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NF1 microduplications: identification of seven nonrelated individuals provides further characterization of the phenotype

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Purpose: Neurofibromatosis, type 1 (NF1) is an autosomal dominant disorder caused by mutations of the neurofibromin 1 (*NF1*) gene at 17q11.2. Approximately 5% of individuals with NF1 have a 1.4-Mb heterozygous 17q11.2 deletion encompassing *NF1*, formed through nonallelic homologous recombination (NAHR) between the low-copy repeats that flank this region. *NF1* microdeletion syndrome is more severe than NF1 caused by gene mutations, with individuals exhibiting facial dysmorphisms, developmental delay (DD), intellectual disability (ID), and excessive neurofibromas. Although NAHR can also cause reciprocal microduplications, reciprocal *NF1* duplications have been previously reported in just one multigenerational family and a second unrelated proband.

Methods: We analyzed the clinical features in seven individuals with *NF1* microduplications, identified among 48,817 probands tested in our laboratory by array-based comparative genomic hybridization.

Results: The only clinical features present in more than one individual were variable DD/ID, facial dysmorphisms, and seizures. No neurofibromas were present. Three sets of parents were tested: one duplication was apparently de novo, one inherited from an affected mother, and one inherited from a clinically normal father.

Conclusion: This is the first report comparing the phenotypes of nonrelated individuals with *NF1* microduplications. This comparison will allow for further definition of this emerging microduplication syndrome.

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Key Words: 17q11.2; developmental delay/intellectual disability (DD/ID); microduplication; neurofibromatosis 1 (NF1); nonallelic homologous recombination (NAHR)

INTRODUCTION

The neurofibromin 1 (*NF1*) gene (OMIM 613113), located on chromosome band 17q11.2, encodes a cytoplasmic protein that regulates several intracellular processes, one of which is the RAS (rat sarcoma)-cyclic adenosine monophosphate pathway. The cells that primarily express neurofibromin are neurons, Schwann cells, oligodendrocytes, and leukocytes. Neurofibromatosis type 1 (NF1, OMIM 162200) is an autosomal dominant disorder caused by various constitutional mutations in *NF1*[1,2] with a frequency in the general population of ~1 in 3,000.[3] The disorder is characterized by neurofibromas, Lisch nodules, and café-au-lait macules. Approximately 5% of individuals with NF1 have a 1.4-Mb heterozygous deletion of 17q11.2 that includes *NF1*.[4] Individuals with *NF1* microdeletion syndrome have a more severe phenotype than those with NF1 due to intragenic mutations, with the microdeletion syndrome characterized by facial dysmorphisms, developmental delay (DD), intellectual disability (ID), and excessive neurofibromas.[5,6]

The *NF1* microdeletions are caused by nonallelic homologous recombination (NAHR) between the low-copy repeats (LCRs) that flank this region.[7] The deletions may be 1.0–1.4 Mb in size depending on the specific LCR mediating the deletion and are classified as types 1–3.[6–11] The most common *NF1* microdeletion, type 1, is a 1.4-Mb deletion mediated by LCRs NF1-repeat (-REP) A and NF1-REP C (**Figure 1**) and is hypothesized to preferentially arise during meiotic NAHR.[8] Type 2 microdeletions have predominantly been seen as a result of mitotic NAHR and are 1.2 Mb in size with breakpoints within *SUZ12* and its pseudogene *SUZ12P* adjacent to NF1-REP C and NF1-REP A, respectively (**Figure 1**).[12–14] The 1.0-Mb type 3 *NF1* microdeletions are the smallest of the three, mediated by LCRs NF1-REP B and NF1-REP C (**Figure 1**).[6,11]

NAHR between LCRs, such as NF1-REPs A, B, and C, lead to both deletion and duplication of the intervening sequence.[15] For many of the recurrent microdeletion syndromes caused by NAHR, reciprocal microduplications have been identified and

