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 group. 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 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. 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 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 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 17–25.

Among the 78 patients described in published reports, seven were familial microdeletions 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 microdeletions 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 microdeletions described.

The data on the percentages of each clinical sign in classic NF1 patients were drawn from published reviews,1 26–29 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 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 2 x 2 table test in 2 x 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.csc.ucsc.edu/).


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 homologous 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 microdeletions patients in comparison with those with classic neurofibromatosis 1, we considered a sample of 92 microdeletions (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 microdeletions 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 microdeletions 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,1 26–29 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 microdeletions 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 microdeletes 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)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 TOP51 4 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>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Deletion type</th>
<th>Clinical signs</th>
<th>Dysmorphic</th>
<th>Learning disability</th>
<th>Cardiovascular malformation</th>
<th>Other features</th>
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<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>
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<tr>
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<td>5</td>
<td>M</td>
<td>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>cen-REP</td>
<td>No</td>
<td>Yes†</td>
<td>No</td>
<td>HCM</td>
<td>MCLS, NYW, CS, HCS, PSH</td>
</tr>
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<td>REP</td>
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<td>IQ48</td>
<td>VSD (upper part)</td>
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<tr>
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<td>6</td>
<td>M</td>
<td>REP</td>
<td>Short stature 10th centile</td>
<td>Yes‡</td>
<td>IQ54</td>
<td>Mirtal insufficiency</td>
<td>MCLS, kypchosiosis, puctus excavatum, genu valgus, pes planus, umbilical hernia</td>
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<td>7</td>
<td>M</td>
<td>REP</td>
<td>No</td>
<td>Yes‡</td>
<td>IQ50</td>
<td>–</td>
<td>–</td>
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<tr>
<td>76</td>
<td>8</td>
<td>F</td>
<td>REP</td>
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<td>Yes†</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**</td>
<td>No</td>
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<td>–</td>
</tr>
<tr>
<td>75</td>
<td>9</td>
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<td>REP</td>
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<td>Yes†</td>
<td>No</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>No</td>
<td>IQ77</td>
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<tr>
<td>7</td>
<td>11</td>
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<td>No</td>
<td>Yes‡</td>
<td>Speech impairment</td>
<td>–</td>
<td>MCLS, amblyopia, thalamic hamartoma</td>
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<tr>
<td>82</td>
<td>23</td>
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<td>REP</td>
<td>Short stature  &gt;97th centile</td>
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<td>–</td>
<td>–</td>
<td>MCLS, NYW, CS, HCS, PSH</td>
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<tr>
<td>77</td>
<td>U</td>
<td>F</td>
<td>REP</td>
<td>Overgrowth  &gt;97th centile</td>
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<td>Speech impairment</td>
<td>–</td>
<td>LD, Learning impairment,</td>
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<tr>
<td>78</td>
<td>U</td>
<td>F</td>
<td>REP</td>
<td>–</td>
<td>Yes‡‡</td>
<td>Speech impairment</td>
<td>–</td>
<td>Delayed motor development, short and broad feet, fifth finger clinodactyly</td>
</tr>
</tbody>
</table>

*Hypertelorism, epicantic folds, low set ears, low posterior hairline.
†Hypertelorism, downsloping eye, strabismus, large and prominent nose with high and broad bridge, bulbous nasal tip, large and low set ears, molar hypoplasia, wide and prominent philtrum, small mouth, small pointed chin.
‡Hypertelorism, long philtrum, broad nose.
§Prominent forehead, hypertelorism, ptosis (O.DX), downsloping 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 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).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 haplo-insufficiency may be involved in the onset of mental retardation (SLC6A4, OMG, RBBDL4, 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 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.

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. In addition, the neuronal amiloride sensitive cation channel 1 (ACC1), 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—are all strongly expressed in the central nervous system.

The Joining to JAZF1 (JUA1) 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 microdeleed 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, although, as pointed out by Tonsgard et al., phenotype evaluation per se is not predictive of the microdeletion.

