LETTER TO JMG

Mental retardation and cardiovascular malformations in *NF1* microdeleted patients point to candidate genes in 17q11.2

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Neurofibromatosis type 1 (*NF1* [MIM 162200]) is a common autosomal dominant disorder that affects 1/3500 individuals and is caused by deletion or point mutations of *NF1*, a tumour suppressor gene mapping to 17q11.2. Its main features include café au lait spots, axillary and inguinal freckling, iris Lisch nodules, neurofibromas, and an increased risk of benign and malignant tumours, particularly optic glioma, neurofibrosarcoma, malignant peripheral nerve sheath tumours (MPNSTs),[1] and childhood myeloid leukaemia.[2]

Over 70% of *NF1* germline mutations cause truncation or loss of the encoded protein.

Approximately 5–20% of all *NF1* patients carry a heterozygous deletion of usually 1.5 Mb involving the *NF1* gene and contiguous genes lying in its flanking regions,[3 4] which is caused by unequal homologous recombination of *NF1* repeats (REPs).[5] Known as the ''*NF1* microdeletion syndrome,'' this condition is often characterised by a more severe phenotype than is observed in the general *NF1* group. In particular, *NF1* microdeleted patients often show variable facial dysmorphisms, mental retardation, developmental delay, and an excessive number of neurofibromas for age.[3 6–12] The severe phenotype of microdeleted patients may be explained by variations in the expression of the genes involved in the rearrangement, which may be caused by different mechanisms, such as gene interruptions, position effects, and decreased gene dosages.

Although *NF1* microdeleted patients generally have different characteristics from those of classic *NF1* patients, it remains difficult to foresee the presence of the deletion at an individual level on the basis of clinical observations. Various studies have reported the clinical characterisation of *NF1* deleted patients and the precise extent of the deletion has been characterised in a subset.[3–5, 13 14] However, no study comparing the incidence of specific clinical signs in *NF1* deleted and classical *NF1* patients has yet been published. The only published comparative study concerned a single clinical sign (the development of an MPNST), for which a correlation between *NF1* microdeletion and a high risk for this tumour was observed.[1]

Our aims in the present study were, first, to verify whether the incidence of specific clinical signs is different in *NF1* microdeleted and general *NF1* patients; and second, to indicate possible correlations between the onset of distinct clinical features and the haploinsufficiency of specific genes involved in the deletions. We considered the extra-*NF1* clinical signs shown by a sample of 92 microdeleted patients (evaluated in this study or described in published reports), and estimated their incidence in comparison with the *NF1* patient group as a whole.

Key points

- *NF1* microdeletion syndrome is determined by haploinsufficiency of the *NF1* gene and its flanking regions; *NF1* microdeleted patients show a more severe phenotype than observed in classical *NF1* patients.
- The aim of this study was to verify the presence of specific clinical signs of *NF1* microdeletion, by combining clinical and genetic evidence from 92 deleted patients, 14 newly characterised and 78 already published.
- Statistical analysis, done by comparing the frequency of 10 clinical signs between NF1 microdeleted patients and the whole NF1 population, showed that the most common extra-*NF1* clinical signs in microdeleted patients were learning disability, cardiovascular malformations, and dysmorphisms.
- Using bioinformatic tools, the deletion gene content of 44 genetically and clinically characterised patients was established. It is proposed that haploinsufficiency of *OMG* and/or *CDK5R1* genes may be implicated in learning disability. In relation to cardiovascular malformations, only *JJAZ1* and *CENTA2* can be considered as plausible candidate genes.
- When present in an *NF1* patient, dysmorphisms, cardiac anomalies, and learning disability are signs indicating *NF1* microdeletion.

METHODS

Patients

In order to generate a database that was as comprehensive as possible, we data-mined the NCBI Entrez Pubmed[15] and Med Miner repository[16] and retrieved all the individually reported cases of patients affected by the *NF1* microdeletion syndrome whose clinical phenotype was also described.

