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Report

Unequal Meiotic Crossover: A Frequent Cause of *NF1* Microdeletions

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Neurofibromatosis type 1 is a common autosomal dominant disorder caused by mutations of the *NF1* gene on chromosome 17. In only 5%–10% of cases, a microdeletion including the *NF1* gene is found. We analyzed a set of polymorphic dinucleotide-repeat markers flanking the microdeletion on chromosome 17 in a group of seven unrelated families with a de novo *NF1* microdeletion. Six of seven microdeletions were of maternal origin. The breakpoints of the microdeletions of maternal origin were localized in flanking paralogous sequences, called "NF1-REPs." The single deletion of paternal origin was shorter, and no crossover occurred on the paternal chromosome 17 during transmission. Five of the six cases of maternal origin were informative, and all five showed a crossover, between the flanking markers, after maternal transmission. The observed crossovers flanking the *NF1* region suggest that these *NF1* microdeletions result from an unequal crossover in maternal meiosis I, mediated by a misalignment of the flanking NF1-REPs.

Neurofibromatosis type I (NF1 [MIM 162200]) is a common autosomal dominantly inherited disorder with an incidence of ~1/4,000 individuals (Huson et al. 1989). In 60%–70% of the patients, a truncating mutation in the *NF1* gene has been found (Heim et al. 1995). In 5%–10% of the cases, an *NF1* gene microdeletion has been described, indicating that this type of rearrangement must be present in ~1/40,000 to ~1/80,000 individuals (Clementi et al. 1996; Cnossen et al. 1997; Rasmussen et al. 1998). So far, 65 patients carrying *NF1* microdeletions have been reported in the literature (Kayes et al. 1994; Wu et al. 1995; Cnossen et al. 1997; Valero et al. 1997; Upadhyaya et al. 1998; López-Correa et al. 1999*a;* Riva et al. 2000). Only a few of those cases are familial (Leppig et al. 1997; Wu et al. 1997). Most patients with *NF1* microdeletions are characterized by the presence of a more severe phenotype with a variable facial dysmorphy (28/39 patients with clinical description have coarse face, hypertelorism, ptosis, and/or a Noonan-like face), severe learning disabilities or mild mental retardation, early and excessive development of cutaneous neurofibromas, and, possibly, a higher incidence of malignancies (Kayes et al. 1994; Wu et al. 1995, 1999; Tonsgard et al. 1997).

In the majority of cases, the microdeletion (17q11.2) is of maternal origin (Lazaro et al. 1996; Upadhyaya et al. 1998). It is thought that the haploinsufficiency of the *NF1* gene, in combination with contiguous, genes may be responsible for this particular phenotype (Leppig et al. 1996).

Most of the *NF1* microdeletions have a size of ~1.5 Mb, and the deletion breakpoints cluster in a flanking duplicated region (NF1-REPs [López-Correa et al. 1999*a;* Dorschner et al. 2000]). These so-called NF1-REPs are paralogous regions of ~100 kb (fig. 1) in a direct orientation, with ~98% of homology (Dorschner et al. 2000).

We genotyped seven families of patients with a de novo *NF1* deletion, to further analyze the molecular mechanisms underlying these deletions. All patients were sporadic cases, and no NF1 manifestations were observed in any of the parents. Each patient fulfilled the NIH criteria for NF1. The patients were evaluated by use of a standard protocol. Clinical features are described in table 1. The presence and the approximate size of the deletion are determined in all patients by use of FISH probes and polymorphic microsatellites located in the *NF1*-gene region (López-Correa et al. 1999*b*).