By comparing a large sample of NF1 microdeleed 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. Conversely, although a high incidence of neurofibromas has been reported in microdeleed 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 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, hypertelorism, mental retardation, and cardiac anomalies in microdeleed 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. 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, 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. 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. Zhu and coworkers 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. The significantly higher incidence of moderate to severe mental retardation in microdeleed 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. CDK5 KO mice have severe cortical lamination defects and suffer from adult mortality and seizures. 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. 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. Interestingly, OMG lies within an NF1 intron, and the gene expression pattern overlaps that of NF1 suggesting that the activity of the two genes might be under coordinated control. The deletion of the entire NF1 gene (and therefore OMG) may deregulate this control mechanism and thus contribute to the mental retardation outcome in microdeleed 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 microdeleed 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 NFI. 19 It has also been reported that neurofibrin plays a role in heart development, 16 and that NFI mutations should be taken into account as a cause of cardiac malformations. Our sample indicates a much higher incidence of cardiovascular malformations in microdeleter patients, thus suggesting a possible contribution to correct cardiac development of at least one of the other deleted genes contiguous to NFI.

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 CNTA2, which encodes a phosphatidylinositol binding protein, 17 and JJAZ1, a zinc finger containing protein, 18 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 NFI patient, should lead to the suspicion of a microdeletion involving the NFI 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 NFI 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, 19 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 microdeleter and classic NFI patients.

ACKNOWLEDGEMENTS
We thank Dr C Gervasini, Dr F Orzan, and P Colapietro for their contribution to FISH analysis of the newly characterised NFI microdeleter 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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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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LETTER TO JMG

Loci for primary ciliary dyskinesia map to chromosome 16p12.1-12.2 and 15q13.1-15.1 in Faroe Islands and Israeli Druze genetic isolates


Primary ciliary dyskinesia (PCD; Immotile cilia syndrome; OMIM 242650) is an autosomal recessive disorder resulting from dysmotility of cilia and sperm flagella. Cilia and flagella function either to create circulation of fluid over a stationary cell surface or to propel a cell through fluid. These related structures are highly complex organelles composed of over 200 different polypeptides. The core or axoneme of cilia and flagella comprises a bundle of microtubules and many associated proteins. The microtubules are formed from α and β tubulin polypeptides and are arranged in a well recognised ‘9+2’ pattern: nine peripheral microtubule doublets in a ring connected around a central pair of microtubules by radial spoke proteins. The peripheral microtubules have dynein motor proteins attached and are connected with each other by nexin links. In human beings, ciliated epithelium can be found lining the respiratory tract, including the sinuses and middle ear, the brain ependyma, the female oviduct, and the male vas deferens. Cilia in the respiratory tract play an important part in airway clearance of respiratory secretions. In primary ciliary dyskinesia, impaired mucociliary clearance causes recurrent respiratory tract infections including chronic otitis media, rhinitis, and sinusitis, often leading to permanent lung damage (bronchiectasis). Patients are also often subfertile due to sperm tail immotility and immotile oviduct cilia. About half of the patients have defects of laterality; usually complete mirror-image reversal of the left-right axis (situs inversus) and this association is known as Kartagener syndrome. Primary ciliary dyskinesia has an estimated incidence of 1 in 20 000 live births.

A locus was mapped in seven families from the Faroe Islands to a 2.8 cM interval on chromosome 16p12.1-12.2, with a maximum lod score of 3.15, obtained using GENEHUNTER. A locus was mapped in three out of four Druze families from the Golan region of Israel to a 17 cM region on chromosome 15q13.1-15.1, with a maximum lod score of 3.2 at (proportion of linked families) = 0.7, using GENEHUNTER.

This study provides further evidence of the genetic heterogeneity underlying primary ciliary dyskinesia and has established a framework to facilitate isolation of two genes for primary ciliary dyskinesia. Determining the molecular basis of primary ciliary dyskinesia will provide insight into the genetic control of cilia assembly and function and the pathway which determines the vertebrate left right axis.

