Clinical variability of type 1 neurofibromatosis: is there a neurofibromatosis-Noonan syndrome?

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Abstract

Detailed clinical, ophthalmological, and molecular studies were performed on a multigeneration family in which there were many subjects with type 1 neurofibromatosis, a common autosomal dominant disorder. Affected family members displayed a wide range of clinical findings including, in two subjects, features seen in Noonan syndrome (triangular facies, downward slanting palpebral fissures, micrognathia, short stature, and learning disability). Subjects have been described previously whose features have overlapped with neurofibromatosis and Noonan syndrome, and it has been suggested that these persons might represent a separate condition. DNA haplotype analysis showed linkage of the neurofibromatosis phenotype seen in this family to the proximal long arm of chromosome 17 in the region where the type 1 neurofibromatosis gene has been mapped. These results imply that the Noonan phenotype seen in some patients with type 1 neurofibromatosis might be the result of variable or variant expression of the neurofibromatosis gene on chromosome 17. The possible role of non-specific factors, such as fetal hypotonia, in producing the neurofibromatosis-Noonan phenotype needs further investigation. The availability of closely linked and intragenic molecular markers for neurofibromatosis could potentially be useful in the diagnosis and characterisation of patients and families with atypical forms of neurofibromatosis.

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both NF-1 and Noonan syndrome.⁵⁻¹⁰ This has led to the designation of the neurofibromatosis-Noonan syndrome. It is still not clear as to whether this syndrome is indeed an independent genetic entity or instead represents variable expression of either NF-1 or Noonan syndrome.

One approach to answering the question of genetic heterogeneity versus variable expressivity in NF-1 is to examine the range of clinical expression in a large family in which the gene for NF-1 is segregating. We have ascertained a family spanning three generations in which multiple members are affected with NF-1. Clinical variability, including the finding of some features of Noonan syndrome in two subjects, is discussed along with results of molecular linkage analysis using chromosome 17 DNA probes.

Methods

The family, whose pedigree is shown in fig 1, was identified through the Neurofibromatosis Clinic of the Department of Medical Genetics, Children's National Medical Center. Of the 15 family members examined, eight were felt to meet clinical diagnostic criteria for NF-1 as defined by the 1987 NIH Consensus Conference.¹¹ After obtaining informed consent, detailed medical histories were obtained and physical examinations were carried out on each family member. Slit lamp examination was performed by an ophthalmologist familiar with the ocular manifestations of NF-1. Finally, samples of peripheral blood were obtained and leucocyte DNA was extracted by methods described previously.¹² An RFLP haplotype was generated using the restriction enzyme-probe combinations shown in table 1. These DNA markers have been shown to flank the NF-1 locus on the proximal long arm of chromosome 17.²

Type 1 neurofibromatosis (NF-1, von Recklinghausen NF) is a genetically determined disorder of neural crest derived tissues. Among genetic conditions, NF-1 is relatively common with an incidence of 1 in 3000 live births. Inheritance is autosomal dominant with 50% of cases representing new mutations. By the use of molecular linkage analysis in multigenerational families, the gene for NF-1 has been mapped to the proximal long arm of chromosome 17.¹² Family studies have also shown that penetrance of the NF-1 gene (the percentage of those who inherit the gene who show any clinical manifestations) is high, approaching 100%.³ The hallmark of NF-1, however, is its wide variability of phenotypic expression even within families.^{3,4}

A number of subjects have been described with a condition which displays features of

Figure 1 Family pedigree indicating subjects who meet clinical criteria for the diagnosis of type 1 neurofibromatosis.

Table 1 Chromosome 17 haplotype analysis.

Locus	Probe	Restriction enzyme
D17S58	EW 301	TaqI/BglII
D17Z1	P3-6	EcoRI
D17S33	HHH 202	RsaI
D17S116	VAW 211	MspI
CRYB1	B8-2	MspI
CRYB1	B11-A	PvuII
D17S117	VAW 212	PstI
D17S120	VAW 215	TaqI/HindIII
D17S55	EW 204	BglII
D17S57	EW 206	MspI
D17S73	EW 207	HindIII/BglII
D17S115	VAW 210	MspI
D17S119	VAW 214	MspI
D17S118	VAW 213	MspI
D17S54	EW 203	TaqI

