of the RAS/MAPK pathway is SHP2 which is encoded by the NS1-related *PTPN11* gene (5). SHP2 is a protein tyrosine phosphatase, which either inactivates pathway repressors (such as Sprouty family members), or dephosphorylates RAS GTPase-activating protein-binding sites of RTKs, prolonging their activation (20).

The SHP2 protein (NS1-type related) assists the RTK binding of guanine nucleotide exchange factors Son of sevenless homolog 1 or 2 (SOS1, SOS2) which are NS4-related and NS9-related, respectively. Both can replace RAS-

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bound GDP with GTP, thus activating RAS (10, 15). NF1 (NF1-related) boosts the GTPase activity of RAS proteins, including Kirsten rat sarcoma oncogene homolog (KRAS) (NS3-related) and neuroblastoma rat sarcoma oncogene homolog (NRAS) (NS6-related), as well as the RIT1 GTPase (NS8-related), inhibiting the RAS/MAPK pathway (8, 12, 14). Activated RAS-GTP, in turn, triggers cytoplasmic kinase rapidly accelerated fibrosarcoma-1 proto-oncogene, serine/threonine kinase (RAF1) (NS5-related) and BRAF (NS7-related) (11, 13, 15). Finally, a protein involved in marking NF1 for ubiquitin-associated degradation is leucine-zipper-like transcription regulator 1 (LZTR1) (NS10-related), which consequently hyperactivates RAS/MAPK signaling (15). Therefore, germline mutations that affect components of the RAS/MAPK pathway are involved in the pathogenesis of both NS and NF1.

It still remains uncertain whether NFNS is a separate entity as previously suggested (1, 2, 22, 23), or rather a phenotypic variation caused by alterations of the *NF1* gene, as proposed by others (24-27). In one study that supports the phenotypic variation hypothesis, the clinical examination of 94 persons with NF1 revealed that 12 of them (including some familial cases) possessed NS features (25).

Here we present a nuclear Greek family with four members. In this family, the father presents typical NF1, the mother suffers from generalized epilepsy, while their two male children present NFNS manifestations. In light of the presented family, we propose a combined genetic and environmental mechanism as a cause of NFNS phenotype.

Patients and Methods

Family data. The extended pedigree of the studied Greek family is shown in Figure 1. The nuclear family members (individuals II-3, II-4, III-3, III-4, Figures 1 and 2) were clinically examined at the Department of Neurology of our University Hospital. The parents (II-3, II-4) were not related to each other, as they originated from distinct areas of Greece. The parents gave their informed consent to the use of their family's clinical and genetic information for research and scientific publication purposes.

Clinical data. The diagnosis of NF1 was set for the father and his two sons, based on the standard criteria for that disease (3). Features of NS and fetal hydantoin syndrome were also examined clinically, based on defined criteria (28, 29). Clinical evaluation of the nuclear family members revealed the following:

Individual II-3 (father): A 40-year-old man with typical characteristics of NF1: many neurofibromas and café-au-lait spots all over his body, an osseous lesion in left parietal bone, and hyperostosis of ulna in both hands (Figure 3). At 39 years, he presented with grand mal epileptic crises. Magnetic resonance imaging revealed an extended space-occupying mass of the right frontotemporal region without any changes after more than 6 months' follow-up.

Individual II-4 (mother): A 34-year-old woman with mild mental retardation. She first presented with generalized epilepsy at the age

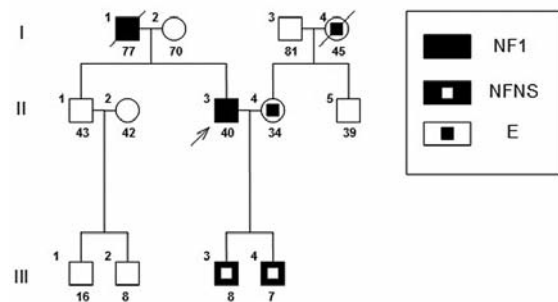


Figure 1. Pedigree of the studied family. NF1: Neurofibromatosis 1; NFNS: neurofibromatosis-Noonan syndrome; E: epilepsy.

of 18 years. She has been under hydantoin treatment ever since. Currently, she is under medication and is free of seizures. Magnetic resonance imaging was normal. Careful clinical investigation excluded clinical manifestations of NF1 syndrome.

Individual III-3 (first child): A boy aged 8 years with NFNS phenotype (Figure 4). He had short stature (below 5th percentile), weak constitution, moderate mental deficiency, ocular hypertelorism, myopia and strabismus, low nasal bridge, highly arched palate, increased width of mouth, low-set ears and slightly hypoplastic nails. He presented with several neurofibromas (one of them remarkably in the tongue) and café-au-lait spots all over his body. He had a large melanin-spotted area, including a neurofibroma in the right side of his back.

Individual IV-4 (second child): A 7-year-old boy, also with NFNS phenotype (Figure 5). He presented with moderately short stature (below 10th percentile), weak constitution, moderate mental deficiency, hypertelorism, low nasal bridge, highly arched palate, increased width of mouth, low-set ears and hypoplastic distal phalanges and nails of the hands. He presented many NF1 signs, including multiple café-au-lait spots and neurofibromas.

Genetic studies. Cytogenetic analysis of white blood cells was performed on both boys. A systematic search for existing mutations in *NF1* and *PTPN11* genes of the father and his two sons was conducted with DNA sequencing.

Results and Discussion

Cytogenetic analysis revealed normal male karyotypes for both boys. DNA sequencing of *NF1* and *PTPN11* genes was conducted. The father and his two sons shared a C-to-T transition changing arginine codon to a stop codon (R1947X) in exon 31 of the *NF1* gene in heterozygosity with the normal allele. Hence, all three presented autosomal dominant NF1. Furthermore, no mutation was detected in the *PTPN11* gene in any of them.