Signs included among the diagnostic criteria for *NF1* were excluded (with the exception of plexiform neurofibroma), as were minor sporadically present signs for which no incidence figures were available.

Abbreviations: DSM-IV, *Diagnostic and Statistical Manual of Mental Diseases*, fourth edition; FISH, fluorescent in situ hybridisation; MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1

This selection led to a total of 21 papers describing individually reported cases for a total of 78 patients. We excluded seven well characterised patients carrying mosaic deletions from both published reports and the newly characterised cohort.

The references of the extracted articles are 3–14 and 17–25.

Among the 78 patients described in published reports, seven were familial microdeleted patients and in two cases the parent showed a mosaic condition. The remaining apparently sporadic patients can be considered founder deletion carriers, although we cannot exclude low grade or tissue specific mosaicism in the asymptomatic parents. Conversely the 14 new *NF1* deleted patients were recruited by means of loss of heterozygosity (LOH) studies and characterised by FISH (fluorescent in situ hybridisation) analysis. Extension of FISH to the patients' parents contributing the deletion allowed us to identify a mosaic deletion in parents of cases 65 and 94, and to exclude low grade mosaicism in the remaining cases.

Both the newly described patients and those described in the published reports fulfilled the NIH diagnostic criteria. We classified microdeleted patients as being affected by mental retardation only in those cases where intelligence quotient (IQ) was reported or where an explicit statement of mild to moderate to severe mental retardation was declared by the investigators. When IQ was known, patients were classified as having mild (IQ = 50–70) or moderate to severe mental retardation (IQ<50) according to the DSM-IV criteria.

With respect to cardiovascular malformations, we referred to large surveys of NF1 patients investigated by conventional methods for the diagnosis of cardiovascular malformations (auscultation, radiography, electrocardiography, echocardiography), as these methods were applied to the NF1 microdeleted patients described.

The data on the percentages of each clinical sign in classic *NF1* patients were drawn from published reviews.[2 26–29] These reference percentages may also include patients carrying the *NF1* deletion, the relative percentage of which is estimated to be 5–20%.[4]

Database construction

The published reports and the recruited patients allowed us to build a common data structure in which to tabulate the information. For each patient, we added any new clinical sign that had not been included previously, thus obtaining a relational database with 103 fields.

The presence of a specific sign was attributed only when it was explicitly reported and formalised in binary fashion (that is, present or not present). When a field could not be completed because of lack of information or an ambiguous interpretation, it was defined as null and was not counted.

Statistical analysis

The analysed features were studied as discrete variables. As the clinical data concerning each feature were not available for all the patients, the total number of patients for whom the data were applicable is given in each data entry. The frequency of each sign was calculated as the ratio between the evaluable patients and the affected patients, and the two patient populations were statistically compared using the χ^2 test in 2×2 tables with one degree of freedom and a 0.1% error probability (confidence range 99.9%).

Electronic database information

The proximal and distal boundaries of each kind of deletion were defined, and the deletion specific gene content was identified, using the integrated maps available on NCBI (http://www.ncbi.nlm.nih.gov/genome/seq/) and UCSC (http://genome.cse.ucsc.edu/).

Information concerning the expression patterns, the presence of specific functional domains in the protein products and their putative cellular role, and the existence of hortologous genes in model organisms was obtained from the following internet pages: LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/), *Human unidentified gene-encoded large proteins analyzed by Kazusa cDNA Project* (HUGE) (http://www.kazusa.or.jp/huge/), SAGE (http://www.ncbi.nlm.nih.gov/SAGE/), BODYMAP (http://bodymap.ims.u-tokyo.ac.jp/), NCI60 cancer microarray project (http://genome-www.stanford.edu/nci60/) and, for the homologous murine sequences, mouse genome informatics (http://www.informatics.jax.org/).