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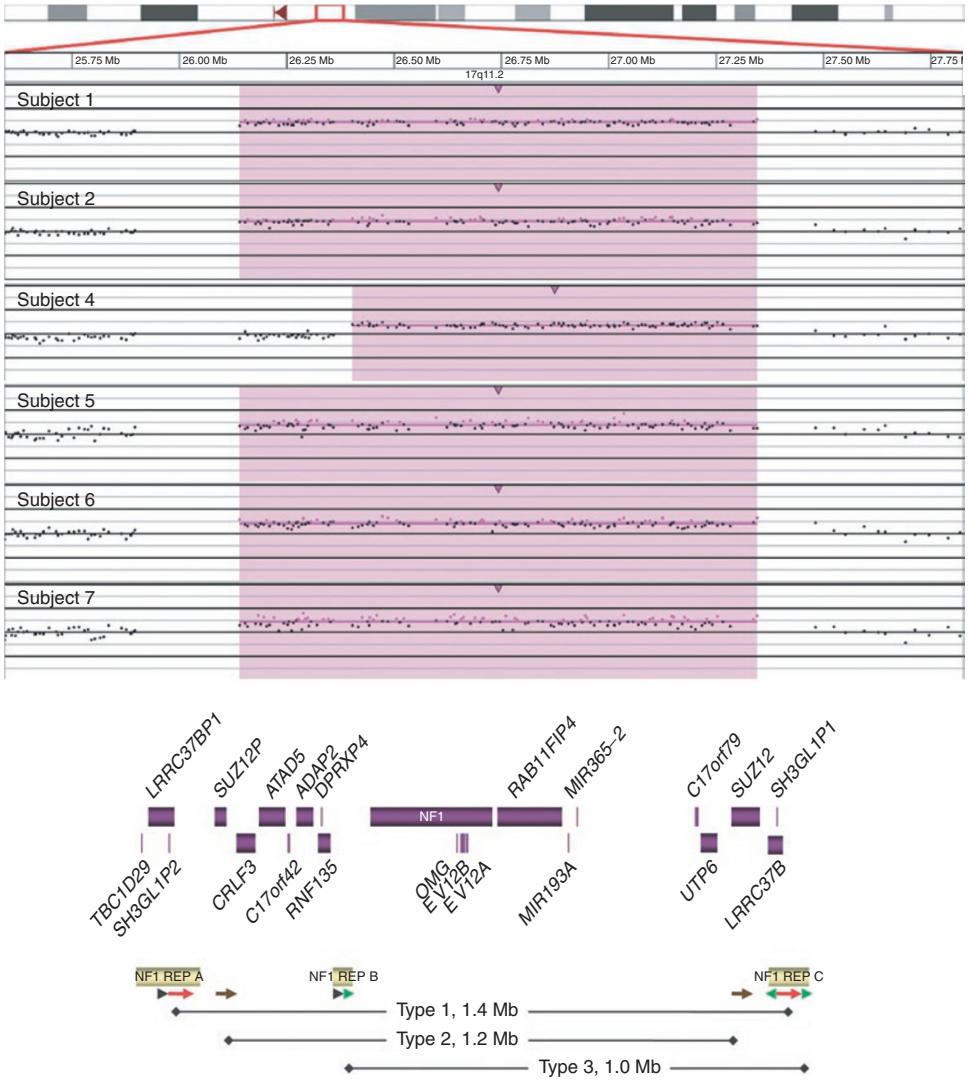

Figure 1 Microduplications encompassing *NF1* detected in the six subjects in this study for whom oligonucleotide microarray analysis was performed. For all microarray plots, probes are arranged on the x-axis according to physical mapping positions, with the most proximal 17q11.2 probes on the left and the most distal 17q11.2 probes on the right. Values along the y-axis represent $\log_2$ ratios of subject:control signal intensities. Genes in the duplication region are shown underneath the plots as purple boxes. The yellow boxes represent the blocks of low-copy repeats in the region, with colored arrows corresponding to areas of homology among the low-copy repeats. Identical colors correspond to homologous regions. The sizes of the three types of *NF1* microdeletions are shown, with nonallelic homologous recombination between the red arrows leading to type 1, brown to type 2, and green to type 3.[11]

