

# Molecular analysis of three patients with interstitial deletions of chromosome band 14q31

Barbara C Byth, M Teresa Costa, Ikuko E Teshima, William G Wilson, Nigel P Carter, Diane W Cox

## Abstract

**Two patients and one three generation family with interstitial deletions of distal chromosome band 14q31 are described. The deletions were initially identified by chromosome analysis; we have used highly informative simple sequence repeat polymorphisms to define the deletions at the molecular level. This analysis also establishes the parental origin of the deleted chromosome. One of the patients was initially described as having a terminal deletion of chromosome 14 from 14q31 to 14qter; we show here that this child has instead an interstitial deletion of band 14q31. The smallest deletion involves a single anonymous DNA marker and is associated with an almost normal phenotype. The two patients with larger deletions have phenotypes similar to those seen in previously described cases of interstitial deletions of chromosome 14, including minor dysmorphic features and developmental delay. Delineation of these deletions allows the ordering of markers within the 14q31 region, in which the gene for the degenerative neurological disorder Machado-Joseph disease is localised.**

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A characteristic constellation of features is found in patients with interstitial deletions of the distal portion of chromosome 14 (14q23→q32). Ten such cases have been described previously.<sup>1-4</sup> A characteristic facies (including bushy eyebrows, epicanthic folds, strabismus, small nose with flat nasal bridge, microcephaly, low set and deformed ears, carp mouth, dental abnormalities, narrow and arched palate, micrognathia) is often observed. In addition, single palmar creases, heart defects, skeletal abnormalities, and genitourinary abnormalities including cryptorchidism have been reported in some cases. The extent of the chromosomal deletions in these patients is probably quite heterogeneous, so to date phenotype-genotype correlations have been impossible. Some of the same features, particularly a similar facial appearance, are seen in some patients reported to have terminal deletions of chromosome 14 (deletion of 14q32.3→qter) which do not overlap with the deletions of band 14q31.<sup>5</sup> We have used simple sequence repeat polymorphisms, principally

(CA)<sub>n</sub> repeats, localised to the distal portion of chromosome 14, to define at the molecular level the extent of the interstitial 14q31 deletions in three patients (HSC 1251, 1141, and 1337).

Analysis of the 14q31 deletions in our patients refines the localisation of a number of markers within the region. The gene responsible for Machado-Joseph disease (MJD) has been localised to this chromosome region by linkage analysis in Japanese<sup>6,7</sup> and Azorean families.<sup>8</sup> MJD is a multisystem neurodegenerative disease inherited as an autosomal dominant trait. The gene, recently cloned,<sup>9</sup> contains a trinucleotide repeat, which is expanded in MJD. The gene lies close to or within the deleted region in two of the patients.

## Materials and methods

### PATIENTS

#### Patient 1 (HSC 1251)

This female patient has been reported previously as having a cytogenetically detectable deletion of all of chromosome band q31 (deletion of 14q24→14q32.1).<sup>2</sup> She had some of the characteristic facial features seen in other cases with interstitial deletions of chromosome 14: bushy eyebrows, downward slanted palpebral fissures, epicanthic folds, protuberant ears, an upturned nose with wide nasal bridge, micrognathia, and dental abnormalities. She also has mild psychomotor retardation. Both parents had normal karyotypes and were available for study.

#### Patient 2 (HSC 1141)

This female patient was initially reported as having a terminal deletion of chromosome 14 (deletion of 14q31.1→qter).<sup>4</sup> She has not been located for repeat karyotype analysis. She had microcephaly, protuberant ears, epicanthic folds, micrognathia, gingival hypertrophy, and a narrow palate. Her motor milestones were normal, but she was diagnosed as having mild developmental delay at 5 years of age. Both parents had normal karyotypes.

#### Patient 3 (HSC 1337)

This patient and his family have not been reported elsewhere. The proband was referred for assessment of developmental delay. He was the product of a normal pregnancy and delivery; birth weight was 4600 g. Early gross motor milestones were normal, but at the age of 5

The Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada  
B C Byth  
D W Cox

Department of Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada  
M T Costa  
I E Teshima

Departments of Paediatrics and Molecular and Medical Genetics, University of Toronto, Ontario, Canada  
I E Teshima  
D W Cox

Department of Pediatrics, University of Virginia Health Sciences Centre, Charlottesville, VA 22908, USA  
W G Wilson

Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK  
N P Carter

Correspondence to: Dr Cox.