Peripheral blood samples were obtained from patients

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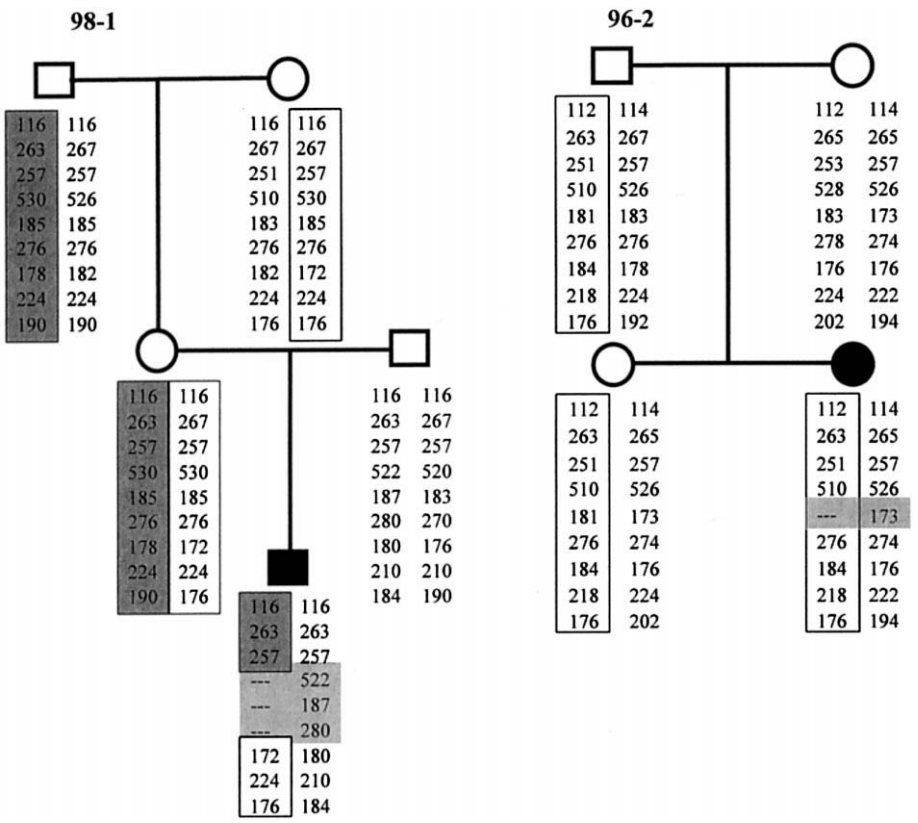

Figure 1 Haplotype reconstruction of nine 17q11.2 markers in family 96-2 (paternal deletion) and family 98-1 (maternal deletion). The hatched box indicates the markers included in the deleted region. The markers analyzed in the patients are, from top to bottom, D17S842, D17S841, D17S1863, 5′NF1-1, IVS38, D17S1800, 3′NF1-4, D17S798, and D17S1880. Note a crossover between the two most telomeric markers and the set of centromeric markers on the maternal chromosome 17 in the sibs of family 96-2.

with NF1 and from family members, after informed consent was obtained. Grandparental blood samples were collected according to the parental origin of the deletion in six families, and blood from one unaffected sib was collected in one family (96-2). We used six markers, located in the sequences flanking the common deletion region, to elucidate the mechanism underlying the deletion; these markers were D17S842 (AFM240xe5), D17S841 (AFM238vb10), and D17S1863 (AFMc003ze1), mapping centromeric to the deletion region, and 3′NF1-4 (primer sequence and alleles of this marker are available, by request, from the authors), D17S1880 (AFMa072zh9), and D17S798 (AFM179xg11), mapping telomeric to the common deleted region. The localization and genetic distances of the markers are derived from resources at the National Center for Biotechnology Information (NCBI), the Whitehead Institute/MIT Center for Genome Research, Mapping, and the Genome Database linkage maps. Markers localized in the deleted region were IVS 38 (in the *NF1* gene), 5′NF1-1 (centromeric to the *NF1* gene) and D17S1800 (telomeric to the *NF1* gene; fig. 1). The microsatellite markers were analyzed by PCR, as described elsewhere (López-Correa et al. 1999*b*). The results are represented in figures 1 and 2. All patients had deletions confirmed by FISH and microsatellite analysis. Six patients had microdeletions of maternal origin, as demonstrated by at least two intragenic markers, and their deletion was flanked by the NF1-REPs (fig. 1). One patient (family 96-2) had a shorter deletion of paternal origin, which included only the *NF1* gene and the three genes embedded in intron 27b (*EVI2A, EVI2B,* and *OMGP* [López-Correa et al. 1999*b*]).

In the group of six patients with deletions of maternal origin, a crossover between flanking centromeric and telomeric markers was observed in five informative cases. In the sixth case, the markers were not informative (X173). Thus, all five informative patients carrying a maternally derived deletion showed a crossover between markers flanking the deletion region. In the patient carrying the smaller deletion of paternal origin, there is no evidence of an interchromosomal recombination in the deletion region on the paternal chromosome. Both sibs inherited the same set of paternal markers flanking the deleted region (fig. 1).

