Journal of Clinical & Experimental Dermatology Research

Zeng et al., J Clin Exp Dermatol Res 2014, 5:2
DOI: 10.4172/2155-9554.1000211

Research Article Open Access

Three Novel Missense Mutations of *NF1* in Neurofibromatosis Type 1 Patient

Kang Zeng, Qi-Guo Zhang, Liu-Ping Liang and Yan-Hua Liang*

Department of Dermatology, Nanfang Hospital, Southern Medical University, Guangzhou, China

Abstract

Neurofibromatosis type 1 is a common neurocutaneous disorder, mostly caused by mutations in the *NF1* gene. To identify the molecular genetic etiology of neurofibromatosis type 1 in two familiar and three sporadic cases of Han Chinese, DNA was isolated from the peripheral blood of eight patients in two NF1 pedigrees, three sporadic cases, and 100 unrelated healthy controls. Mutation screening for coding and exon-intron boundary sequences of *NF1* gene was performed. Three novel missense mutations, c.601T>A in exon 4, c.871G>T in exon 6, and c.1448A>G in exon 10, were identified. These mutations provided new data for the spectrum of *NF1* mutations causing neurofibromatosis type 1.

Keywords: Neurofibromatosis 1; Mutation; Chinese

Introduction

Neurofibromatosis type 1 (NF1; OMIM#162200) is a common neurocutaneous disorder that is characterized by multiple cafe-au-lait, skinfold freckling, Lisch nodules, and neurofibromas. Mutations in the *NF1* gene, which encodes the neurofibromin protein, have been identified as the pathogenic gene of *NF1* [1]. The incidence has been estimated to be 1 in 3500 individuals [2]. The rare manifestations include malignancies, plexiformneurofibromas, opticglioma, learning difficulties, juvenile xanthogranuloma and skeletal ab-normalities [3]. About 50% of all malignant peripheral nerve sheath tumors (MPNSTs) arise as neurofibromatosis type 1 associated lesion [4].

Café-au-lait (CAL) spots occur in more than 90% of NF1 cases, and present as well-circumscribed, light-brown, homogenous patches that range from 1 to 2 mm to greater than 20 cm in diameter, with the majority being under 10 cm [5]. Neurofibromas, another one of the hallmark signs of NF1, can occur on the whole body and vary in shape and size. Generally, the 'cutaneous'or 'dermal' tumors are dome-shaped, soft, fleshy, and skin colored to slightly hyperpigmented, and the 'subcutaneous' tumors are of the firm, nodular variety [6]. Beside skin, neurofibromas can also occur in the gynecologic tract, including the cervix, resulting in cervical stenosis and lower abdominal pain [7].

NF1 gene, mapped on chromosome 17q11.2, spans approximately 280 kb of genomic DNA, contains 60 exons and encodes neurofibromin composed of 2818 amino acid residues [8]. Neurofibromin, as a GTPase activating protein (GAP) for Ras, is widely expressed in many tissues. To date, more than 1,000 different *NF1* mutations have been reported by the Human Gene Mutation Database. Most of these *NF1* mutations lead to truncated protein products.

In this study, we performed germline mutation analysis for *NF1* gene in two Chinese families and three sporadic cases with neurofibromatosis 1.

Materials and Methods

Subjects

Two unrelated NF1 families (eight affected members in total) and three sporadic cases were diagnosed at the Department of Dermatology, Nanfang Hospital, Southern Medical University, Guangzhou, China. Two experienced dermatologists made the diagnosis based on clinical and histopathological findings independently. This study was approved by the ethical committee of Southern Medical University Review Board, with participants giving their informed consent. Peripheral blood samples were collected from all available individuals and 100 unrelated population-match controls.