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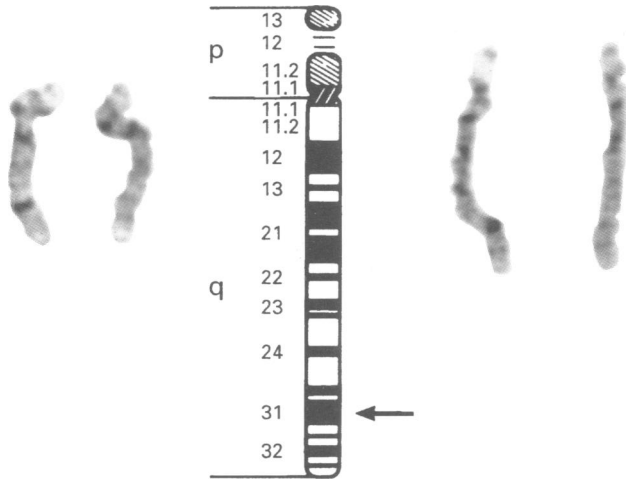


Figure 1 Ideogram of chromosome 14 with a pair of chromosomes 14 and *del(14)* from patient 3 on either side; in each pair the normal 14 is to the left and the *del(14)* is to the right. The arrow indicates the band that is partially deleted.

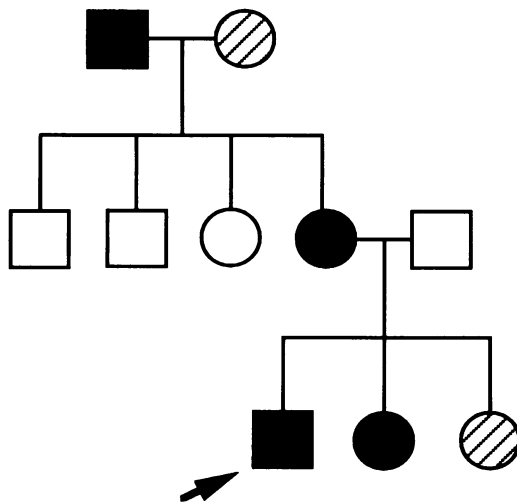


Figure 2 Pedigree of patient 3 (indicated by the arrow). Filled symbols indicate the presence of the deletion. Shaded symbols indicate subjects tested and shown to be of normal karyotype.

years he was lagging in all areas especially in speech and language skills. Physical examination was normal except for cupped ears. Chromosome analysis showed an interstitial deletion of part of chromosome band 14q31

(fig 1). The same abnormality was found in a younger sister with a similar phenotype, and in his mother and maternal grandfather (fig 2). The mother had delayed speech development and learning difficulties; she functions in the borderline normal IQ range. Her three sibs are intellectually normal, two having completed university education. Her physical examination was normal except for cupped ears. The grandfather completed elementary school and worked successfully as a tradesman until retirement. He had cupped ears but no other dysmorphic features. DNA samples were obtained from both parents and from the maternal grandparents.

#### ANALYSIS OF SIMPLE SEQUENCE REPEAT POLYMORPHISMS

Fourteen markers localised to the distal portion of chromosome 14 were used in the analysis (table). These markers are polymorphic (CA)<sub>n</sub> repeats, with the exceptions of the AACT marker, which is a complex dinucleotide repeat (TA)<sub>n</sub>(GA)<sub>n</sub>,<sup>15</sup> and of D14S140 which is a tetranucleotide repeat.<sup>13</sup> References to primer sequences are given in the table. Genomic DNA (10 ng) was amplified in 10 µl PCR reactions containing the following reagents: 50 mmol/l KCl, 10 mmol/l Tris-Cl pH8.4, 1 or 2 mmol/l MgCl<sub>2</sub>; 200 µmol/l dCTP, dGTP, dTTP, 25 µmol/l dATP; 2 µCi α-[<sup>35</sup>S]-dATP; 0.5 µmol/l each primer and 0.5 U Amplitaq (Perkin Elmer). The amplification conditions were 30 cycles of: denaturing 94°C/30 seconds, annealing 30 seconds, extension 72°C/30 seconds. The PCR products were resolved on 6% sequencing gels and visualised by autoradiography. The D14S140 marker was analysed without the inclusion of α-[<sup>35</sup>S]-dATP on 5% non-denaturing polyacrylamide gels stained with ethidium bromide.

