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CASE REPORT

CASE REPORT FOR TWO SIBLINGS CARRYING NEUROFIBROMATOSIS TYPE 1 WITH A RARE *NF1*: c.5392C>T MUTATION

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ABSTRACT

Neurofibromatosis type 1 (NF1) is a neurocutaneous syndrome caused by mutations on the *NF1* gene, which is located at chromosome 17q11.2. Although an autosomal dominant inheritance pattern is well-established, about half of new cases are the result of *de novo NF1* mutations. Neurofibromatosis type 1 has an incidence rate of 1/2600-3000 individuals, making it a major public health problem. The product of the *NF1* gene, the neurofibromin protein, is known to play a critical role in cellular differentiation and in tumor suppression. Due to widespread expression of neurofibromin in numerous tissues, particularly in cutaneous and nervous systems, *NF1* mutations cause a wide variety of clinical symptoms, including cutaneous and ocular lesions such as café au lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, iris Lisch nodules, choroidal freckling and internal tumors. In this article, we report the cases of two siblings with NF1, a 21-year-old male and his 24-year-old sister, who have the same c.5392C>T mutation on the *NF1* gene (p.Gln1798 Ter). Café au lait macules and freckling were the prominent clinical features in both siblings. However, a plexiform neurofibroma was also observed on the left arm of the sister, which is known to carry potential risk for malignant transformation. Although the mutation was previously described once, to the best of our knowledge, no case report has been published since then.

Keywords: Familial; Mutation; Neurofibromatosis type 1 (NF1).

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a neurocutaneous genetic disorder with a well-established autosomal dominant inheritance pattern and characterized by multiple café au lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, choroidal freckling and iris Lisch nodules. While osteopenia, osteoporosis and scoliosis are major skeletal features, dsyplasia of the long bones, especially tibia and fibula, is a rare but distinctive feature of NF1. Vascular system involvement of NF1 can cause renal artery stenosis, coarctation of the aorta, or other vascular lesions associated with hypertension. Most NF1 patients have normal intellectual functioning but learning disabilities, behavioral problems, and features of autism spectrum disorder can be seen [1]. While NF1 has a high penetrance, it is characterized by highly variable clinical expressivity. In some patients, skeletal changes and benign tumors of the neurocutaneous system with malignant potential are the cause of morbidity and mortality, while other patients present only with café au lait spots. Its incidence is approximately 1/2600-3000 individuals [2,3]. Approximately half of the cases are familial. Although there is no clear genotype-phenotype correlation, the severity of the phenotype is thought to be correlated with the reading frame truncation degree [4]. Neurofibromatosis type 1 is caused by mutations on the *NF1* gene, located at chromosome 17q11.2. Neurofibromin is widely expressed in a variety of tissues, including the brain, kidney, spleen, thymus, functioning as tumor suppressor by inhibiting the activity of the *RAS* gene [5], regulating cell proliferation, survival and growth.

MATERIALS AND METHODS

Case 1. A 21-year-old male patient presented to our dermatology clinic due to presternal papulopustular eruption, consistent with bacterial folliculitis. Dermatological

examination revealed multiple café au lait macules along with a few asymptomatic subcutaneous soft papules on his shoulders and deltoid regions. Bilateral inguinal and axillary freckling were also present (Figure 1). Lisch nodules on both irides were seen in his ophthalmological examination. Based upon the presence of axillary and inguinal freckling, café au lait macules, and Lisch nodules, the patient was diagnosed with NF1. Further clinical examination and anamnesis did not reveal any other sign or symptom of NF1.

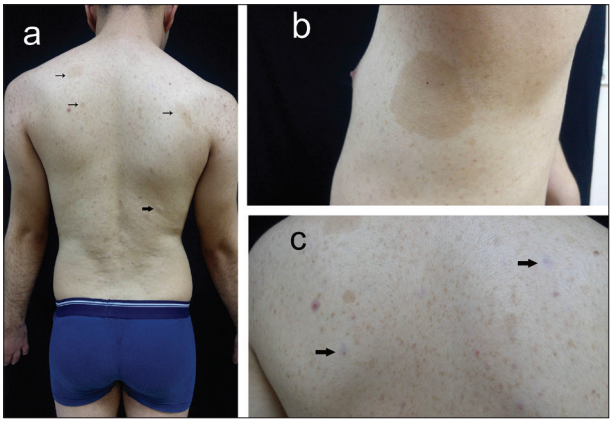

Figure 1. Dermatologic findings of patient 1. a) Numerous light brown colored patches-café au lait spots (thin arrows) on the back of the patient along with soft papules (bold arrow). b) Axillary freckling with an axillary café au lait macule. c) Close-up view of soft subcutaneous papules with a bluish hue (bold arrows).