The sequence homologies identified in *Mus Musculus* by means of a BLAST search were confirmed using an analysis in MGI and *e! The Mouse Genome Sequencing Consortium Mouse Genome Browser*, in which the hortologous regions have been mapped. The rat data were drawn from *Rat genome data* (http://www.informatics.jax.org/rat/).

The functional domain analysis for the proteins encoded by the studied genes was undertaken using the tools and links in the expert protein analysis system (EXPASY) molecular biology server (http://www.expasy.ch/).

RESULTS

Clinical evaluation of NF1 patients

In order to verify the presence and incidence of specific clinical signs in *NF1* microdeleted patients in comparison with those with classic neurofibromatosis 1, we considered a sample of 92 microdeleted patients (14 novel clinical descriptions and 78 from published reports).

Table 1 shows the clinical signs and symptoms on which it was possible to make the comparative analysis. Among the clinical signs found to be more frequent in *NF1* microdeleted patients than in the classic *NF1* patients, there was a significant difference ($p<0.001$, that is, 99.9%) in the incidence of dysmorphic features, hypertelorism, mental retardation, and cardiovascular malformations (table 1).

When available, we also extracted information on the extent of the deletion when molecular cytogenetic characterisation had been undertaken. Of the 92 microdeleted patients, 44 underwent microsatellite or FISH and long range polymerase chain reaction (PCR) analysis, including 28 for whom the information was retrieved from published reports,[4 5 13 14] 14 described in the present study, plus two previously reported cases that had been precisely characterised by our group using FISH.[3]

Table 2 lists the clinical features of the 14 previously unreported microdeleted patients, including those who differed from the *NF1* classical phenotype in the statistical analysis. Four patients had short stature or retarded growth, one had macrocephaly, and one was microcephalic. Only one patient had excessive growth. Nine patients had dysmorphisms, only two had mild mental retardation, and three had cardiovascular diseases. Examples of patients with facial dysmorphisms from the newly described microdeleted group are shown in fig 1.

The 44 finely characterised patients were then grouped on the basis of the extent of their deletion to explore possible genotype–phenotype correlations. Thirty seven patients carrying REP deletions made it possible to explore phenotypical variability within a subset having the same deletion: dysmorphic features, mental retardation, and cardiovascular anomalies were present in, respectively, 34 of 37 patients (92%), 12 of 26 (46.1%), and 7 of 37 (19%).

Eight patients with unusual sized deletions (one or both endpoints not falling within the *NF1* REPs) were a further main resource for the genotype–phenotype correlation study of *NF1* patients carrying different deletions. They included

Table 1 Presence of specific clinical signs in 92 NF1 microdeleted patients *v* NF1 patients according to published reports

	NF1 microdeleted patients			NF1 patients	
Clinical signs	**Total evaluable**	**Total affected**	**%**	**%**	**Discordance* (χ^2 value)**
Plexiform neurofibroma	88	25	28	25 to 30	No (0.36 to 0.13)
Macrocephaly	63	20	32	40 to 50	No (1.6 to 6.48)
Facial dysmorphisms†	88	69	78	5 to 15	**Yes (1065.8 to 264.6)**
Hypertelorism	64	27	42	15	**Yes (48.60)**
Learning disability	63	36	57	4 to 8	**Yes (702 to 300.1)**
Seizures	56	5	9	3.8 to 6	No (7.11 to 1.5)
Cardiovascular malformations‡	61	11	18	2.1	**Yes (120.39)**
Deafness	82	2	2	5.3	No (2.05)
Scoliosis	60	9	15	10 to 30	No (2.5 to 7.5)
Pectus excavatum-carinatum	58	10	17	20	No (0.45)