Key points

- Primary ciliary dyskinesia is an autosomal recessive disorder characterised by recurrent sinopulmonary infections, bronchiectasis, and subfertility, due to dysfunction of the cilia. Fifty percent of patients with primary ciliary dyskinesia have defects of laterality (situs inversus) and this association is known as Kartagener syndrome. Primary ciliary dyskinesia has an estimated incidence of 1 in 20 000 live births.
- To identify loci for this genetically heterogeneous condition, genome wide linkage scans and subsequent fine mapping were performed in two different isolated populations with distinct ciliary ultrastructural defects, from the Faroe Islands and the Druze community in northern Israel.
- A locus was mapped in seven families from the Faroe Islands to a 2.8 cM interval on chromosome 16p12.1-12.2, with a maximum lod score of 3.15, obtained using GENEHUNTER. A locus was mapped in three out of four Druze families from the Golan region of Israel to a 17 cM region on chromosome 15q13.1-15.1, with a maximum lod score of 3.2 at (proportion of linked families) = 0.7, using GENEHUNTER.

Abbreviation: PCD, primary ciliary dyskinesia
found in a patient with Kartagener syndrome and normal cilia ultrastructure. The exact role of DNAH11 in primary ciliary dyskinesia has yet to be elucidated. Two other genes for primary ciliary dyskinesia have been localised: CILD2 to 19q23 and another locus to 15q24 (M P Witt, personal communication).

To identify loci for PCD, genome wide linkage scans and subsequent fine mapping were performed in two different isolated populations with distinct ciliary ultrastructural defects, from the Faroe Islands and the Druze community in northern Israel.

The Faroes comprise a small group of islands in the North Atlantic Ocean located near Iceland, Scotland, and Norway with a relatively young population of mixed Norwegian, Danish, and British ancestry. The islands were colonised at the same time as Iceland, around 1100 years ago and the population size remained small until rapid expansion in the 1800s. The current population size is estimated at 48 000 and has arisen predominantly by indigenous expansion. The Druze community, a small middle eastern religious sect, was established slightly later, in the eleventh century. Members live predominantly in defined geographical areas of southern Syria, southern Lebanon, and northern Israel and they remain isolated mainly for religious reasons. Both populations are also isolated for geographical and cultural reasons, in the Faroe Islands associated with limited immigration and in the Druze with a high level of consanguinity.

With relatively few founders, a limited population size, and recent expansion, the Faroese and Druze populations are suitable for genetic mapping of a heterogeneous recessive disease. This has been demonstrated in the Faroe Island population, in which enrichment of disease incidence associated with specific gene mutations due to founder effects has been shown for cystic fibrosis and glycerol storage disease IIIA. Similar findings have been reported for several conditions in the well studied Druze population including cystic fibrosis, Krabbe disease and mucolipidosis. However the Druze population has not always proved to be genetically homogeneous.

We report here the identification of two new PCD loci in these genetic isolates, located on chromosomes 16p12.1-12.2 and 15q31.1-15.1 in the Faroe Island and Druze population respectively.

**SUBJECTS AND METHODS**

This study was carried out using the two family sets shown in fig 1. Informed consent was obtained in accordance with protocols approved by the University College London Hospital NHS Trust ethical committee. The diagnosis of affected subjects was made according to established clinical criteria and 15q13.1-15.1 in the Faroe Island and Druze population, in which enrichment of disease incidence has been shown for cystic fibrosis and glycerol storage disease IIIA. Similar findings have been reported for several conditions in the well studied Druze population including cystic fibrosis, Krabbe disease and mucolipidosis. However the Druze population has not always proved to be genetically homogeneous.

We report here the identification of two new PCD loci in these genetic isolates, located on chromosomes 16p12.1-12.2 and 15q31.1-15.1 in the Faroe Island and Druze population respectively.