Mutation analysis

The genetic diagnosis was carried out at the central laboratory of Nanfang Hospital of the Southern Medical University in Guangzhou, China. The peripheral blood samples were collected from all patients of two families and three sporadic cases. DNA was extracted using the E.Z.N.A. ™Blood DNA Kit (Omega, Norcross, GA, Cat. No. D3392). All coding exons and exon-intron boundary sequences of *NF1* gene were amplified per previous reports [9]. Polymerase chain reaction (PCR) was carried out in a 25 uL volume, containing 20 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mMKCl, 3.0 mM $MgCl_2$, 0.01% gelatine, 0.2 mM dNTPs, 10 pmol of each primer, and 0.75 U of Hot Stars Taq (QIAgene, Germany). The PCR programme was set as below: Hot Stars Taq activation at 94°C for 5 min, followed by 40 cycles, each having denaturation at 94°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 45 s, except for the gradient decreasing of the annealing temperature by 0.5°C per cycle from 65°C to 54°C in the first 22 cycles, and final extension at 72°C for 5 min. Direct sequencing was performed with a Big Dye Direct Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed on the ABI 3130 genetic analyzer (Applied Biosystems, U.S.A.). The new variants were then analyzed in 200 normal chromosomes to exclude the possibility of polymorphisms.

Results

Clinical findings

The clinical and genetic findings were summarized in Table

***Corresponding author:** Yan-Hua Liang, Department of Dermatology, Nanfang Hospital, Southern Medical University,1838 North Guangzhou Avenue, Guangzhou, Guangdong 510515, China,Tel: +86-20-61641989; Fax: +86-20-61641989; E-mail: liangdoctor@163.com

Received January 21, 2014; **Accepted** February 18, 2014; **Published** February 25, 2014

Citation: Zeng K, Zhang QG, Liang LP, Liang YH (2014) Three Novel Missense Mutations of *NF1* in Neurofibromatosis Type 1 Patient. J Clin Exp Dermatol Res 5: 211 doi:10.4172/2155-9554.1000211

Patient	Age/ gender	Age at onset, years		Mutation			
		café-au-lait spots	Neurofibromas	Type	Location	Nucleotide change	Protein change
Probandin family 1	3.5/F	At birth	no	Missense	Exon 10	c.1448A>G	D485G
I:2 in family 1	55/F	At birth	5	Missense	Exon 10	c.1448A>G	D485G
II:1 in family 1	31/M	At birth	6	Missense	Exon 10	c.1448A>G	D485G
III:3 in family 1	28/F	At birth	6	Missense	Exon 10	c.1448A>G	D485G
Probandin family 2	29/F	At birth	3	Missense	Exon 4	c.601T>A	F201I
Sporadic case 1	46/M	At birth	25	Missense	Exon 10	c.1448A>G	D485G
Sporadic case 2	40/M	At birth	20	Missense	Exon 10	c.1448A>G	D485G
Sporadic case 3	23/F	At birth	5	Missense	Exon 6	c.871G>T	E291X

Table 1: Summary of clinical features and NF1 mutations.

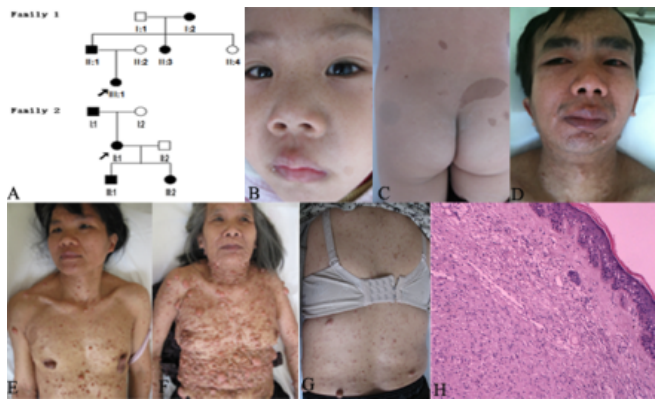

Figure 1: Clinical and histopathological features of two NF1 families.

Family pedigrees **(A)**. Freckling dots on the face **(B)** and café-au-lait spots **(C)** involving intrunk and buttocks of the proband in Family 1. Neurofibromatoses increased in size and number along with age inter-family members **(D-F)** in Family 1 with the same mutation. Only the clinical photos were taken from the proband of Family 2 **(G)**, showing small and scaterred cutaneous tumors or subcutaneous nodules over the body surface. Histological examination of skin biopsies with neurofibromas observed the typical features of neurofibromas and spindle cell proliferations, with a mucinous background and many mast cells **(H)**.

1. Numerous CAL spots and neurofibromas were observed in all patients. The size and number of CAL spots and neurofibromas varied between individuals inter- and intra-families. Brain MRI didn't present abnormalities (data not shown).Surgical intervention was given to sporadic patient 1. Sporadic patient 3 kept 100 mg/day aspirin orally. Other patients didn't accept any surgical intervention or pharmacologic agents.