characterized. For example, 3q29 microdeletion syndrome, characterized by variable phenotypes that include ID and mild dysmorphic features (OMIM 609425),[16] has a reciprocal microduplication that is also characterized by variable phenotypes (OMIM 611936).[17] Two more well-characterized examples are Williams syndrome (OMIM 194050), due to a microdeletion at 7q11.23, and its reciprocal microduplication syndrome (OMIM 609757);[18,19] and Smith–Magenis syndrome (OMIM 182290), due to microdeletion at 17p11.2, and Potocki–Lupski syndrome (OMIM 610883), caused by the reciprocal microduplication.[20,21] Analysis of the phenotypic consequences of the reciprocal *NF1* microduplications, however, has been reported in detail in just one multigenerational family. Grisart et al.[22] reported seven members of a multigeneration family segregating the 1.4-Mb reciprocal microduplication of the type 1 *NF1* microdeletion. The phenotype of the five affected individuals included DD, mild ID, mild facial dysmorphisms, dental enamel hypoplasia, and early-onset baldness. Two family members carrying the duplication were unaffected. This microduplication has also been reported in one case from a series of 2,513 patients undergoing clinical microarray-based comparative genomic hybridization (aCGH); the individual was referred for DD/ID, failure to thrive, and microcephaly.[23]

Here we report the characterization of seven nonrelated individuals with *NF1* microduplications to help clarify the clinical significance of these copy-number alterations.

MATERIALS AND METHODS

Subject ascertainment

During the period encompassing March 2004 through April 2011, we tested 48,817 probands submitted to Signature Genomic Laboratories for microarray analysis due to physical and intellectual disabilities and/or dysmorphic features. The most common indications for study were ID, DD, or multiple congenital anomalies. Informed consent was obtained to publish clinical information, or clinicians submitted de-identified information for publication.

aCGH

All 17q11.2 duplications were initially identified by aCGH using various microarray platforms. Targeted bacterial artificial chromosome–based microarray analysis was originally performed on DNA from subjects 1, 2, and 3 as previously described.[24] Whole-genome bacterial artificial chromosome–based microarray analysis was originally performed on DNA from subject 4 as previously described.[17] Oligonucleotide-based microarray analysis was originally performed on DNA from subjects 5, 6, and 7 using a custom 12-plex 135K-feature whole-genome oligonucleotide microarray (SignatureChip Oligo Solution v2.0, custom-designed by Signature Genomic Laboratories, Spokane, WA, and manufactured by Roche NimbleGen, Madison, WI) using previously described methods.[25] DNA from subjects 1, 2, and 4 were rerun on the SignatureChip Oligo Solution v2.0 12-plex to refine the size of the duplication. Results were visualized using custom microarray analysis software (Genoglyphix, Signature Genomic Laboratories).

Fluorescence *in situ* hybridization (FISH)

FISH was performed on interphase nuclei using bacterial artificial chromosome clone RP11–353O18 from the *NF1* region on 17q11.2 to visualize the duplications as previously described.[26]

RESULTS

Molecular analysis

We identified seven individuals with *NF1* microduplications (**Figure 1**). All seven microduplications span the entire *NF1* gene; five of the microduplications may represent the reciprocal duplication of the more common 1.4-Mb type 1 or 1.2-Mb type 2 *NF1* microdeletion resulting from NAHR between NF1-REP A and NF1-REP C or its neighboring sequence.[10,13] The microduplication in subject 4 is likely reciprocal to the 1.0-Mb type 3 *NF1* microdeletion, caused by NAHR between NF1-REP B and NF1-REP C;[11] subject 3's microduplication was detected on a targeted bacterial artificial chromosome array that could not distinguish between these duplication types. All seven duplications were visualized by FISH (**Figure 2**). FISH of the smallest 1.0-Mb microduplication in subject 4 showed three signals in only 26/50 (52%) of interphase cells, whereas three signals were seen in at least 70% of interphase cells with the other six duplications. A failure to visualize the duplication in less than 100% of cells is not likely due to mosaicism; with tandem duplications, FISH probes hybridize close to each other, which can make it more difficult to visualize three separate signals, so that fewer cells will show all of the signals as the probes get closer to each other with smaller duplications, such as in subject 4.