The order of the markers was derived from linkage analysis,<sup>11,16</sup> from physical mapping studies in somatic cell hybrids,<sup>17</sup> and from the study of a person with a balanced translocation in which D14S48 was proximal to D14S68 and D14S73.<sup>18</sup> The order of markers D14S68, D14S73, and D14S67 used here was derived from analysis of loss of heterozygosity in renal cell tumours.<sup>19</sup> D14S140 was placed by analysis of recombinant subjects in four pedigrees from the CEPH reference collection (CEPH 1332, 28, 35, and 45) (Byth and Cox, unpublished data).

#### Results of molecular analysis of deletion patients

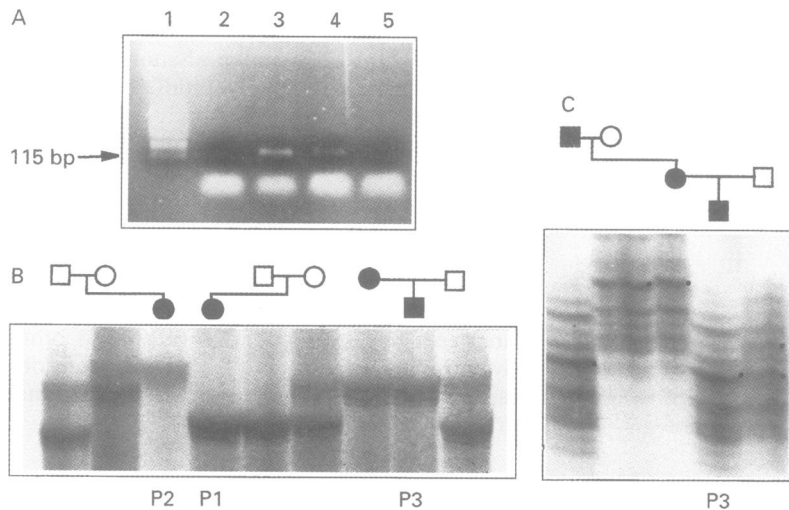
| Marker  | Reference | Patient 1  |    | Patient 2 |   | Patient 3 |   | Mother of patient 3 |   |
|---------|-----------|------------|----|-----------|---|-----------|---|---------------------|---|
|         |           | p          | m* | p         | m | p         | m | p                   | m |
| D14S43  | 10        | +          | +  | +         | + | +         | + | ND                  |   |
| D14S76  | 11        | 0          |    | +         | + | +         | + | ND                  |   |
| D14S61  | 11        | +          | +  | +         | + | 0         |   | ND                  |   |
| D14S53  | 12        | 0 (FS+ -)† |    | +         | + | +         | + | +                   | + |
| D14S74  | 11        | 0          |    | -         | + | +         | + | ND                  |   |
| D14S55  | 12        | 0 (FS+ -)  |    | 0         |   | +         | + | ND                  |   |
| D14S48  | 12        | 0          |    | -         | + | 0         |   | 0                   |   |
| D14S140 | 13        | -          | +  | 0         |   | +         | + | 0                   |   |
| D14S68  | 11        | -          | +  | -         | + | 0         |   | 0                   |   |
| D14S73  | 11        | 0 (FS+ -)  |    | 0         |   | +         | - | -                   | + |
| D14S67  | 11        | -          | +  | -         | + | +         | + | +                   | + |
| D14S81  | 11        | +          | +  | +         | + | +         | + | ND                  |   |
| PI      | 14        | +          | +  | +         | + | 0         |   | +                   | + |
| AACT    | 15        | +          | +  | +         | + | ND        |   | ND                  |   |

\* p = paternal allele; m = maternal allele; + denotes presence and - absence of allele; 0 denotes an uninformative result; ND = typing not done.