*The discordant values between the two groups of patients and the relative clinical signs are given in bold.
†Including the following signs, each observed in at least one patient: coarse face, flat occiput/brachycephaly, facial asymmetry, prominent forehead, frontal bossing, ptosis, downslanting deep set eyes, eversion of the lateral eyelid, epicanthic folds, strabismus, large nose, prominent nose, high nasal bridge, broad nasal bridge, broad nose, bulbous nasal tip, large ears, low set ears, malar hypoplasia, wide philtrum, prominent philtrum, small mouth, thick lips, micrognathia, small pointed chin, low posterior hairline.
‡Including: atrial septal defect, ventricular septal defect, patent ductus arteriosus, pulmonary stenosis, dilated aortic valve, hypertrophic cardiomyopathy, mitral valve prolapse.

three patients (BL, 106-3, BUD)[3 5 14 18] carrying large deletions that extended centromerically to REP-P and telomerically to REP-M, all of whom suffered from mental retardation; two (BL and 106-3) also had dysmorphic features, but only BL had hypertelorism. Patients 113-1 and TOP[5 14] had small deletions where the telomeric endpoint lies within REP-M but the centromeric endpoint was localised 5′ of the *NF1* gene: both showed mental retardation and facial dysmorphisms (including hypertelorism in patient 113-1). Atypical deletions included case 118 (present study)—who suffered from seizures and in whom the telomeric boundary was between *NF1* intron 6 and exon 10b, whereas centromerically

Table 2 Clinical features of the 14 newly described patients carrying NF1 microdeletion characterised by refined fluorescent in situ hybridisation (FISH) analysis

				Clinical signs				
Patient	**Age (years)**	**Sex**	**Deletion type**	**Growth defects**	**Dysmorphic**	**Learning disability**	**Cardiovascular malformation**	**Other features**
119	4	M	REP	No	No	No	–	
118	5	M	cen-REP	No	No	No	–	Optic glioma, seizures
93	6	M	REP	90th centile, macrocephaly	Yes*	No	HCM	Broad neck, 3 NFs
65	6	M	REP	Height 3rd centile, microcephaly 2nd centile	Yes†	IQ48	VSD (upper part)	Small hands/feet, short fingers
116	6	M	REP	Short stature 10th centile	Yes‡	IQ54	Mitral insufficiency	MCLS, kyphoscoliosis, pectus excavatum, genu valgus, pes planus, umbilical hernia
72	7	M	REP	No	Yes§	IQ50		Small hands/feet, short fingers
76	8	F	REP	No	Yes¶	No	–	–
94	8	F	REP	No	Yes**	No	–	–
75	9	F	REP	No	Yes††	No	–	–
85	11	M	REP	No	No	IQ77	–	MCLS
7	11	M	REP	No	Yes‡‡	Speech impairment	–	MCLS, amblyopia, thalamic hamartoma
82	23	F	REP	Short stature	No	No	–	Hearing impairment, Noonan-like
77	U	F	REP	Overgrowth >97th centile	Yes§§	Speech impairment, LD	–	NFs, required special education, short and broad fingers and toes
78	U	F	REP	–	Yes¶¶	Speech impairment	–	Delayed motor development, short and broad feet, fifth finger clinodactyly

*Hypertelorism, epicanthic folds, low set ears, low posterior hairline.
†Hypertelorism, downslanting eye, strabismus, large and prominent nose with high and broad bridge, bulbous nasal tip, large and low set ears, malar hypoplasia, wide and prominent philtrum, small mouth, small pointed chin.
‡Hypertelorism, long philtrum, broad nose.
§Prominent forehead, hypertelorism, ptosis (O.DX), downslanting eyes; strabismus, large and prominent nose with high and broad bridge and bulbous nasal tip, large and low set ears, wide and prominent philtrum, low posterior hairline.
¶Coarse face, hypertelorism.
**,††Epicanthic folds.
‡‡Hypertelorism, broad and wild nasal bridge, broad nasal tip.
§§Simple facial features.
¶¶Epicanthic folds, bulbous nose, narrow high palate, low forehead.
cen-REP, deletion extending centromerically to REP-P; CVM, cardiovascular malformations; F, female; HCM, hypertrophic cardiomyopathy; LD, learning disabilities; M, male; MCLS, multiple cafè-au-lait spots; NF, neurofibroma; REP, NF1 repeat mediated deletion; U, unknown; VSD, ventricular septal defect.

