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), and childhood myeloid leukaemia. 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, which is caused by unequal homologous recombination of NF1 repeats (REPs). Known as the “NF1 microdeletion syndrome,” this condition is often characterised by a more severe phenotype than is observed in the general NF1 population. In particular, NF1 microdeleted patients often show variable facial dysmorphisms, mental retardation, developmental delay, and an excessive number of neurofibromas for age. 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 microdeleter 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.

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.

Our aims in the present study were, first, to verify whether the incidence of specific clinical signs is different in NF1 microdeleter 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 microdeleter 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, dysmorphism, 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 and Med Miner repository 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 Disorders, 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 17–23. 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 NTH 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. These reference percentages may also include patients carrying the NF1 deletion, the relative percentage of which is estimated to be 5–20%.

**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 $\chi^2$ test in 2x2 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.csc.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/), BODemap (http://bodemap.ims.u-tokyo.ac.jp/), NCi60 cancer microarray project (http://genome-www.stanford.edu/nci60/) and, for the homologous gene-encoded large proteins analyzed by Kazusa cDNA Project, KAZUSA Database information (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/). 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/)

**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 I, 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, 14 described in the present study, plus two previously reported cases that had been precisely characterised by our group using FISH.

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 microdelet 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
three patients (BL, 106-3, BUD)\textsuperscript{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 hypetelorism. Patients 113-1 and TOP\textsuperscript{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 hypetelorism 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 1 Presence of specific clinical signs in 92 NF1 microdeleted patients vs NF1 patients according to published reports](http://jmg.bmj.com/)

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>NF1 microdeleted patients</th>
<th>NF1 patients</th>
<th>Discordance* ($\chi^2$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total evaluable</td>
<td>Total affected</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Plexiform neurofibroma</td>
<td>88</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>63</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Facial dysmorphisms\textsuperscript{†}</td>
<td>88</td>
<td>69</td>
<td>78</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>64</td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td>Learning disability</td>
<td>63</td>
<td>36</td>
<td>57</td>
</tr>
<tr>
<td>Seizures</td>
<td>56</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Cardiovascular malformations\textsuperscript{‡}</td>
<td>61</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Deafness</td>
<td>82</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>60</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Pectus excavatum-carinatum</td>
<td>58</td>
<td>10</td>
<td>17</td>
</tr>
</tbody>
</table>

*The discordant values between the two groups of patients and the relative clinical signs are given in bold.
\textsuperscript{†}Including the following signs, each observed in at least one patient: coarse face, flat occiput/brachycephaly, facia asymmetry, prominent forehead, frontal bossing, ptosis, downslanting deep set eyes, eversion of the lateral eyelid, epicantihc folds, strabismus, large nose, prominent nose, high 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.
\textsuperscript{‡}Including: atrial septal defect, ventricular septal defect, patent ductus arteriosus, pulmonary stenosis, dilated aortic valve, hypertrophic cardiomyopathy, mitral valve prolapse.

![Table 2 Clinical features of the 14 newly described patients carrying NF1 microdeletion characterised by refined fluorescent in situ hybridisation (FISH) analysis](http://jmg.bmj.com/)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Deletion type</th>
<th>Growth defects</th>
<th>Dysmorphic</th>
<th>Learning disability</th>
<th>Cardiovascular malformation</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>4</td>
<td>M</td>
<td>REP</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>118</td>
<td>5</td>
<td>M</td>
<td>cen-REP</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>93</td>
<td>6</td>
<td>M</td>
<td>90th centile, macrocephaly</td>
<td>Yes\textsuperscript{†}</td>
<td>No</td>
<td>No</td>
<td>HCM</td>
<td>–</td>
</tr>
<tr>
<td>65</td>
<td>6</td>
<td>M</td>
<td>Height 3rd centile, microcephaly 2nd centile</td>
<td>Yes\textsuperscript{†}</td>
<td>IQ84</td>
<td>VSD (upper part)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>116</td>
<td>6</td>
<td>M</td>
<td>REP</td>
<td>Short stature 10th centile</td>
<td>Yes\textsuperscript{†}</td>
<td>IQ54</td>
<td>Mitrail insufficiency</td>
<td>–</td>
</tr>
<tr>
<td>72</td>
<td>7</td>
<td>M</td>
<td>REP</td>
<td>No</td>
<td>Yes\textsuperscript{§}</td>
<td>IQ50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>76</td>
<td>8</td>
<td>F</td>
<td>REP</td>
<td>No</td>
<td>Yes\textsuperscript{‡}</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>94</td>
<td>8</td>
<td>F</td>
<td>REP</td>
<td>No</td>
<td>Yes\textsuperscript{**}</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>75</td>
<td>9</td>
<td>F</td>
<td>REP</td>
<td>No</td>
<td>Yes\textsuperscript{††}</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>85</td>
<td>11</td>
<td>M</td>
<td>REP</td>
<td>No</td>
<td>No</td>
<td>IQ77</td>
<td>–</td>
<td>MCLS</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>M</td>
<td>REP</td>
<td>No</td>
<td>Yes\textsuperscript{‡‡}</td>
<td>Speech impairment, LD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>82</td>
<td>23</td>
<td>F</td>
<td>REP</td>
<td>Short stature &gt;97th centile</td>
<td>Yes\textsuperscript{§§}</td>
<td>Speech impairment, LD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>77</td>
<td>U</td>
<td>F</td>
<td>REP</td>
<td>Overgrowth &gt;97th centile</td>
<td>Yes\textsuperscript{‡‡}</td>
<td>Speech impairment, LD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>78</td>
<td>U</td>
<td>F</td>
<td>REP</td>
<td>–</td>
<td>Yes\textsuperscript{**}</td>
<td>Speech impairment</td>
<td>–</td>
<td>Delayed motor development, short and broad feet, fifth finger clinodactyly</td>
</tr>
</tbody>
</table>