Case 2. After a few weeks, a 24-year-old female patient, the elder sister of the first case, presented at our clinic due to the recent diagnosis of her brother with NF1, although she did not have any specific complaints. Several asymptomatic light brown patches, café au lait spots, were visible on her abdomen, in the right lower quadrant, and on her right flank [Figure 2(a) and 2(b)]. The flexor surfaces of her right arm and forearm were covered with flesh-colored, painless non indurated continuous plaques [Figure 2(a)]. Her left arm was also hypertrichotic [Figure 2(c)]. A biopsy from the plaques revealed prominent immunohistochemical staining with S100, indicating a neural sheath tumor. Further opthalmological, neurological and orthopedic examinations revealed no additional pathology. The family medical history showed similar skin lesions in their paternal grandfather, father, uncle, aunt, cousins and brother. The pedigree is shown in Figure 3.

Mutation Analysis. Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Next-generation sequencing (NGS) was performed on a MiSeq (Illumina Inc., San Diego, CA, USA), following the manufacturer's instructions for the *NF1* gene. The procedure for the preparation of libraries was performed according to the manufacturer's instructions. The hg19 (GRCh37) reference sequence was used as a reference for identifying genetic variants. The variant call formats (VCF) files were analyzed by Variant studio (Illumina Inc.) and Geneticist Assistant (SoftGenetics, State College, PA, USA) software program. The sensitivity of this test was determined as 99.0% for 5.0% single nucleotide polymorphism (SNP) allele fraction rate with 1000×

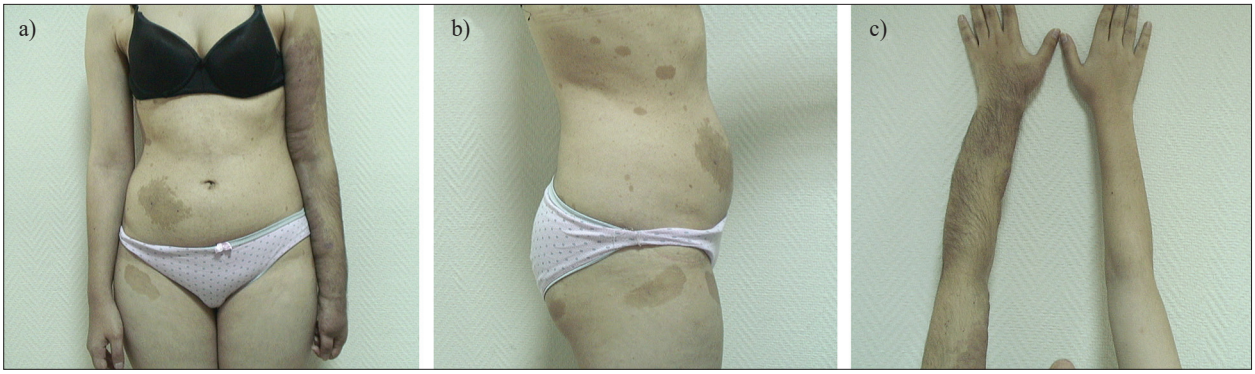

Figure 2. Dermatologic findings of patient 2. a, b) Numerous café au lait spots on the trunk and proximal part of the patient's thigh (black arrows), brown-colored soft plaques covered with terminal hair on the patient's left arm (white arrows). c) Close-up view of the arms showing hypertrichosis on the left arm.

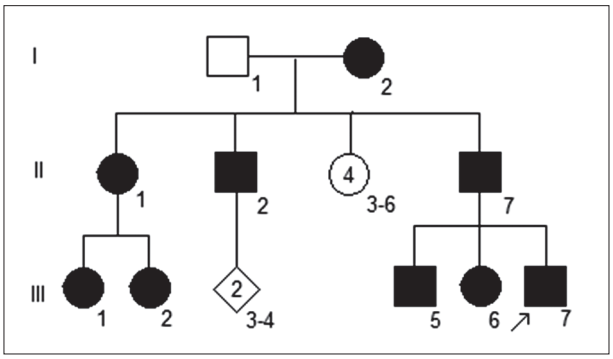

Figure 3. Pedigree of the family, affected family members are indicated as black circles. Mutation analysis was performed only for III-6 and III-7 (proband).

coverage. The specificity of this test was determined as 99.0% for 0.5% SNP allele fraction rate with 1000× coverage. All identified variants are evaluated with respect to their pathogenicity and causality and categorized into classes according to the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines [6]. Heterozygous class 1 c.5392C>T, p.Gln 1798Ter mutation in exon 38 of the *NF1* gene has been detected with a mutation surveyor program. Detected variants were also analyzed and confirmed by Sanger sequencing (Figure 4) according to the manufacturer's protocols. Briefly, the amplicons were analyzed by direct sequencing with ABI PRISM® 3500 (Life Technologies, Waltham, MA, USA). Analysis of sequence results was done by the Mutation Surveyor program (SoftGenetics). The primer sequences and polymerase chain reaction (PCR) conditions will be provided by the corresponding author upon request.