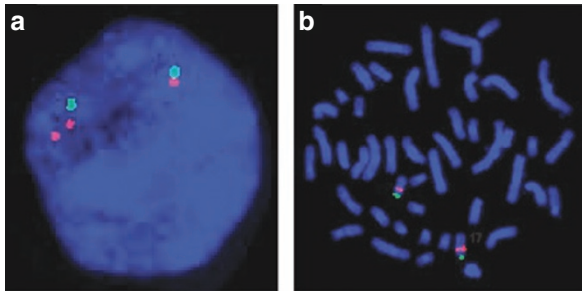

Figure 2 Representative (a) interphase and (b) metaphase fluorescence *in situ* hybridization images showing microduplication of 17q11.2 in subject 7. Bacterial artificial chromosome probe RP11–353O18 from 17q11.2 is labeled in red, and bacterial artificial chromosome probe RP13–640F18 from the 17q subtelomere is labeled in green as a control. On interphase cells, three red signals are present, indicating duplication. Metaphase fluorescence *in situ* hybridization excluded an unbalanced translocation.

For the three subjects with parental follow-up, one microduplication (subject 4) was apparently de novo, one (subject 2) was inherited from a clinically normal father, and one (subject 7) was inherited from a mother who had similar dysmorphic features to those of her son (including flat midface, apparently short palpebral fissures, and short nose with mildly broad and flattened tip, but with a more normal mouth than her son's small mouth), congenital unilateral microphthalmia, and language delay but normal cognition in adulthood. For the remaining subjects, parental samples were unavailable for testing.

In addition to the duplication at 17q11.2, aCGH for subject 1 identified a 15q11.2 deletion between breakpoint (BP) 1 and BP2 proximal to the Prader–Willi/Angelman syndrome critical region. This deletion has been implicated as a risk factor for a variety of neurocognitive disorders including behavioral problems and idiopathic generalized epilepsy.[27–31] It is often inherited from a normal or mildly affected parent and has also been seen in normal control individuals.[27,28,30,31] Additional clinically significant copy-number alterations were not identified in any of the remaining subjects.

Clinical features

Clinical features seen in more than one individual with this microduplication include DD, facial dysmorphisms, variable ID, and seizures. Other major features seen in single cases were microcephaly, macrocephaly, autism, cleft lip and palate, polymicrogyria, and iris coloboma (**Table 1**). Neurofibromas were not noted in any of these individuals.

Case–control comparison

To further evaluate the likelihood of the pathogenicity of *NF1* microduplications, we compared the frequency of these microduplications in our population of individuals undergoing clinical aCGH testing with that in reported control groups. No *NF1* microduplications have been reported in two different series of

Table 1 Summary of clinical features of individuals with *NF1* microduplications in this and previous studies

Subject	1	2	3	4	5	6	7	Grisart et al.[22]	Lu et al.[23]
Age at diagnosis	13 y	4 y	11 y	Newborn	2 m	21 y	3 y		NS
Sex	F	M	M	M	M	M	M	6M, 1F	F
Duplication type	1 or 2	1 or 2	1, 2, or 3	3	1 or 2	1 or 2	1 or 2	1	1 or 2
Inheritance	Unknown	Pat	Unknown	De novo	Unknown	Unknown	Mat	Mat and Pat	NS
Growth	Short stature, microcephaly	Macrocephaly	FTT	NS	NS	Normal	Height >97th percentile Weight 95th percentile OFC 70th percentile	Short stature (2/7), microcephaly (2/7)	FTT, microcephaly
Neuro-development	DD	Severe delays, ID	Global delay	NA	NS	DD (walking at 2 y), nonverbal, ID, likely autism	DD (walking at 28 m); speech delay (words at 26 m)	DD (5/7), speech delay (3/7), ID (2/7)	DD/ID
Neurology	Seizures	Seizures	Seizures	Polymicrogyria	NS	DTRs 3+	Normal MRI	Seizures (1/7)	NS
Dysmorphic features	NS	+, Including high palate and ankyloglossia	+	Bilateral CL/P; left iris coloboma	+	No facial dysmorphisms	+, Including unilateral lacrimal pit	Mild dysmorphic features (2/7)	NS
Sparse hair/ premature balding	NS	—	—	NA	NS	—	—	Premature balding (5/7), sparse facial hair (4/7)	NS
Tooth anomalies	NS	NS	NS	NA	NS	—	Small lower teeth, widely spaced	Dental enamel hypoplasia (5/7)	NS
Other		Pectus excavatum, adducted thumbs, hypospadias, chronic lung disease; twin brother (not tested) with cleft palate deceased at 3 m with sepsis; father not affected	Dizziness; mitral valve prolapse; sib with similar phenotype (not tested)			Small hands and feet; diabetes; hypertension; hypercholesterolemia; GERD	Irregular linear hypopigmented skin lesion; one faint CAL; strong adult body odor; mother has similar facies, unilateral microphthalmia, language delay	1 M, 1 F not affected	