*Hypertelorism, epicantihc folds, low set ears, low posterior hairline.
\textsuperscript{†}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.
\textsuperscript{‡}Hypertelorism, long philtrum, broad nose.
\textsuperscript{§}Prominent forehead, hypertelorism, ptosis (O.DX), downslanting eyes, strabismus, large and prominent nose with high and board bridge and bulbous nasal tip, large and low set ears, wide and prominent philtrum, low posterior hairline.
\textsuperscript{**}Coarse face, hypertelorism.
\textsuperscript{††}Hypertelorism, broad and wild nasal bridge, broad nasal tip.
\textsuperscript{‡‡}Simple facial features.
\textsuperscript{§§}Epicantihc folds, bulbous nose, narrow high palate, low forehead.
\textsuperscript{cen-REP}, deletion extending centromerically to REP-P; CVM, cardiovascular malformations; F, female; HCM, hypertrophic cardiomyopathy; LD, learning disabilities; M, male; MCLS, multiple cafe-au-lait spots; NF, neurofibroma; REP, NF1 repeat mediated deletion; U, unknown; VSD, ventricular septal defect.
it extended beyond REP-P—and case 155-1, 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); the only exception was patient BUD, who had a deletion extending beyond the most telomeric ACCN1 gene (fig 2).

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 JAZ1). 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.

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.

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 neuron specific activator of cyclin
dependent kinase 5 (CDK5) required for the proper development and functioning of the central nervous system.\(^3\)\(^5\)\(^6\) In addition, the neuronal amiloride sensitive cation channel 1 (ACCNI), 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\(^2\)\(^3\)\(^4\)\(^5\)—are all strongly expressed in the central nervous system.

The joined to JAZF1 (JJA1) 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 microdepleted 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,\(^1\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) although, as pointed out by Tonsgard et al.,\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) phenotype evaluation per se is not predictive of the microdeletion.

By comparing a large sample of NF1 microdepleted 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.\(^3\)\(^4\) Conversely, although a high incidence of neurofibromas has been reported in microdepleted 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 are certain 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, hypertelorism, mental retardation, and cardiac anomalies in microdepleted patients (table 1). With regard to dysmorphisms, an ascertained 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.\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) 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,\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\) 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.\(^3\)\(^4\) 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.\(^3\)\(^4\) Zhu and coworkers\(^3\)\(^4\) 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.\(^3\)\(^4\)\(^13\)\(^14\) The significantly higher incidence of moderate to severe mental retardation in microdepleted 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.\(^3\)\(^4\)\(^5\)\(^15\)\(^16\)Cdk5r KO mice have severe cortical lamination defects and suffer from adult mortality and seizures.\(^3\)\(^4\)\(^17\)\(^18\) 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.\(^3\)\(^4\)\(^19\) 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.\(^2\)\(^3\)\(^4\)\(^5\)\(^6\) Interestingly, OMG lies within an NF1 intron, and the extent of expression pattern overlaps that of NF1 suggesting that the activity of the two genes might be under coordinated control.\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) The deletion of the entire NF1 gene (and therefore OMG) may deregulate this control mechanism and thus contribute to the mental retardation outcome in microdepleted 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 microdepleted patients.
Cardiovascular malformations
In recent years, cardiovascular involvement has been observed in patients with neurofibromatosis type 1. Specifically, Friedman et al. have underlined the importance of cardiovascular anomalies that should be investigated in all patients with a diagnosis of NF1. It has also been reported that neurofibromin plays a role in heart development, and that NF1 mutations should be taken into account as a cause of cardiovascular malformations. Our sample indicates a higher incidence of cardiovascular 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 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 the genes that are possibly involved in heart development. We did, however, consider CENTA2, which encodes a phosphatidylinositol binding protein, and JJAZ1, a zinc finger containing protein, 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
Dysmorphism, 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 haplinsufficiency.

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, 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 microdeleter 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.

ACKNOWLEDGEMENTS
We thank Dr C. Gervasi, Dr F. Orzan, and P. Colapietra for their contribution to FISH analysis of the newly characterised NF1 microdeleted patients. This work was supported by a 2002 grant from FIRST to PR and by a 2002 grant from AIRC to LL.

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Received 22 September 2003
Accepted 2 November 2003

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

F Brancati, E M Valente, N P Davies, A Sarkozy, M G Sweeney, M LoMonaco, A Pizzuti, M G Hanna, B Dallapiccola

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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*J Med Genet* 2004 41: 41

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