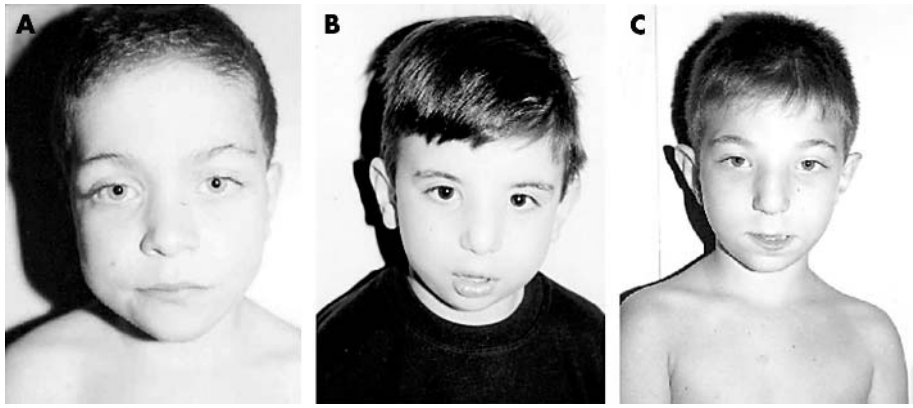

Figure 1 Facial appearance of three patients with NF1 microdeletions: case 116 at age 6 years (A), case 65 at age 6 (B), and case 72 at age 7 (C). See table 2 for details of the facial dysmorphisms.

it extended beyond REP-P—and case 155-1,[5] whose deletion ranged from the 5′ of the *NF1* gene to a breakpoint region (also shared by BL and 106-3), and who had dysmorphic features and mental retardation.

Deletion gene content analysis in NF1 patients

On the basis of the deletion characterisation of 44 patients (16 analysed in our laboratory and 28 described by other investigators), we identified a critical genomic interval including all but one of the characterised deletions (fig 2)[5 30]; the only exception was patient BUD, who had a deletion extending beyond the most telomeric ACCN1 gene (fig 2).[14]

The genomic interval comprises 21 genes with a known function, 10 with an unknown function, and 30 with predicted functions supported by mRNA or EST alignments with the genomic contig. The genes with a known function are shown in fig 2.

As dysmorphisms, mental retardation, and cardiovascular malformations were found to be commonly present in the *NF1* microdeleted subgroup in comparison with the *NF1* non-deleted patients, we searched the deleted region for candidate genes that might be involved in producing clinical signs such as mental retardation and cardiovascular malformations, defined on the basis of the target tissue or organ—that is, the central nervous system and the heart. By combining database screening and published findings concerning gene expression patterns and function, we identified six genes where haploinsufficiency may be involved in the onset of mental retardation (*SLC6A4*, *OMG*, *RHBDL4*, *ZNF207*, *CDK5R1*, and *ACCN1*), and two possible candidates for cardiovascular malformations (*CENTA2* and *JJAZ1*). In particular, the oligodendrocyte-myelin glycoprotein (*OMG*) gene, which maps within the REP interval (fig 2), encodes for a protein that has been recently shown to be a potent inhibitor of neurite outgrowth.[31]

The solute carrier family 6 (serotonin neurotransmitter transporter) member 4 gene (*SLC6A4*) (fig 2) maps centromerically to REP-P; its product is a transporter involved in the uptake of the serotonin neurotransmitter by presynaptic neurones or glial cells.[32]

The remaining candidate genes for mental retardation are shared by the non-REP deletions extending telomerically to REP-M (fig 2).