Results

CLINICAL FINDINGS

The clinical manifestations noted on examination of the various family members are shown in table 2. The patriarch of the family (I.1, fig 2) was 69 years of age and, overall, was the most severely affected. On physical examination he had multiple café au lait spots, cutaneous and plexiform neurofibromas, and axillary freckling. In addition, he had macrocephaly, a triangular face, short stature, excess nuchal skin, ptosis, downward slanting palpebral fissures, prominent nasolabial folds, scoliosis, decreased muscle tone, and a history of learning disability. There was no physical evidence of cardiovascular disease. Among his children, the oldest son (II.1) died at the age of 34 from metastatic complications of a soft tissue sarcoma. Family photographs show cutaneous neurofibromas and café au lait spots suggesting the diagnosis of NF-1. The other second generation family members affected with NF-1 (II.4, II.5, and II.6) had on examination multiple café au lait spots, neurofibromas, and axillary freckling, but none had the Noonan phenotype, any neuromuscular abnormalities, nor a history of learning disability. Interestingly, subject II.6 was the only family member noted to have Lisch nodules on slit lamp examination. This finding has been

reported by other groups to be present in most NF-1 patients after infancy.

Of the three affected children, two (III.4 and III.5) had manifestations of NF-1 and facial features similar to their affected mother. The youngest child (III.7), however, had a phenotype almost identical to that of his grandfather with triangular facies, prominent forehead, low set ears, and downward slanting palpebral fissures. Also like his grandfather, he showed significant muscular hypotonia and developmental delay (fig 3). There was no evidence of a cardiovascular abnormality.

LINKAGE ANALYSIS

The results of the chromosome 17 DNA haplotype analysis are shown in fig 4. In the first generation, eight DNA markers were found to be fully informative. In each case, the affected subjects in generation II inherited from their father the intact chromosome 17 region containing the NF-1 locus. The unaffected son inherited the alternative chromosome 17 segment from his father.

In the second generation, four markers were found to be fully informative, and, again, the grandpaternally derived chromosome 17 region containing the NF-1 mutation was inherited by the children who showed clinical features of NF-1, while the unaffected child inherited an alternative segment. In subjects III.4 and III.5, two meiotic crossover events occurred in regions surrounding but not including the NF-1 locus. Multipoint linkage analysis indicated relative odds of 63:1 in favour of linkage of the NF-1 phenotype in this family to DNA sequences from the proximal long arm of chromosome 17. This verifies, at the molecular level, the clinical diagnosis of NF-1.

Discussion

This study confirms the variability of clinical expression of NF-1. Even within families (where presumably the same mutation is

Table 2 Clinical manifestations.

	I.1	II.1	II.4	II.5	II.6	III.4	III.5	III.7
Age (y)	69	NA*	35	27	30	6	4	1
Height (cm)	153	NA	151	148	160	104	102	73
Height (%)	<5	NA	<5	<5	<5	<5	40	<5
Weight (kg)	49	NA	61	61	64	18	17	8
Weight (%)	<5	NA	60	60	30	25	40	<5
Head circumference (cm)	58	NA	55	56	58	53	52	48
Head circumference (%)	95	NA	50	75	95	95	75	75
Café au lait spots	+	+	+	+	+	+	+	+
Cutaneous neurofibromas	+	+	+	+	+	+	+	+
Plexiform neurofibromas	+	+	+	+	+	+	+	+
Axillary freckling	+	NA	+	+	+	+	+	+
Lisch nodules	+	NA	+	+	+	+	+	+
Triangular facies	+	+	+	+	+	+	+	+
Ptosis	+	+	+	+	+	+	+	+
Slanted palpebral fissures	+	+	+	+	+	+	+	+
Broad neck	+	NA	+	+	+	+	+	+
Scoliosis	+	NA	+	+	+	+	+	+
Malignancy	+	+	+	+	+	+	+	+
Hearing loss	+	+	+	+	+	+	+	+
External ear anomaly	+	NA	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	+	+
Developmental delay	+	+	+	+	+	+	+	+
Learning disability	+	+	+	+	+	+	+	NA

NA = not available (dead).

<5 = between -2 and -3 SD of mean.

Figure 2 Subject I.1. Multiple neurofibromas and café au lait spots are visible along with prominence of the forehead, triangular facies, prominent nasolabial folds, and scoliosis.

Figure 3 Subject III.7 (grandson of I.1). Triangular facies, prominent forehead, café au lait spots, and muscular hypotonia are evident.

segregating), affected subjects can have markedly different phenotypes. Two subjects in our family show craniofacial features of Noonan syndrome along with muscular hypotonia and developmental delay. Neither of them has any

evidence of cardiovascular disease nor pectus deformity of the chest. Although macrocephaly and prominence of the forehead were seen in many of the other family members, none showed a Noonan phenotype, evidence of neuromuscular abnormality, or learning disability.