The R1947X mutation results in protein synthesis of truncated NF1 protein (30). It is a rather common mutation, located in a CpG dinucleotide mutational hotspot (31, 32), previously reported in multiple Caucasian and East Asian patients with NF1 (30, 33-40).

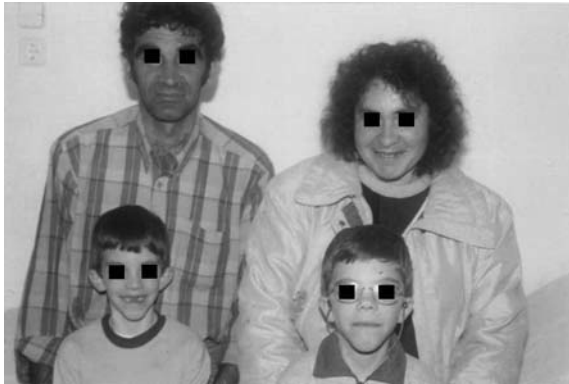


Figure 2. Photograph of the four nuclear family members.



Figure 4. Older child (III-3), neurofibromatosis-Noonan syndrome. a: En face, b: profile, c: tongue, d: back.



Figure 3. Father, neurofibromatosis 1. a: en face, b: profile, c: back.



Figure 5. Younger child (III-4), neurofibromatosis–Noonan syndrome. a: En face, b: profile, c: back, d: hand.

In addition to clinical signs of NF1, both boys displayed certain NS features, such as short stature and characteristic facial appearance, including hypertelorism and strabismus, low nasal bridge, increased width of mouth, and low-set ears. Nevertheless, the children did not present with some other major signs of NS, such as short or webbed neck.

Careful clinical observation and family history suggested that the children actually had fetal hydantoin syndrome (29). The diagnosis was based on the hallmark presence of hypoplastic nails in both boys and the fact that their epileptic mother had received hydantoin treatment during both pregnancies. In conclusion, the NFNS phenotype in the described children was caused by a combination of a genetic factor and an epigenetic/environmental factor: the inherited NF1-causing mutation and the exposure to the anticonvulsant hydantoin during fetal development respectively.

Some authors have previously proposed that NFNS is a separate entity (1, 2, 22, 23), but this hypothesis is not

supported by accumulating evidence. Some other possible explanations have been suggested by others in order to explain the combination of the two phenotypes. The first obvious hypothesis, namely the mere coincidence of two relatively frequent dominant conditions, is not justified statistically, since there are far more observed cases than predicted by chance alone. Indeed, in a series of 94 patients with NF1, 13% of them had NS features (25). Nevertheless, only rarely have mutations been found in both *NF1* and *PTPN11* genes in patients with NFNS (41, 42).

A second offered hypothesis that NFNS represents associated disorders due to mutation(s) at closely linked loci has been weakened by results of linkage analysis, which places the NS genes on various chromosomal loci, with none of them residing on chromosome 17p11.2 where the *NF1* gene is located (19, 43). In an extended family of four generations in which eight members had NFNS, two had NF1 only and two had NS only, linkage analysis

showed tight linkage of the NF1 phenotype to the *NF1* gene, whereas the NS phenotype was linked to a distinct locus on 17q (44).

A third possibility with many supporters is that NFNS is a phenotypic variant of the NF1 spectrum of phenotypes, caused by mutations in the *NF1* gene. This possibility seems plausible, since there is evidence of families with members with NF1 and NFNS which are linked to 17p11.2, and several mutations in the *NF1* gene have been found in patients with NFNS (23-27, 45, 46), as in the two boys of our report.

In light of the presented family, we favor a fourth explanation, namely that NFNS phenotype may result as a combined effect of a genetic factor (mutation in the *NF1* gene) and an epigenetic factor (environmentally derived hydantoin in our cases). As far as we know, our report is the first to provide evidence supporting this novel hypothesis.

Hallmarks of NS include short stature, mental retardation (25%), characteristic facial appearance, webbing of neck and cryptorchidism (28). The differential diagnosis for patients with NS-like phenotype includes XO/XY mosaicism, fetal hydantoin syndrome, fetal mysoline syndrome and fetal alcohol syndrome (28). In addition to NS features, patients with fetal hydantoin syndrome may have cleft lip and/or palate, hypoplasia of distal phalanges, with characteristically small nails, and of course a maternal history of anticonvulsant treatment during pregnancy (29).

It seems that NS-like facial appearance may be produced by a number of genetic or other causes (all of which promote weakness of fetal facial muscles) and thus may represent the end result of a non-specific insult during fetal development (1). Along these lines, it is suggested that the NS phenotype seen in some patients with NF1 may be due to dysgenesis or other developmental alterations of the central nervous system resulting in muscular hypotonia, as previously suggested (47). In the presence of hypotonia, development of craniofacial structures will be altered, leading to hypoplasia of the midface and micrognathia. When coupled with craniofacial changes known to be common in NF1 (prominent forehead, hypertelorism and broad nasal tip), the resulting NS-like facial phenotype is easy to envisage.

In our family, the two young patients had both NF1- and NS-like phenotypes, but the delineation of these phenotypes was possible because each one could only have been derived from a distinct parent. The father clearly presents NF1 and transmitted the mutant *NF1* gene to his sons, while the epileptic mother was under hydantoin treatment throughout both pregnancies. This observation raises the possibility that the observed NFNS cases are in reality caused by a genetic and an epigenetic factor. Therefore, the clinical history of NFNS needs to be re-addressed carefully examining this possibility.

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