A good candidate for mental retardation is the cyclin dependent kinase 5 regulatory subunit 1 gene (*CDK5R1*), which encodes a neurone specific activator of cyclin

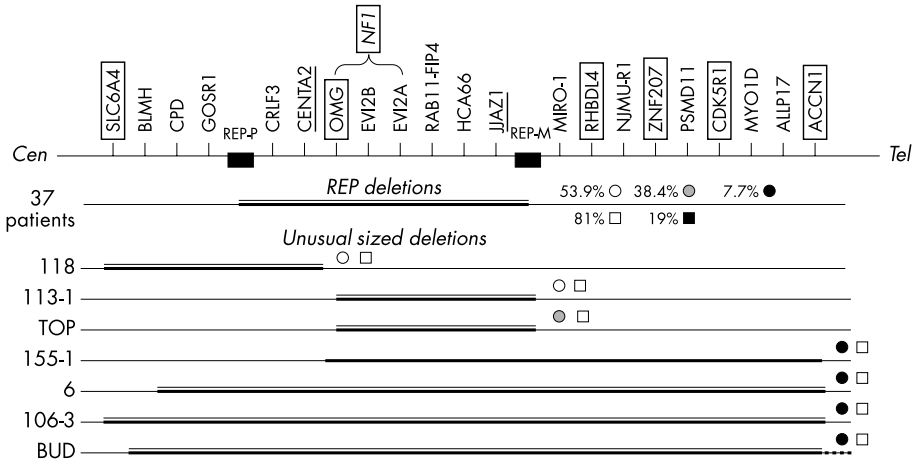

Figure 2 Mapping to 17q11.2 region, from SLC6A4 gene to ACCN1 gene, of REP and unusual deletions from 44 *NF1* microdeleted patients. All the genes with a known function are shown in the upper line: the candidate genes for mental retardation and cardiovascular malformations are respectively boxed and underlined; the black boxes represent *NF1* REP-P and REP-M. The white, grey, and black circles at each deletion interval indicate absent, mild, and moderate to severe mental retardation, respectively. The white and black squares indicate the absence and presence of cardiovascular malformations. The frequencies of the conditions related to the above clinical signs are also given for the group of patients (n = 37) carrying an REP deletion. For the unusually sized deletions, the specific patient codes are indicated on the left.

dependent kinase 5 (*CDK5*) required for the proper development and functioning of the central nervous system.[33 34] In addition, the neuronal amiloride sensitive cation channel 1 (*ACCN1*), zinc finger protein 207 (*ZNF207*), and rhomboid veinlet-like 4 (*RHBDL4*) genes—which respectively encode a neurone specific member of the degenerin/epithelial sodium channel (*DEG/ENaC*) superfamily, a zinc finger protein, and a protein homologous to the *D melanogaster* transmembrane Rhomboid protein[35–38]—are all strongly expressed in the central nervous system.

The Joined to JAZF1 (*JJAZ1*) and centaurin-α 2 (*CENTA2*) genes, which are significantly expressed in the heart and candidates for cardiovascular anomalies, were found to be included in the REP deletion interval (fig 2).

DISCUSSION

In this study we considered the clinical signs not included among the NIH consensus diagnostic criteria in a sample of 92 microdeleted patients, and compared their incidence with that given for classical *NF1* patients. We also established the gene content of 44 deletions of known extent, and sought to identify distinct clinical sign–genotype correlations.

Over the last few years, several papers have reported a more severe phenotype in patients carrying a microdeletion than in those affected by mutational neurofibromatosis,[1 3 5 8–12] although, as pointed out by Tonsgard *et al*,[10] phenotype evaluation per se is not predictive of the microdeletion.

By comparing a large sample of *NF1* microdeleted patients with the published data on classical *NF1* patients, we attempted to define the differences in the incidence of the selected clinical signs between the two populations. When selecting the clinical characteristics, we excluded all the signs and symptoms that are diagnostic criteria for *NF1*, in order to identify those that might highlight the candidate genes in *NF1* microdeletion syndrome. One exception to this rule was plexiform neurofibroma, for which we considered the latest emerging correlations between microdeletions and the development of malignancy in the tumour.[1] Conversely, although a high incidence of neurofibromas has been reported in microdeleted patients, we did not include the age dependent sign of neurofibroma development because of the heterogeneity of the sample and the frequent lack of information about neurofibroma onset.