Craniofacial dysmorphism has been appreciated in NF-1 for many years. Some patients are said to have a characteristic facial appearance with prominence of the forehead, telecanthus, or ocular hypertelorism with a broad nasal tip.^{13,14} As noted previously, many authors have reported patients with typical features of NF-1 (café au lait spots or neurofibromas) who also have features of Noonan syndrome. Findings seen in these patients included ptosis, downward slanting palpebral fissures, short neck with low posterior hair line, abnormally shaped or set ears, short stature, and congenital heart disease. Significantly, hypotonia and mental retardation/developmental delay have been noted in many patients with the neurofibromatosis-Noonan phenotype.⁸

Four possibilities have been suggested to explain the findings in these patients.⁷ The first, coincidental occurrence of NF-1 and Noonan syndrome, seems unlikely because of the frequency with which the neurofibromatosis-Noonan phenotype has been reported.⁷ In considering the possibility that the neurofibromatosis-Noonan phenotype reflects biological variability of Noonan syndrome, it should be noted that while many subjects with Noonan syndrome can have café au lait spots, lentigines, or other pigmentary changes, other major manifestations of NF-1 (that is, neurofibromas and optic nerve gliomas) are rare or not seen at all. The two most likely explanations, therefore, are that either the NF-Noonan phenotype reflects variable expressivity of the gene for NF-1 or that the neurofibromatosis-Noonan syndrome is a distinct disorder.

Meinecke¹⁵ reported a 5 year old boy with the neurofibromatosis-Noonan phenotype whose father had typical NF-1 without Noonan-like features. He argued that the NF-Noonan phenotype represented variable or variant expression of the NF-1 gene and may not be a separate genetic entity. This view is supported by the findings in our study. Molecular linkage analysis in this family shows cosegregation of chromosome 17 markers and the NF-1 phenotype. These findings, along with the presence of typical physical features of NF-1, make it likely that the Noonan phenotype seen in two of our patients is the result of variable expression of the NF-1 gene.

IS THERE A NEUROFIBROMATOSIS-NOONAN SYNDROME?

Kaplan and Rosenblatt¹⁴ suggested that some patients with NF-1 had a facial appearance which resembled the Noonan phenotype. Allanson *et al*⁸ suggested that a Noonan-like facial appearance may be produced by a number of conditions which have in common in utero weakness of facial muscles, and thus

Figure 4 Chromosome 17 DNA haplotype analysis. Informative probes are indicated on the left. The corresponding alleles and resulting haplotype are shown for each family member. The chromosome containing the NF-1 allele is indicated by shading. Note the area of meiotic recombination in III.4 and III.5 which surrounds but does not include the NF-1 locus.

may represent the end result of a non-specific insult during fetal development. Hypotonia and other neurodevelopmental abnormalities have been identified in a significant number of children with NF-1.^{3,16} Preliminary data based on neurobehavioural examination of young children with NF-1 show that children with significant muscular hypotonia are at increased risk for developmental disability and mental retardation.¹⁶

Many of the features of Noonan syndrome seen in patients with NF-1 may be caused by dysgenesis or other developmental alterations of the central nervous system resulting in muscular hypotonia. In the presence of hypotonia, development of craniofacial structures will be altered leading to hypoplasia of the midface and micrognathia. When coupled with craniofacial changes known to be common in NF-1 (prominent forehead, ptosis, ocular hypertelorism, and broad nasal tip), it is easy to see how the resulting facial phenotype could suggest Noonan syndrome.

The answer to the question of whether there really is a neurofibromatosis-Noonan syndrome will depend on the further characterisation at the molecular level of the defect in NF-1, and especially in those patients with a Noonan phenotype. Preliminary investigation suggests that the NF-1 gene on chromosome

17 is quite large.^{17,18} This would account for the frequent occurrence of NF-1 and the high percentage of patients with new mutations. A large gene size could also potentially explain variability of clinical expression. In a fashion similar to disorders of the type I collagen¹⁹ and dystrophin²⁰ genes, the resulting clinical phenotype may vary with the type of alteration (deletion versus single base change) and its location within the NF-1 gene. Finally, since the phenotype in NF-1 can vary considerably even within families, the potential role of other genetic or non-genetic factors on expression of the mutation in the NF-1 gene needs to be considered.

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