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Clinical findings and mutation analysis of NF1 patients in Turkey

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ABSTRACT

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that is caused by mutations of the NF1 gene. NF1 is clinically characterized by neurofibromas, pigmentation anomalies, and an increased risk of malignant tumors. The mutation rate of NF1 is one of the highest known for human disorders: approximately 50% of all affected individuals are sporadic cases and carry *de novo* mutations Therefore mutation analysis of NF1 may be an important tool in early diagnosis and genetic counseling. This is the first large NF1 study performed in Turkey. The data collected in this study enabled us to overview the genetic and clinical aspects of NF1 molecular diagnostics. The patients, who were clinically diagnosed for NF1, were included in this study. These patients were clinically evaluated, and subgroup of them genotyped or DNA sequenced for mutations in NF1, either to confirm the clinical diagnosis or to identify pathogenic mutations. The mutation detection rate was 52%, based on analysis of only genomic DNA. We observed that frameshift mutations were the largest proportion of the identified mutations (38.5%). The frequency of microdeletions was 26.9% and the splice site and nonsense mutations were 11.5% in this cohort. Turkish NF1 patients have similar NF1 germline mutations compared to other populations. Considering that some of these detected mutations belonged to the patients who did not fulfill the NIH criteria for NF1 diagnosis, mutation analysis of NF1 is an important tool in early diagnosis and genetic counseling.

1. Introduction

Neurofibromatosis type 1 (*NF1*; OMIM 162200) is an autosomal dominant disorder affecting approximately 1 in every 3500 births. NF1 is caused by mutations in the *NF1* gene that is located on chromosome 17q11.2 (Shen et al., 1996). The *NF1* gene spans 350 kb of genomic DNA, and contains 60 exons. The germline mutation rate for the *NF1* gene is one of the highest reported in humans. Approximately 50% of all affected individuals carry *de novo* mutations (Li et al., 1995). The mutational spectrum of the *NF1* gene illustrates the need for multiple, complementary techniques to detect pathogenic lesions (Pasmant et al., 2012; Messiaen et al., 2000). *NF1* is a tumor suppressor gene that encodes the protein neurofibromin. Neurofibromin is a Ras guanosine triphosphatase (GTPase) activating protein that inhibits Ras signaling, and thus, acts as a regulator of signaling for cell proliferation and differentiation (Trovo-Marqui and Tajara, 2006). There are no clear genotype–phenotype correlations with the exception of findings from two cases. However, affected patients with large deletions in the *NF1* gene have a more severe clinical phenotype (Tonsgard et al., 1997).

NF1 patients heterozygous for the 3 bp inframe deletion c.2970_2972delAAT in the *NF1* gene lack cutaneous neurofibromas (Upadhyaya et al., 2007). Because of clinical overlap between NF1 and Legius syndrome, genetic analysis of the *NF1* gene is necessary to confirm the clinical diagnosis. If a pathogenic mutation is identified, genetic testing including presymptomatic testing and prenatal diagnosis can be offered to family members. The main clinical features of NF1 are

Abbreviations: NF1, neurofibromatosis type 1; GTPase, Ras guanosine triphosphatase; NIH, National Institutes of Health; SSCP, single strand conformation polymorphism; HRM, High Resolution Melting Curve; CLS, café-au-lait spots; CN, cutaneous neurofibromas; LN, lisch nodules; AF, axillary freckling; IF, inguinal freckling; PN, plexiform neurofibroma; SD, skeletal dysplasia; OG, optic glioma; RMS, rhabdomyosarcoma; MPNST, Malignant Peripheral Nerve Sheath tumor

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café-au-lait spots (CLS), skin fold freckling, cutaneous neurofibromas (CN), and lisch nodules (LN). A diagnosis of NF1 is clinically confirmed by the presence of two or more of the above mentioned diagnostic criteria, as established by the National Institutes of Health (NIH) in 1988 (DeBella et al., 2000; Anon, 1988). Hacettepe University Faculty of Medicine, a reference center in Turkey for neurological diseases, organizes the multidisciplinary management of NF1 patients. "The NF Study Group of Hacettepe University" has been established in 2003. The study group comprises physicians from all related clinical departments for both pediatric and adult patients, and also researchers from the basic sciences involved in molecular studies of NF1. The departments include pediatric neurology, pediatric oncology, dermatology, ophthalmology, orthopedics, neurosurgery, neurology, genetics, and medical biology. This study describes the clinical and genetic findings of NF1 in patients from Turkey.

2. Material and methods

2.1. Patients

A national NF1 database consisting of 217 probands and their 203 affected and 329 unaffected relatives was established through the recruitment efforts of the NF Study Group of Hacettepe University in Turkey. Of our patient cohort, DNA from familial cases as well as available family members was genotyped and 50 sporadic/familial patients were analyzed for mutations. Testing was performed to confirm the clinical diagnosis or to identify the pathogenic mutation. Clinical findings of patients with NF1 were highly variable and symptoms develop over time. The study protocol was approved by the Institutional Ethics Committee of Hacettepe University, ANKARA (Report number: FON06/16-39, Date: 11.05.2006). Written informed consent was obtained from all participants.