Four Druze Israeli families were studied comprising 7 affected and 22 unaffected individuals (Three are shown in fig 1B). The parents in three families, UCL122, UCL124, and UCL125, were first cousins, while the fourth family, UCL123, was non-consanguineous. In contrast to the Faroe Island population, Druze patients showed an unusually high incidence of situs inversus (six out of seven affected children). Electron microscopy defined an ultrastructural phenotype of partial absence of the inner dynein arms (fig 2C). Nasal brushings were processed for ultrastructural studies using established techniques and over 100 suitably oriented cross sections of cilia throughout the section were examined to confirm this structural defect. The four families were located in two neighbouring Druze villages comprising 7500 inhabitants, in the mountainous region of the Golan Heights. Families UCL122 and UCL124 were from one village and families UCL123 and UCL125 were from the other.

All of the patients with primary ciliary dyskinesia who were recognised within the two populations were ascertained and there is considerable population enrichment for primary ciliary dyskinesia in both the Faroe Island and Druze populations. The prevalence of primary ciliary dyskinesia in the Faroe Islands is around 1:4500 whilst the local prevalence amongst the Druze population studied is as high as 1:1000.

Genome wide scans of 10 cM density were performed in these two populations using four of the Faroe Island pedigrees (UCL96, 97, 98, and 109) and all four Druze pedigrees (UCL122, 123, 124, and 125). Genomic DNA was extracted from white blood cells using standard methodology. Over 350 hundred microsatellite markers from the Génethon map were genotyped in each family by polymerase chain reaction and fluorescent based semi-automated methodolology using methods previously described. Alleles were assigned using Genescan and Genotyper software (Applied Biosystems). Linkage analysis was performed under the assumption of autosomal recessive inheritance with a penetrance of 0.9, a disease allele frequency of 0.007 with equal allele frequencies (0.1) for each marker and a phenocopy rate of 0.0001. This disease allele frequency reflects the worldwide incidence of primary ciliary dyskinesia of 1:20 000. Analysis was also performed using the disease allele frequencies that reflected more closely the actual population frequencies we have determined for each population as described above: 0.015 for the Faroe Island families (incidence 1:4500) and 0.03 for the Druze families (incidence 1:1000). Multipoint analyses were performed using the GENEHUNTER program.

For subsequent fine mapping, six novel microsatellites were determined in the laboratory from the genomic sequence of BAC clones spanning the selected region. The primer pairs used to amplify these polymorphisms were: 5'-TGTTCCTAGACGTTGTCAGC-3'/5'-ACTGTACCCCTTCAAA CG-3' (16/1425), 5'-TGGCGAGCAATGGGCACGC-3'/5'- GCTCTCAAACTCCTCAGTC-3' (16/10321), 5'-CAGGGCTGTCGTAGTAATTG-3'/5'-CTTATCACCCTGACTACCT-3' (15/14), 5'-GCTCTCTATGCAATCTTCCAC-3'/5'-GAAATGTGTTTCCCGCTC TC-3' (15/20), 5'-CCATCGCGCATCAGAAGG-3'/5'- GTGCCCCTTATC-3' (15/23) and 5'-GAGTACACCACACCAGAAAG-3'/5'-CTCACGACAGTTGTCAGA-3' (15/36).

Haplotypes were manually constructed using the most parsimonious allele configuration to minimise recombinants. Génethon marker order and genetic distances were determined from the deCODE Genetics high resolution genetic map. Additional markers were positioned in the deCODE framework map based on their relative position within the human genome sequence available from the UCSC Genome Browser, April 2003 Build 33 sequence freeze.
Figure 1  Pedigrees consistent with linkage on chromosome 16p12.1-12.2 and 15q13.1-15.1. (A) Faroe Island and (B) Druze Israeli haplotypes. Filled symbols indicate individuals affected with primary ciliary dyskinesia, asterisks indicate patients with situs inversus, and a double line indicates first cousin marriage. Genetic markers used in the initial genome scan are shown in bold and the box indicates the minimum critical interval encompassing each locus. For each marker, alleles are coded numerically such that the same allele number correlates to the same allele size across families. The frameworks of microsatellite markers used were as follows with genetic distances indicated in Kosambi cM:

**A**

- D16S3046, 2.59
- D16S3076, 0.003
- D16S403, 0.1
- D16S412, 0.5
- D16S417, 1.3
- 16/4125, 0.4
- 16/10221, 0.16
- D16S401, 0.01
- D16S3133, 0.31
- D16S3093, 0.99
- D16S3131, 1.55
- D16S3068, 1.55
- D16S3093, and D15S975, 2.09
- D15S1002, 6.02
- D15S1048, 1.57
- D15S165, 1.99
- D15S1031, 0.22
- 15/14, 1.38
- D15S1010, 1.19
- 15/20, 0.87
- D15S144, 0.57
- D15S1007, 1.09
- D15S1040, 0.96
- 15/23, 0.92
- D15S1232, 0.88
- D15S971, 0.27
- 15/36, 1.12
- D15S118, 4.02
- D15S1012. Genetic distances were taken from the DeCODE map except for markers 16/4125, 16/10221, D16S309, 15/14, D15S10010, 15/20, 15/23, D15S1232 and 15/36. These were positioned relative to the DeCODE marker framework using Human Genome Project draft sequence information as described in the text.
Physical distances were taken from this genomic sequence map.

RESULTS
A region consistent with linkage in the four Faroe Island families was identified on chromosome 16p12.1-13.1. No evidence for linkage at any of the known PCD loci was found. Fine mapping was undertaken in the entire seven family resource which included the three additional families UCL135, UCL139, and UCL140. Linkage analysis using GENEHUNTER was performed across a 9 cM region between markers D16S3046 and D16S3093. This gave a maximum multipoint lod score of 3.15 between markers D16S412 and 16/10321. Using a disease allele frequency calculated from the actual recorded Faroe Island population disease incidence rather than worldwide incidence of primary ciliary dyskinesia, the maximum lod score obtained was 3.13. This is probably a conservative estimate of the true degree of linkage as the families were only analysed as unrelated pedigrees. Haplotype analysis indicated the key recombinant events defining the critical region on chromosome 16p12.1-12.2 (fig 1A). A paternal recombination in family UCL98 at D16S403 defined the telomeric limit of the region. The centromeric limit was defined by a maternal recombination event in family UCL139 at D16S3133. The D16S403-D16S3133 critical interval spans 2.8 cM which corresponds to a physical distance of 2.37 Mb.

Although no statistically significant evidence of a common disease associated haplotype amongst the families could be proven, three markers in the D16S403-D16S3133 interval did show a suggestion of linkage disequilibrium with a higher frequency of certain alleles on disease chromosomes compared to control chromosomes. The control data were taken from genotypes on the families' obligate normal chromosomes and on chromosomes from a small set of unrelated Faroe control individuals. The three markers were 16/4125, D16S401, and D16S3133, with p<0.1 for all three using Fisher's exact test. At 16/4125 allele 2 was present on 3/15 (20%) of disease and 1/25 (4%) of control chromosomes; D16S401 allele 2 was present on 6/15 (40%) of disease and 7/25 (28%) of control chromosomes; and D16S3133 allele 1 was present on 8/15 (53%) disease and 3/13 (23%) of control chromosomes. All other markers in the entire D16S3046-D16S3093 interval were non-significant. It was also noted that the affected child in family UCL98 is homozygous for the haplotype 3-2-2 at markers 16/4125-16/10321-D16S401.

Data from one additional Faroe Island pedigree were not included in the present study. Exclusion from the chromosome 16 locus was indicated in this family suggesting the possibility of locus heterogeneity amongst Faroe Islanders. However clinical data on the individual who appears to be recombinant across the region if affected remains incomplete and the affection status of this individual remains uncertain at present.