CAL, café-au-lait macule; CL/P, cleft lip and palate; DD, developmental delay; DTR, deep tendon reflex; F, female; FTT, failure to thrive; GERD, gastroesophageal reflux disease; ID, intellectual disability; m, months; M, male; Mat, maternally inherited; MRI, magnetic resonance imaging; NA, not applicable; NS, not specified; OFC, occipitofrontal circumference; Pat, paternally inherited; sib, sibling; y, years; +, present; —, not present.

published control cohorts with a total of 10,355 individuals.[32,33] Despite the absence of this microduplication in control cohorts, there was no significant difference between the control and patient population frequencies (0/10,355 vs. 7/48,817, one-tailed $P = 0.26$, Fisher's exact test), possibly due to the rarity of this microduplication and the need for an even larger control cohort.

DISCUSSION

This is the first report comparing the phenotypes of nonrelated individuals with *NF1* microduplications. Microduplication of 17q11.2 may be associated with a nonspecific phenotype; ID/DD and dysmorphic features were the only clinical features common to a majority of cases in our study and previous studies (**Table 1**).[22,23] The only other features present in more than one nonrelated individual in our cohort and the cases in the literature are short stature, failure to thrive, microcephaly, and seizures (**Table 1**).[22,23] One of our subjects had small teeth, while the family reported by Grisart et al.[22] had dental enamel hypoplasia. Premature balding, present in the previously reported family, was not present in our cohort, which may be due to the relative younger ages of the subjects.

Similar to other genomic disorders that have heterogeneous clinical phenotypes,[34–36] our cohort displays phenotypic variability. In addition, there have been healthy carriers of this microduplication in the family reported by Grisart et al.[22] and in a father in our cohort. This suggests that there may be reduced penetrance or the expressivity may be modified by other genetic and nongenetic factors. Differing sizes of the microduplications could account for some of the variable phenotypes in our study population. One gene in the region between NF1-REP A and NF1-REP B, which is variably included in these microduplications, depending on whether they are type 1/2 or type 3, is ring finger protein 135 (*RNF135*, OMIM 611358). *RNF135* loss-of-function mutations, as well as an NF1-REP A to NF1-REP B deletion including this gene, have been implicated in an overgrowth syndrome where symptoms include tall stature or a large head circumference at least 2 standard deviations (SD) above the mean, dysmorphic features, and variable additional features, including learning disabilities. Inclusion of this gene in the common *NF1* microdeletion is hypothesized to contribute to the taller stature seen in these individuals.[37] It is interesting to hypothesize that if a decrease in function of this gene leads to overgrowth, then possibly an increase in function, through duplication of the region, could lead to short stature and/or microcephaly, such as that seen in our cohort. Individuals with type 1/2 microduplications may be more likely to have short stature and/or microcephaly, although one subject in our cohort with this microduplication had tall stature (subject 7, height +2.9 SD at 4 years) and another had macrocephaly (subject 2). An NF1-REP A to NF1-REP B microduplication has been described in a patient with a clinical diagnosis of Rubinstein–Taybi syndrome and his healthy sister.[38] Growth parameters were not available for this family, although growth retardation is a feature of Rubinstein–Taybi syndrome. The authors hypothesized that if the haploinsufficiency of *RNF135* could contribute to an overgrowth syndrome, then duplication of *RNF135* could contribute to the skeletal anomalies and ID seen in their patient.[38] However, it is also possible that Rubinstein–Taybi syndrome could be unrelated to the microduplication in this family and may be attributable to an unidentified mutation, such as one in *EP300,* which was not sequenced in this proband.