The proband of family 1 (Figure 1A), a 3.5-year-old girl, present freckling dots on 6 her face (Figure 1B), and numerous CAL spots involving trunk, extremities and buttocks (Figure 1C). Her father (II:1, Figure 1D), aunt (II:3, Figure 1E), and grandmother (I:2, Figure 1F) had more severe neurofibromas and CAL spots. In this family, all patients had CAL spots after birth, and neurofibromas were recognized when aged at 5-6 years, aggrevated at teenage age.

Two males and two females were affected by *NF1* in family 2 (Figure 1A). All patients had CAL spots at birth, and the neurofibromas

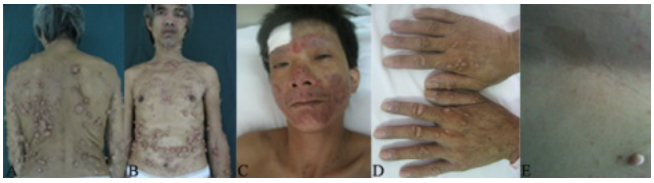

Figure 2: Clinical findgins of two sporadic NF1 patients.

Sporadic patient 1 **(A and B)** present the most severe condition in this study, with numerous tumors from 1cm to 10cm in diameter covering 70 percentages of the body surface.Sporadic patient 2 only show a few café-au-lait spots and skin-colored small nodules **(E)**, with striking lesions including puritic, red papules on the face **(C)** and depigmented firm nodules on both hands **(D)**.

began growing from 3 years old, with disease progression at teenage age (representative Figure 1G). On histological examination of skin biopsies with neurofibromas, the typical features of neurofibromas and spindle cell proliferations was observed, with a mucinous background and many mast cells (Figure 1H).

Sporadic patient 1 was a 46 year-old male, who had onset at the age of 20s. However, the neurofibromas increased rapidly, covering over 70 percentages of the body surface at visit in our clinic in 2012 (Figures 2A and 2B). The populated tumors lead to sleeping difficulties and mobilities. Tumor size varied from 1 cm to 10 cm in diameter.

Sporadic patient 2, a 40-year male, recognized CAL spots and skin-colored nodules at the age of 20 years. On examination, 11 CAL spots sized from 2 cm to 8cm were observed, and most of nodues are subcutaneous and small (Figure 2E). He visited us for eczema, including pleomorphic and symmetrical lesions such as the puritic, red papules on the face (Figure 2C), patches and depigmented firm nodules on both hands (Figure 2D).

Sporadic patient 3, a 23-year female present 2-8 mmdiametered freckling on the face, up to 10 cm diametered CAL spots, and small nodules on her trunck (data now shown due to her disagreement in the public). Chest X-ray indicated a shadow of low density at the right upper lung, suggesting space-occupying tumors (data now shown). Ophthalmoscopic examination found Listch nodules at both sides, without any ophthalmic comfortlessness.

Mutation detection of the *NF1* gene in patients with NF1

Direct sequencing of the entire coding sequence of *NF1* identified three novel *NF1* missense mutations, c.1448A>G (D485G) in family 1 (Figure 3A), c.601T>A (F201I) in family 2 (Figure 3C), c.871G>T (E291X) in sporadic patient 3 (Figure 3E). All patients in the same family carried the same mutations, and sporadic patient 1 and 2 was found to bring the same substitution mutation c.1448A>G (Figure 3A) as that observed in family 1. All mutations were not disclosed in 200 normal chromosomes of the healthy controls.

Discussion

In this study, we report two families and three sporadic cases affected by NF1, according to the revised NF1 criteria [3,10]. Most patients in the study showed typical *NF1* with superficial neurofibromas, large CAL spots and freckling on the axillary region. The proband of family 1 present only CAL spots and freckling, with a positive NF1 family history and more spreading nodules in her family members. Plexiformneurofibromas usually develop from birth to late adolescence (about 18 years of age) and are found in 30-50% of NF1 patients [3]. In a cohort study, 41 children, ranging in age from 1 month to 14 years,