Three out of four of the Druze families were consanguineous and a homozygosity mapping approach was used. Genome scan markers were scored for homozygosity in the affected individuals in families UCL122, UCL124, UCL125, and for consistency of linkage in the non-consanguineous family UCL123. Four regions of excess homozygosity were identified on chromosomes 1q32, 3p25, 5q33, and 15q13. Fine mapping using additional markers at each region resulted in exclusion of all regions except that on chromosome 15q13. Linkage analysis using GENEHUNTER across a 25 cM region between markers D15S975 and D15S1012 gave a maximum multipoint lod score between markers D15S1031 and D15S118 of 3.2 allowing for heterogeneity, at x (proportion of linked families) = 0.73 which suggested that three out of four families were linked. Using a disease allele frequency calculated from the actual recorded Druze population disease incidence rather than worldwide incidence of primary ciliary dyskinesia, the maximum lod score obtained dropped to 3.0. Haplotype analysis confirmed linkage in families UCL122, UCL123, and UCL125, but not in UCL124. No evidence for linkage of family UCL124 to any of the known PCD loci was found. The key recombination events defining the critical region were determined (fig 1B). The centromeric and telomeric limits of the region were defined by loss of homozygosity at D15S1048 in the affected child in UCL125 and a maternal recombination in family UCL122 at D15S1012 respectively. The D15S1048-D15S1012 critical interval on chromosome 15q13.1-15.1 spans a region of 17 cM, corresponding to a physical distance of 9.1 Mb. Interestingly the non-consanguineous family UCL123 was homozygous by descent for alleles at the markers D15S1031, 15/14, D15S1010, 15/23, and D15S1232, identifying these as regions of potential interest if the families harbour a single ancestral mutation. However the significance is reduced since there was no evidence of allele sharing amongst affected Druze patients.

DISCUSSION
These results provide statistically significant support for loci for primary ciliary dyskinesia on chromosomes 16p12.1-12.2 and 15q13.1-15.1. Since primary ciliary dyskinesia is a genetically and clinically heterogeneous disorder the selection of patients with an identical ultrastructural phenotype and families from isolated populations should minimise the risk of genetic heterogeneity. The incidence of inbreeding is often high in these populations which may allow the application of homozygosity or linkage disequilibrium mapping to identify and refine the disease gene localisation. This is a powerful approach and has proved successful in mapping many autosomal recessive diseases including.
primary ciliary dyskinesia, even in the presence of heterogeneity. This study uses PCD family groups from two genetically isolated populations from the Faroe Islands and the Golan Heights Druze community, both of which were founded and have expanded relatively recently. There is significant enrichment for primary ciliary dyskinesia in both these populations presumably due to isolation and inbreeding, the incidence being around 1:4500 in the Faroe Islands and 1:1000 in the Druze population studied.

In both populations the initial assumption was that the existence of a single ancestral Faroe Island or Druze mutation would account for all the patients in each group. However, in the current study such linkage disequilibrium has not been detected. At the Faroeos locus no common haplotype was observed among the affected subjects even amongst families UCL196, UCL197, and UCL109, who may be interrelated, and the allele sharing observed was not statistically significant. Identification of linkage disequilibrium by additional marker typing at both loci would be useful to further refine the critical interval and pinpoint the localisation of the disease gene.

The extent of linkage disequilibrium encompassing a disease gene allele is dependent on course on a number of factors including natural selection and physical features such as the local recombination and gene conversion rate. Population scale effects include the population size, demographic and admixture history. A large population under mutation drift equilibrium displays low levels of linkage disequilibrium while high linkage disequilibrium correlates with a small population due to genetic drift. If the population is reduced, then allelic diversity is lowered and genetic drift is increased. Simulation studies on human populations predicts that slow early growth, such as that which occurred in the Faroeos population, can be a key factor in increasing the extent of linkage disequilibrium in an isolated population. The age of the disease and marker alleles can be regarded as one of the most important considerations; the exact timing of linkage disequilibrium in an isolated population is 50.

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We have demonstrated locus heterogeneity in the PCD Druze families since only three of the four families are linked to the 15q locus. Moreover the lack of allele sharing suggests allelic heterogeneity. The intense inbreeding within this community could allow different mutations on different haplotypes of a disease gene to expand within the population giving rise to allelic heterogeneity. It has been suggested that multiple mutations in a single specific gene in a small geographic area and in inbred families may in fact be a common phenomenon. Such allelic heterogeneity has been well described in previous molecular genetic studies in the Druze population. Three different iduronidase mutations were found to cause Hurler syndrome in Druze patients from the Galilee region of Israel and five different mutations in the arylsulfatase A gene causing metachromatic leucodystrophy, occurring on at least three different haplotypes, were reported in the same region. Two different mutations and three different genotypes were noted in a small Druze kindred with Wilson’s disease.