The genetic distance between markers D17S841/

Table 1

Clinical Features in Patients with an *NF1* Microdeletion

	Family						
Feature	96-3	96 - 2[a]	96-1	97-1	98-1	99-1	X173
Age (years)	5	14	27	19	13	23	7
Sex	F	F	F	F	M	M	F
Familial history	−	−	−	−	−	−	−
Parental origin	M	P	M	M	M	M	M
CLS	+	+	+	+	+	+	+
Neurofibromas	+	−	+++	+	−	+++	−
Plexiform neurofibroma	−	−	−	+[b]	+[c]	−	−
Macrocephaly	+	−	+	−	−	−	−
Facial dysmorphism	+	−	+	+	+	+	+
Madelung deformity	−	−	+	+	−	−	−
Scoliosis	−	−	+	−	−	−	−
Pectus excavatum/carinatum	−	−	+	−	+	+	−
Overgrowth	+	−	+	+	+	+	−
Learning difficulties	NA	−	+	+	+	+	+
Other		Epilepsy; unilateral deafness	MPNST				Hypertrichosis

Note.—Dysmorphic features observed in the reported patients are coarse face, hypertelorism, and ptosis. Note that the oldest patient developed a malignant peripheral nerve-sheath tumor (MPNST) and that two of the patients have a large plexiform neurofibroma. NA = not ascertained. A minus sign (−) denotes presence, and a plus sign (+) denotes presence. No. of plus signs indicates level of severity.

[a] Patient with a paternal deletion.

[b] Facial.

[c] Leg.

D17S842 and D17S1880/D17S798 has been estimated as ~4 cM (NCBI, the Whitehead Institute/MIT Center for Genome Research, and the Genome Database linkage–radiation hybrids maps). The expected number of meiotic recombinations in six individuals would be ~0.24. The probability of detecting by chance five recombinations in six informative meioses is $<5.8 \times 10^{-7}$ (binomial distribution; this assumes interference). This finding is highly suggestive of an unequal crossover in maternal meiosis I, at the breakpoint of the deletion and mediated by the NF1-REPs, as being the mechanism leading to the deletion.

It has been observed that large DNA deletions resulting in the loss of 1–5 Mb occur with a higher frequency in specific regions of the human genome. The breakpoints of those microdeletions appear to occur at hot-spot regions. Low-copy repeats (REPs, duplicons, or paralogous sequences) have been shown to flank these regions, facilitating the presence of homologous recombination. It has been proposed that pericentromeric regions are particularly prone to this kind of microdeletion. Some of the microdeletion syndromes showing flanking repeats are Prader-Willi/Angelman syndrome on chromosome 15q11.2 (Amos-Landgraf et al. 1999), velo-cardio-facial syndrome and DiGeorge syndrome on 22q11 (Edelmann et al. 1999), Williams-Beuren syndrome on 7q11.2 (Perez Jurado et al. 1998), and hereditary neuropathy with liability to pressure palsies (HNPP [Lopes et al. 1996]) and Smith-Magenis syndrome (SMS; Chen et al. 1997) on chromosome 17p11.2.

Different reports have been published recently concerning the pathogenesis of these deletions, most specifically regarding the occurrence of inter- versus intrachromosomal rearrangements between the REPs. Concerning the microdeletions located at 15q11.2, 22q11.2, and 7q11.2, no consistent sex-specific occurrence of inter- or intrachromosomal rearrangements have been detected (Carrozzo et al. 1997; Baumer et al. 1998; Robinson et al. 1998).

The molecular rearrangement resulting in the deletion (in the case of HNPP) and duplication (in the case of Charcot-Marie-Tooth disease type A1) of the 1.5-Mb region at 17p11.2 has been proposed to occur by two different and sex-dependent mechanisms. Rearrangements of paternal origin, which are mostly duplications, are generated by interchromosomal recombination, whereas deletions and duplications of maternal origin result from an intrachromosomal rearrangement (Lopes et al. 1997, 1998).