2.2. Clinical data

Detailed clinical information of patients was obtained through a questionnaire, which was prospectively completed by a dermatologist, a pediatric neurologist, or a clinical geneticist. The questionnaire included clinical features of NF1 including tumors and other neurological problems. Clinical data were stored in our in-house developed database. An overview of the different major clinical criteria of NF1 presented in index patients is provided in Table 1.

Table 1
Clinical data of patients with NF1.

Characteristics	N (%)
Number of studied patients	217 (100%)
Familial cases	91 (41.9%)
Sporadic cases	126 (58.1%)
Cases with tumors	44 (20.3%)
Cases without tumors	173 (79.7%)
Symptoms and signs	
Café-au-lait spots (CLS)	200 (92.2%)
Axillary freckling (AF)	112 (51.6%)
Inguinal freckling (IF)	55 (25.3%)
Lisch nodules (LN)	63 (29%)
Cutaneous neurofibroma (CN)	55 (25.3%)
Plexiform neurofibroma (PN)	27 (12.4%)
Optic glioma (OG)	31 (14.3%)
Skeletal dysplasia (SD)	30 (13.8%)
Hamartoma	6 (2.8%)
Other tumors	
Cranial nerve tumor, brain tumors, Rhabdomyosarcoma (RMS), Malignant Peripheral Nerve Sheath tumor (MPNST), Myxoid liposarcoma, Periferic and lymph node tumors	34 (15.7%)

2.3. Mutation analysis

DNA was isolated from peripheral blood cells or chorionic villus samples according to standard procedures (Miller et al., 1988). During the 14 years of *NF1* molecular diagnostics, initially, Linkage Analysis was used and later, several other techniques were implemented to analyze *NF1* for genetic variants such as single strand conformation polymorphism (SSCP) and High Resolution Melting Curve (HRM) analysis.

2.4. DNA sequencing

All coding exons and exon/intron boundaries (including 30 bp of each intron) of the *NF1* gene were amplified by polymerase chain reaction (PCR) using genomic DNA. PCR amplicons were purified and directly sequenced using the Big Dye Terminator 3.1 Kit (PE Applied Biosystems, Foster City, CA) and ABI-PRISM 3130 Genetic Analyzer (PE Applied Biosystems) according to manufacturer's instructions. A complete list of primers can be provided upon request.

3. Results

Clinical features of patients with NF1 are shown in Table 1. Café-au-lait spots (CLS) were the most common finding observed in a majority (92%) of patients. Axillary freckling (AF), inguinal freckling (IF), cutaneous neurofibroma (CN), plexiform neurofibroma (PN), and lisch nodules (LN) on the iris were observed in 51.6%, 25.3%, 25.3%, 12.4%, and 29% of patients, respectively. Glioma or glioma-suspected lesions were identified in 31 (14.3%) patients. Skeletal dysplasia (SD) was found in 30 (13.8%) patients, and different types of tumors were identified in 34 (15.7%) patients. The age at diagnosis of NF1 among these 217 patients recruited through our hospital ranged from 6 months to 17 years (median 7.7 years). The age at first diagnosis of NF1 with CLS was 6 months (median 6.3 years), with axillary freckling (AF) was 2 years (median 6 years), with inguinal freckling (IF) was 3 years (median 6.3 years), or with lisch nodules (LN) was 3 years (median 9 years). The age at first diagnosis of NF1 with cutaneous neurofibroma (CN), plexiform neurofibroma (PN), optic glioma (OG), and skeletal dysplasia (SD) was 3 years with medians of 10.0, 9.9, 7.4 and 9.8 years, respectively. NF1 with hamartoma had an age of first diagnosis at 3 years with a median age of 8.6 years.

DNA from 50 patients was used for mutation analysis. A number of known and novel mutations were identified in our study, as shown in Table 2. A complete list of polymorphisms that were identified can be provided upon request.

4. Discussion

The NF Study Group in Hacettepe University aims to diagnose NF1 through a coordinated multi-disciplinary approach, and arrange family meetings to bring together patients and professionals. This work was performed either to confirm the clinical diagnosis or to identify pathogenic mutations for further analysis in families, including prenatal diagnosis using segregation analysis (Miller et al., 1988). The data collected during this period enabled an overview of the genetic and clinical aspects of NF1 molecular diagnostics in Turkish patients. Mutation analysis in 50 patients and phenotypic characterization of 217 patients was performed to determine the mutational spectrum of the *NF1* gene in Turkish patients and to find a possible genotype-phenotype correlations.

We found that the mutation detection rate in index patients (52%) using genomic DNA was similar to that in the literature but slightly lower than that found by Messiaen et al. (2000). Thirteen patients did not fulfill NIH diagnostic criteria for NF1, but were nevertheless included in our study. Exclusion of this group would have likely resulted in a higher mutation detection rate. Higher mutation detection rates are