Both critical regions on chromosome 16p12.1-12.2 and 15q13.1-15.1 contain no obvious candidate genes based on known or predicted function or association with axonemal function. The disease genes may therefore encode proteins with a novel structural or regulatory role in cilia or flagella, associated with distinct outer (Faroe Island) and inner (Druze) dynein arm functions. From published reports and expressed sequence tag, protein and gene prediction annotation derived from the UCSC Genome Browser, RefSeq, NCBI Map Viewer, Ensembl transcript and human cDNA collections, the chromosome 16p12.1-12.2 critical region contains 15 distinct genes verified with strong experimental support, and three predicted genes. Using the same criteria the chromosome 15q13.1-15.1 critical region contains 25 well supported genes and 15 predicted genes.

With the identification of two new loci for primary ciliary dyskinesia, the two candidate intervals defined on chromosomes 16p12.1-12.2 and 15q13.1-15.1 provide a framework for the isolation of the putative genes causing primary ciliary dyskinesia. The evolutionary conservation of cilia and flagella structure and function and of the genes encoding axonemal proteins has previously been used to facilitate isolation of human PCD genes. Along with advances in the human genome project there has been huge progress with the genome projects for the flagellated PCD model organisms Chlamydomonas and Trypanosoma. Their genome sequence information and annotation is publicly available (http://genome.jgi-psf.org/chlr1/chlr1.home.html, http://parsum1.path.cam.ac.uk/overview.htm) and it is now possible to incorporate cross species comparative genomics into the bioinformatics based prioritisation of PCD candidate genes.

Another postgenomic approach applied to the study of cilia structure and function is also likely to prove useful in the search candidate genes for primary ciliary dyskinesia. Ostrowski and colleagues recently reported results from the first proteomic analysis of the human ciliary axoneme, aimed at characterising all the constitutive protein components. In this study one and two dimensional gel electrophoresis of cilia from human bronchial epithelial biopsy produced a map of over 200 putative axonemal proteins, yielding many potentially novel components of this complex organelle. Our own bio-informatic analysis has indicated that none of the available protein sequences from this study correspond to genes products predicted to map to either the 16p12.1-12.2 or the 15q13.1-15.1 loci. Ciliary actin identified in the proteomic set does have domain similarity to both a cardiac specific and a skeletal muscle specific actin isoform located in the critical region on chromosome 15 but there does not appear to be a functional correlation. Proteomic studies such as this have enormous promise and are highly appropriate for analysis of the cilium. Techniques for more direct analysis of complex protein mixtures using methods based on mass spectrometry and the development of protein “chips” are actively evolving fields which will further advance this approach.

In conclusion, this study reinforces the value of isolated human populations in gene mapping of heterogeneous disorders. Determining the molecular basis of primary ciliary dyskinesia will provide insight into the genetic control of cilia assembly and function and the pathway which determines the vertebrate left-right axis.

ACKNOWLEDGEMENTS

We are grateful to the populations of the Faroe Islands and Israel Golan Heights and to all the individuals who participated in this study. We thank Drs Michael Dunn, Amanda Walne, Martin Gray and Ms Laure Boudery for clinical ascertainment and technical support. We thank Drs Christopher O’Callaghan and Wendy Stannard for the electron micrograph of a normal axoneme.


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Received 8 September 2003

Revised version received 24 October 2003

Accepted 20 November 2003

REFERENCES


VENTURINI M, GUARNIERI P, NATACCI F, et al. Mental retardation and cardiovascular malformations in NF1 microdele tated patients point to candidate genes in 17q11.2 (J Med Genet 2003; 41:35–41). We would like to offer our sincere apologies to the authors of this paper for a number of errors that were introduced regarding the incorrect use of the term “learning disability”. The instances of “learning disability” in the Key points box, and in Tables 1 and 2 should be “mental retardation”. These errors are much regretted and the corrected versions are shown here.