We were aware that we may have underestimated the difference in the incidence of the selected clinical signs between classic *NF1* and *NF1* deleted patients because the more recently identified and characterised patients with deletions are included in the general *NF1* population evaluated in previous published reports.

Dysmorphic features

The results of our study suggest a significantly higher frequency of dysmorphisms, hypertelorisms, mental retardation, and cardiac anomalies in microdeleted patients (table 1). With regard to dysmorphisms, an ascertainment bias needs to be considered because the patients sent for microdeletion analysis are commonly affected by a visibly more severe phenotype which includes dysmorphic traits, whereas these may be present but not reported in non-deleted *NF1* patients. This has also been shown recently in relation to other well known microdeletion syndromes such as William's and Velocardio facial syndromes.[39] *NF1* gene haploinsufficiency is probably not the only cause of dysmorphisms, which are likely to involve other genes in the complex pathways regulating the correct development of the body as a whole. It is currently impossible to correlate a single gene to such a complex phenotype.

The only distinctive dysmorphic sign that was possible to compare with non-deleted patients was hypertelorism (table 1), although it may escape evaluation in the non-deleted patients. It is easily detectable and therefore likely to be reported more often than other signs. We agree with Tonsgard on the difficulty of defining a specific dysmorphic sign for *NF1* microdeletion syndrome,[10] despite the consistent general impression of a coarse and dysmorphic face. For all of these reasons, we believe that no conclusions can be drawn concerning the higher incidence of dysmorphisms in *NF1* deleted patients.

Mental retardation

Another sign that was more represented in *NF1* deleted patients was mental retardation. It is worth noting that *NF1* patients carrying large deletions have an increased frequency of structural brain anomalies revealed by neuroimaging studies, as shown by Korf and coworkers.[40] As these anomalies are not usually seen in *NF1* patients, it is hypothesised that mental retardation may at least partially reflect abnormal brain development rather than defective brain function caused by neurofibromin haploinsufficiency.[40] Zhu and coworkers[41] have shown that the cerebral cortex of *NF1*-null mouse embryos develops abnormally, thus suggesting the involvement of neurofibromin in CNS development. *NF1* patients rarely have a severe mental retardation (the incidence is similar to that found in the general population, at 3–5%), but often show a wide range of lesser mental retardation and cognitive defects.[42 43] The significantly higher incidence of moderate to severe mental retardation in microdeleted patients probably reflects the haploinsufficiency of one or more contiguous genes in addition to *NF1*.

We identified six candidate genes for mental retardation in the deletion intervals, of which *OMG* and *CDK5R1* are particularly interesting because of their known function in CNS development. *CDK5R1* encodes a neurone specific activator of cyclin dependent kinase 5.[44] *Cdk5r* KO mice have severe cortical lamination defects and suffer from adult mortality and seizures.[33 34] Moreover, an active CDK5-p35 complex is present in Golgi membranes, and antisense oligonucleotide suppression of *Cdk5* or p35 blocks the formation of membrane vesicles from the Golgi apparatus in young cultured neurones.[45] These results suggest that Cdk5-p35 plays a role in membrane trafficking during the outgrowth of neuronal processes.

It has recently been shown that *OMG* is a potent inhibitor of neurite outgrowth that acts by binding to the Nogo receptor, a protein associated with myelin.[31] Interestingly, *OMG* lies within an *NF1* intron, and the fact that its expression pattern overlaps that of *NF1* suggests that the activity of the two genes might be under coordinated control.[46] The deletion of the entire *NF1* gene (and therefore *OMG*) may deregulate this control mechanism and thus contribute to the mental retardation outcome in microdeleted patients.