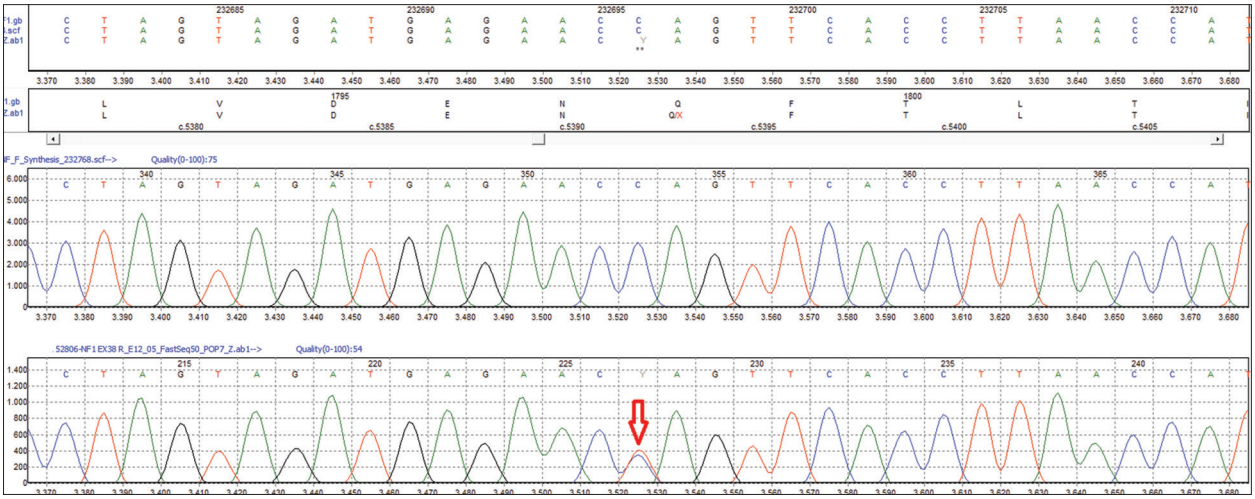

Figure 4. Electropherogram of the Sanger sequencing confirmed the mutation c.5392C>T, p.Gln1798Ter in exon 38 of the *NF1* gene. The arrow indicates the position of the c.5392C>T mutation. The proband and his eldest sister were heterozygotes.

DISCUSSION

The *NF1* gene spans 287 kb of chromosome 17q11.2 and comprises 57 constitutive and four alternatively spliced exons (9a,10a-2, 23a and 48a) [7]. To date, the Human Gene Mutation Database has documented more than 2450 disease-causing NF1 variants, of which 745 are missense/nonsense [8]. In the Varsome database (https:// vas=rsome.com/gene/nf1), 26.1% of reported class 1 *NF1* variants (328/1254) are nonsense mutations [9]. In our analysis, a C>T transition at nucleotide 5392 in exon 38, causing a premature termination codon at codon 1798 instead of a glutamine codon, was detected.

Recently, Zhu *et al.* [10] reported a study of *NF1* germline variants in patients with congenital pseudoarthrosis of the tibia (CPT). Screening 55 NF1 patients with CPT they found 44 *NF1* variants, of which 25 were novel. They described a c.5392C>T, p.(Gln1798Ter) inherited mutation in one patient, but they did not present the patient's clinical characteristics [10].

Here, we present two cases of NF1, a brother and a sister with c.5392C>T, p.Gln1798Ter mutation on the *NF1* gene. Neurofibromatosis type 1 has great clinical variability even between affected individuals of the same family with the same mutation. Our represented cases of siblings also show inter-individual variation; they have different features of skin pigmentation, and the sister has an additional neural sheath tumor. The prominent lesions of the brother were relatively benign café au lait macules, but the sister also had a plexiform neurofibroma, estimated to have a lifetime risk of 5.0% for malignant transformation [11]. We speculate that modifier genes, as well as epigenetic and environmental changes, might be the cause of the clinical variability.

Although NF1 is a monogenic disorder, its clinical variability is similar to multifactorial disorders and may be affected by epigenetic and environmental changes, modifier genes as well as second somatic mutations during tumorigenesis. As NF1 is expressed in a large variety of tissues, possible modifier genes have variable effects, such as actin cytoskeleton remodeling, cell signaling, intracellular trafficking, ubiquitylation, membrane localization, cell adhesion and neural differentiation [12]. In twin studies, it has been shown that plexiform neurofibromas tended to be less concordant, and it is explained by the two-hit hypothesis as many NF1-related tumors necessitate a second mutation on the wild-type *NF1* allele [13]. Consistent with this view, the major phenotypic difference is plexiform neurofibroma between our cases.

To the best of our knowledge, this is the first case report with detailed clinical findings of NF1 with *NF1*: c.5392C>T mutation. Although *NF1*: c.5392C>T is a nonsense mutation, predicted to cause premature termination and a truncated neurofibromin protein, the clinical course of NF1 in our patients and their family is benign. Even though there is great intra-familial and inter-familial variability in NF1 phenotypes, we can speculate that with the effect of epigenetic and environmental changes and modifier genes, the *NF1*: c.5392C>T mutation did not cause a severe *NF1* phenotype in this family.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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