In contrast, in this study, the maternal *NF1* deletions occurred by an interchromosomal recombination in all five informative cases, whereas the paternal deletion occurred by a different mechanism. The observed maternal deletions probably occur by misalignment of the flanking repetitive elements in homologous chromosomes at meiosis I (interchromosomal recombination). The paternal deletion showed a different size and therefore was not

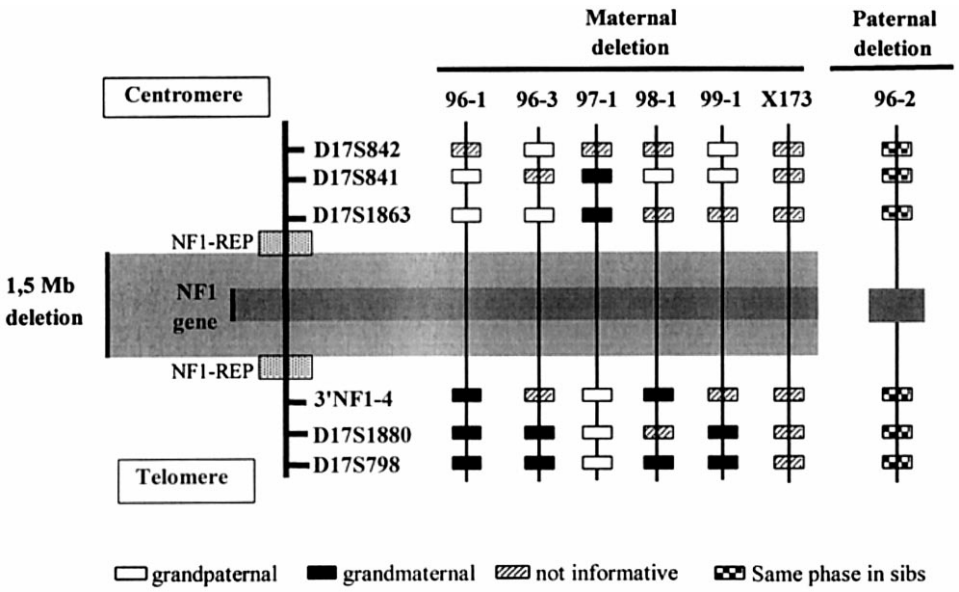

Figure 2 Six markers located at the flanking region of the deletion. The light-gray shadow indicates the large deletion region, of 1.5 Mb; the dark-gray shadow indicates the shorter deletion, of ~300 kb (only the *NF1* gene), in 96-2. In patient X173, markers were not informative. Note that four of five patients have the centromeric region of grandpaternal origin and the telomeric region of grandmaternal origin; in 97-1 the opposite phenomenon is found.

mediated by a recombination between the flanking NF1-REPs. This paternal deletion might have originated by a different mechanism. Nevertheless, one patient with a large paternal deletion of ~1.5 Mb flanked by NF1-REPS has been described elsewhere (Dorschner et al 2000). It is not known whether this large paternal microdeletion is mediated by unequal crossover between NF1-REPs, and it would be worthwhile to test this hypothesis.

The presence of an interchromosomal recombination theoretically predicts the formation of a duplication product on the other homologous chromosome involved in the unequal crossover. Zygotes carrying such hypothetical duplication are likely to be formed at the same frequency as are zygotes with deletions. So far, no duplications involving the critical region 17q11.2 have been described. Whether the duplication product is viable and gives rise to a recognizable phenotype is still unknown.

This reciprocal phenomenon has been described at the 17p11.2 region. Patients with SMS have a common contiguous gene–deletion syndrome in 17p11.2 (Chen et al. 1997). Recently, a group of six patients with a mild phenotype has been reported with duplications of the SMS deletion region; those duplications are preferentially of paternal origin and mostly arise from an interchromosomal recombination event. The duplication is probably the reciprocal recombination product of the SMS deletion and represents a paradigm of the homologous recombination mechanism (Potocki et al. 2000).

In our study, six of seven microdeletions were of maternal origin, with apparently the same deletion size. Five of these six arose by an unequal crossover, during meiosis I, as a result of a homologous recombination between NF1-REPs flanking the deletion region. A sex-dependent occurrence of interchromosomal rearrangements in *NF1* is hypothesized. A larger series of patients is needed for confirmation of this observation and in order to study the mechanism in the much rarer microdeletions of paternal origin, especially those with the common 1.5-Mb microdeletion region.

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Electronic-Database Information

Accession number and URLs for data in this article are as follows:

Genome Database, The, http://gdbwww.gdb.org/
NCBI, Human Genome Resources, http://www.ncbi.nlm.nih.gov/genome/guide/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim/ (for NF1 [MIM 162200])
Whitehead Institute/MIT Center for Genome Research, Mapping, http://www-seq.wi.mit.edu/mapping.html

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