Although *NF1* and *RNF135* are two genes in this microduplication region that are currently associated with human disease, and it is possible that a change in the dosage of one or both of these genes is contributing to the abnormal phenotypes we see in our study, other genes in the region may also contribute. *SUZ12* (also known as *JJAZ1*, OMIM 606245) is critical in embryonic development,[39] and *OMG* (OMIM 164345) is an important inhibitor of neurite overgrowth.[40] It is possible that either of these genes can contribute to phenotypes seen in *NF1* microduplications, but this is yet to be explored in detail.[22]

Variable phenotypes in these individuals may also be due to factors elsewhere in the genome. For example, subject 1 carried another copy-number variant, a BP1-BP2 15q11.2 deletion proximal to the Prader–Willi/Angelman syndrome critical region. It has been hypothesized that this deletion can predispose individuals to a variety of neurocognitive disabilities, including the developmental delay and seizures present in this subject.[27,29,30] This deletion may contribute to the resulting phenotype in this proband along with the duplication of *NF1*, consistent with a "two-hit" model recently proposed to explain phenotypic variability and reduced penetrance with recurrent 16p12.1 microdeletions[41] and hypothesized to hold true for other genomic disorders.[42] In other individuals, the phenotype may be impacted by environmental, genetic, or epigenetic variants that are undetectable by aCGH. In addition, sequencing was not performed to characterize the duplication end points in these individuals, so it is possible that unrecognized complexity is present at the breakpoints that could affect phenotypic expression.

Because the duplications identified in our subjects and the previous study[22] are flanked by LCRs, NAHR may be the causative mechanism for the rearrangements. NAHR can take place between paralogs on the same chromatid (intrachromatid) resulting in only a deletion of the gene, not a duplication. It has been noted that interchromatid NAHRs for certain regions of the genome are rarer than intrachromatid events, possibly explaining the higher presence of deletions as compared with duplications.[43] Another hypothesis regarding the uncommon occurrence of microduplications as compared with microdeletions may be underdiagnosis of microduplications because the phenotypes tend to be more mild, although there are notable exceptions to this paradigm, such as 17p12 duplications containing *PMP22*, which cause the relatively severe Charcot–Marie–Tooth syndrome type 1a, whereas the reciprocal deletion causes the milder hereditary neuropathy with liability to pressure palsies.[44] In our patient population undergoing clinical aCGH testing, we identified 13 individuals with likely LCR-mediated *NF1* microdeletions during this same time period

of identifying 7 microduplications, which is not a statistically significant difference in frequency (two-tailed $P = 0.26$, Fisher's exact test). It is also interesting to note that two of the three subjects for which the mode of inheritance was established had inherited the *NF1* microduplication. Although the numbers are small for statistical analysis, this result is in contrast to *NF1* microdeletions, where the vast majority are de novo in origin, with 47 of 56 cases (3/3 for type 3) being de novo in one study.[6] It can be speculated that the less severe phenotypes associated with microduplication will yield an increased incidence of inheritance or increased frequency in the general population, although the latter was not found.

Here we report the first series of nonrelated individuals with microduplications of the *NF1* microdeletion syndrome region. The presence of LCRs flanking the duplications suggests that these rearrangements were likely caused by NAHR through recombination of the LCRs. The absence of clinical features in our subjects as reported in the previous multigenerational cohort suggests variable expressivity, which may be modified by the presence of additional genetic and/or nongenetic factors. Our results contribute to the emerging picture of *NF1* microduplication syndrome, although additional subjects are needed to fully understand the effects of this microduplication on the phenotype.

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DISCLOSURE

K.J.M., R.A.S., J.A.R., B.S.T., and L.G.S. are employees of Signature Genomic Laboratories, PerkinElmer, Inc. All other authors declare no conflict of interest.

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