We also compared the presence and severity of mental retardation with the different deletion intervals with precisely mapped end points. As summarised in fig 2, 38.6% of the patients carrying an REP deletion have mental retardation, but only 7.7% have moderate to severe mental retardation. On the other hand, all of the four patients with a deletion extending telomerically to the REP-M are affected by moderate or severe mental retardation, which may indicate that haploinsufficiency of one or more genes distally to REP-M, such as CDK5R1, plays a critical role in brain function or development, thus accounting for the onset of mental retardation in patients carrying such deletions. The hypothesis that severe mental retardation is indicative of a deletion extending telomerically to REP-M needs to be confirmed by parallel clinical and genetic characterisations of a larger number of microdeleted patients.

Cardiovascular malformations

In relation to cardiovascular involvement, several papers have recently highlighted the presence of cardiac and cardiovascular anomalies in patients with neurofibromatosis; in particular, Friedman *et al* have underlined the recurring cardiovascular anomalies that should be investigated in all patients with a diagnosis of *NF1*.[47] It has also been reported that neurofibromin plays a role in heart development,[48] and that *NF1* mutations should be taken into account as a cause of cardiac malformations. Our sample indicates a much higher incidence of cardiac malformations in microdeleted patients, thus suggesting a possible contribution to correct cardiac development of at least one of the other deleted genes contiguous to *NF1*.

All the 11 patients with cardiovascular malformations carry a REP deletion, thus indicating the possible presence within this region of one or more genes involved in the development of the cardiovascular system. Currently, the available functional data concerning the genes included in REP intervals do not allow us to identify genes that are possibly involved in heart development. We did, however, consider *CENTA2*, which encodes a phosphatydilinositide binding protein,[49] and *JJAZ1*, a zinc finger containing protein,[50] as candidates because of their high level of expression in heart tissue.

Further in silico and expression studies are in progress to identify genes with a known or unknown function that map in the interval of typical and atypical deletions and may be involved in heart development.

Conclusions

Dysmorphisms, cardiac anomalies, and mental retardation are signs which, when present in an *NF1* patient, should lead to the suspicion of a microdeletion involving the *NF1* and contiguous genes. On the basis of our data, the more severe phenotype is probably caused by the loss of other contiguous genes as well as by *NF1* haploinsufficiency.

It should also be considered that, in addition to the deletion itself, the variation in the level of expression of the genes involved in the rearrangements may also be caused by additional mechanisms, such as gene interruption and the position effect of genes flanking the deletions. Modulation of the overall clinical phenotype associated with specific polymorphisms has been described in Velo cardiofacial syndrome,[51] and additional genetic factors are probably involved in the clinical phenotypic variations observed in patients carrying a similar REP deletion.

As the number of the microdeleted patients carrying REP and non-REP deletions increases, more specific genotype–phenotype correlations can be inferred and may validate the differences we observed in the incidence of specific signs between microdeleted and classic *NF1* patients.

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ECHO

Severe infantile hyperkalaemic periodic paralysis and paramyotonia congenita: broadening the clinical spectrum associated with the T704M mutation in SCN4A

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An Italian kindred is described with nine individuals affected by hyperkalaemic periodic paralysis associated with paramyotonia congenita (hyperPP/PMC). Periodic paralysis was particularly severe, with several episodes a day lasting for hours. The onset of episodes was unusually early, beginning in the first year of life and persisting into adult life. The paralytic episodes were refractory to treatment. Patients described minimal paramyotonia, mainly of the eyelids and hands. All affected family members carried the threonine to methionine substitution at codon 704 (T704M) in exon 13 of the skeletal muscle voltage gated sodium channel gene (SCN4A). The association between T704M and the hyperPP/PMC phenotype has been only recently revealed. Nevertheless, such a severe phenotype has never been reported so far in families with either hyperPP or hyperPP/PMC. These data further broaden the clinical spectrum of T704M and support the evidence that this mutation is a common cause of hyperPP/PMC.

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