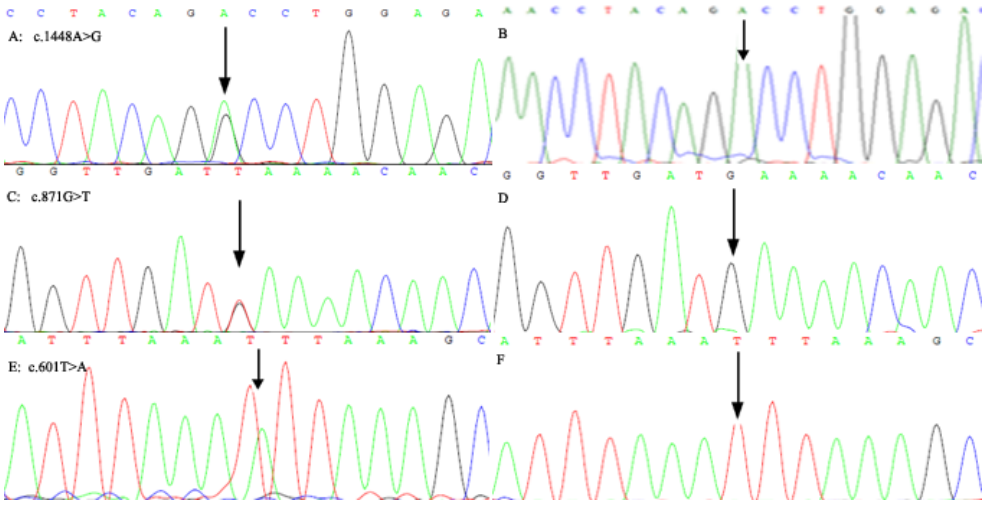

Figure 3: Mutation analysis.

Left three sequence diagrams showed mutation genomic sequences, respectively described as c.1448A>G(D485G) **(A)**, c.601T>A (F201I) **(C)**, and c.871G>T (E291X) **(E)**, compared to the wildtype sequences **(B, D, F)** in unrelated healthy controls. Arrows pointed to the position of nucleiotide acid changes.

had six or more CAL spots at their initial visit, 24 (58.54%) of whom developed neurofibromatosis type 1 eventually. Judged from her age at 3.5 years, and disease progression from the age of 5 or 6 years in her family members, she was speculated to develop neurofibromas in following years if no managements were taken. Recently, several clinical trials showed the effect of imatinib by reduction of sizes and numbers of tumors. After early genetic diagnosis, this girl may administrate imatinib to control the disease development.

The less frequent manifestations of NF1 include malignancies, plexiformneurofibromas, opticglioma, learning difficulties, juvenile xanthogranuloma and skeletal abnormalities. Many NF1 patients have been genotyped but few genotype-phenotype correlations have been identified. In this study, sporadic patient 1 and 2 present the same mutation seen in family 1, c.1448A>G, respectively had mild to severe neurofibromatos. Furthermore, patients with c.1448A>G mutation in family 1 also had mild (proband), moderate (her father and aunt), and severe (her grandmother) disease conditions. Given the same *NF1* mutation, the phenotypic variabilities among inter- and intra-family patients suggested age and other environmental factors may be important modifiers for the disease development.

NF1 genotype-phenotype correlations are difficult to identify because of the variabilities of the NF1 phenotypes, its strong age dependency, the complex clinical features and the heterogeneities of pathogenic *NF1* mutations [11]. At present, no apparent correlation between clinical NF1 phenotype and type of mutations was realized apart from mental retardation and/or learning disabilities, mild facial dysmorphology, and considerable early-onset cutaneous neurofibromas associated with large deletions encompassing the whole *NF1* gene [9,12-14]. A recent retrospective study suggested a potential genotype-phenotype correlation, with *NF1* splice-site mutations being associated with an increased tendency to develop neoplasms, mostly composed of central neural system (CNS) gliomas and malignant peripheral nerve sheath tumors (MPNSTs) [15].

The novel mutation c.601 in the *NF1* gene, reported herein, has been identified in exon 4. Recently, Gabriele et al. reported a novel frameshift insertion mutation in exon 4 (c.654 ins A) of *NF1* gene [16]. Other reports also identified mutations in exon 4, indicating a new hot spots of *NF1* mutations [12,17,18]. Our data suggested that, even if the exon 4, exon 6, and exon 10 of the *NF1* gene are not located in the two functional domains of the protein, Cys-Ser rich domain encoded by exons 11-17 and GAP related domain encoded by exons 21-27a, mutations in these regions may affect the structure and function of NF1 protein, eventual affecting its physiological functions.