**CORRECTION**

doi: 10.1136/jmg.2003.014761corr1

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

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>NF1 microdele tated patients</th>
<th>NF1 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total evaluable</td>
<td>Total affected</td>
</tr>
<tr>
<td>Pectori form malformations</td>
<td>88</td>
<td>25</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>63</td>
<td>20</td>
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<td>Facial dysmorphisms</td>
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<tr>
<td>Hypertelorism</td>
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<td>27</td>
</tr>
<tr>
<td>Mental retardation</td>
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<td>36</td>
</tr>
<tr>
<td>Seizures</td>
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</tr>
<tr>
<td>Cardiovascular malformations</td>
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<td>11</td>
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<td>Deafness</td>
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<td>2</td>
</tr>
<tr>
<td>Scoliosis</td>
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<td>9</td>
</tr>
<tr>
<td>Pectus excavatum-carpinatum</td>
<td>58</td>
<td>10</td>
</tr>
</tbody>
</table>

*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/broadcephaly, facial asymmetry, prominent forehead, frontal bossing, ptosis, downsloping 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.
Table 2  Clinical features of the 14 newly described patients carrying NF1 microdeletion characterised by refined fluorescent in situ hybridisation (FISH) analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
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<th>Deletion type</th>
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<th>Cardiovascular malformation</th>
<th>Other features</th>
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<td>119</td>
<td>4</td>
<td>M</td>
<td>REP</td>
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<td>No</td>
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<td>–</td>
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<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>
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<tr>
<td>93</td>
<td>6</td>
<td>M</td>
<td>REP</td>
<td>Height 90th centile, macrocephaly</td>
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<td>No</td>
<td>HCM</td>
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<tr>
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<td>6</td>
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<td>REP</td>
<td>Height 3rd centile, microcephaly 2nd centile</td>
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<td>IQ48</td>
<td>VSD (upper part)</td>
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<td>REP</td>
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<td>IQ54</td>
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<td>72</td>
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<td>Yes§</td>
<td>IQ50</td>
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<tr>
<td>94</td>
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<td>F</td>
<td>REP</td>
<td>No</td>
<td>Yes**</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>75</td>
<td>9</td>
<td>F</td>
<td>REP</td>
<td>No</td>
<td>Yes††</td>
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<td>–</td>
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<tr>
<td>85</td>
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<td>U</td>
<td>F</td>
<td>REP</td>
<td>Overgrowth &gt;97th centile</td>
<td>Yes††</td>
<td>Speech impairment</td>
<td>–</td>
</tr>
<tr>
<td>78</td>
<td>U</td>
<td>F</td>
<td>REP</td>
<td>–</td>
<td>Yes**</td>
<td>Speech impairment</td>
<td>–</td>
</tr>
</tbody>
</table>

*Hypertelorism, epicantal 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, molar 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.  
††Epicantal folds.  
‡‡Epicantal folds, bulbous nose, narrow high palate, low forehead.  
Simple facial features.  
Epicantal 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 cafe–au–lait spots; NF, neurofibroma; REP, NF1 repeat mediated deletion; U, unknown; VSD, ventricular septal defect.

Key points

- NF1 microdeletion syndrome is determined by haplo-insufficiency 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 mental retardation, 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 mental retardation. 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 mental retardation are signs indicating NF1 microdeletion.

CORRECTION

doi: 10.1136/jmg.2003.008433corr

Owing to the change over of typesetters at the BMJ Publishing Group Ltd last year, we would like to apologise for an error that was published in the paper by Baser et al (J Med Genet 2003;40:758–760). In Table 2 the headings ‘Classical NF2’ and ‘Somatic mosaic’ should be indented as they are sub-sections of the heading ‘Nonsense or frame-shift’. 