Acknowledgements

This work was supported by a grant from National Natural Science Foundation of China (81371724) to Yan-Hua Liang.

References

1. Tong HX, Li M, Zhang Y, Zhu J, Lu WQ (2012) A novel NF1 mutation in a Chinese patient with giant café-au-lait macule in neurofibromatosis type 1 associated with a malignant peripheral nerve sheath tumor and bone abnormality. Genet Mol Res 11: 2972-2978.
2. Rasmussen SA, Friedman JM (2000) NF1 gene and neurofibromatosis 1. Am J Epidemiol 151: 33-40.
3. Gutmann DH, Aylsworth A, Carey JC, Korf B, Marks J, et al. (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278: 51-57.
4. Stricker TP, Henriksen KJ, Tonsgard JH, Montag AG, Krausz TN, et al. (2013) Expression profiling of 519 kinase genes in matched malignant peripheral nerve sheath tumor/plexiformneurofibroma samples is discriminatory and identifies mitotic regulators BUB1B, PBK and NEK2 as overexpressed with transformation. Mod Pathol 26: 930-943.
5. Yang CC, Happle R, Chao SC, Yu-Yun Lee J, Chen W (2008) Giant café-au-lait macule in neurofibromatosis 1: a type 2 segmental manifestation of neurofibromatosis 1? J Am Acad Dermatol 58: 493-497.
6. Boyd KP, Korf BR, Theos A (2009) Neurofibromatosis type 1. J Am Acad Dermatol 61: 1-14.
7. Lastra RR, Bavuso N, Randall TC, Brooks JS, Barroeta JE (2012) Neurofibroma of the cervix presenting as cervical stenosis in a patient with neurofibromatosis type 1: a case report. Int J Gynecol Pathol 31: 192-194.
8. Gabriele AL, Ruggieri M, Patitucci A, Magariello A, Conforti FL, et al. (2011) A novel NF1 gene mutation in an Italian family with neurofibromatosis type 1. Childs Nerv Syst 27: 635-638.

9. Leppig KA, Kaplan P, Viskochil D, Weaver M, Ortenberg J, et al. (1997) Familial neurofibromatosis 1 microdeletions: cosegregation with distinct facial phenotype and early onset of cutaneous neurofibromata. Am J Med Genet 73: 197-204.

10. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, et al. (2007) Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet 44: 81-88.

11. Pasmant E, Vidaud M, Vidaud D, Wolkenstein P (2012) Neurofibromatosis type 1: from genotype to phenotype. J Med Genet 49: 483-489.

12. Kayes LM, Burke W, Riccardi VM, Bennett R, Ehrlich P, et al. (1994) Deletions spanning the neurofibromatosis 1 gene: identification and phenotype of five patients. Am J Hum Genet 54: 424-436.

13. Wu BL, Austin MA, Schneider GH, Boles RG, Korf BR (1995) Deletion of the entire NF1 gene detected by the FISH: four deletion patients associated with severe manifestations. Am J Med Genet 59: 528-535.

14. Wu BL, Boles RG, Yaari H, Weremowicz S, Schneider GH, et al. (1997) Somatic mosaicism for deletion of the entire NF1 gene identified by FISH. Hum Genet 99: 209-213.

15. Alkindy A, Chuzhanova N, Kini U, Cooper DN, Upadhyaya M (2012) Genotype-phenotype associations in neurofibromatosis type 1 (NF1): an increased risk of tumor complications in patients with NF1 splice-site mutations? Hum Genomics 6: 12.

16. Kebudi R, Tuncer S, Upadhyaya M, Peksayar G, Spurlock G, et al. (2008) A novel mutation in the NF1 gene in two siblings with neurofibromatosis type 1 and bilateral optic pathway glioma. Pediatr Blood Cancer 50: 713-715.

17. Griffiths S, Thompson P, Frayling I, Upadhyaya M (2007) Molecular diagnosis of neurofibromatosis type 1: 2 years experience. Fam Cancer 6: 21-34.

18. Jeong SY, Park SJ, Kim HJ (2006) The spectrum of NF1 mutations in Korean patients with neurofibromatosis type 1. J Korean Med Sci 21: 107-112.