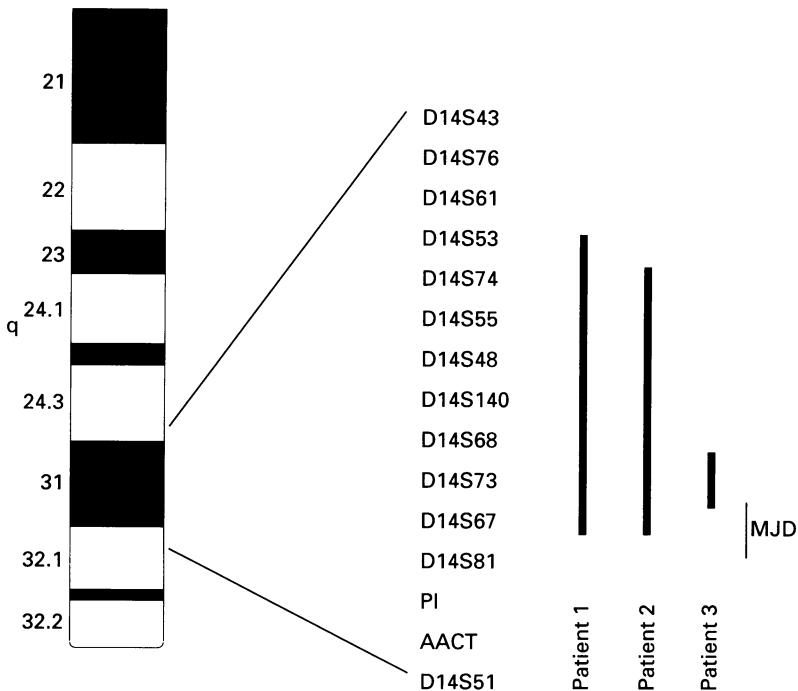
† FS indicates the use of PCR analysis of flow sorted chromosomes for patient 1. Results are given first for the normal chromosome, second for the deleted chromosome.

#### ANALYSIS OF FLOW SORTED CHROMOSOMES FROM PATIENT 1

Flow sorted chromosomes were obtained for patient 1. The deleted and normal chromosomes 14 were separated using bivariate flow karyotype analysis in which the chromosomes were stained with both chromomycin A3 and Hoeschst 33258. The chromosomes were sorted directly into PCR tubes containing 30 µl of sterile distilled water by using a dual laser flow cytometer (FACStar Plus, Becton Dickinson) as described previously.<sup>20</sup> Aliquots of approximately 300 chromosomes were used



**Figure 3** (A) Analysis of flow sorted chromosomes from patient 1 at the D14S67 locus. Lane 1: DNA size marker; lane 2: patient's deleted chromosome 14; lane 3: patient's normal chromosome 14; lane 4: positive control (genomic DNA); lane 5: negative control (no DNA). The size of the amplified product (115 bp) is indicated. This locus is absent from the patient's deleted chromosome 14 and present on her normal chromosome 14. (B) Results of heterozygosity analysis in the three patients at the D14S48 locus. Patient 2 has a deletion of paternal origin at this locus; patients 1 and 3 are uninformative. (C) Results of heterozygosity analysis of patient 3, his parents, and maternal grandparents at the D14S73 locus. Patient 3 and his mother have deletions of, respectively, maternal and paternal origin. The major allelic bands are indicated by dots.



**Figure 4** Diagram of the distal portion of chromosome 14q showing the extent of the deletions in the three patients and the possible location of the MJD gene from Sasaki *et al.*<sup>7</sup> and St George-Hyslop *et al.*<sup>8</sup>.

in the PCR amplification. The PCR conditions were essentially the same as described above, except that 35 cycles of amplification were performed, the  $\alpha$ -[<sup>35</sup>S]-dATP was omitted, and the concentration of dATP increased to 200  $\mu$ mol/l, and the products were analysed on agarose gels.

### Results

The results of the analyses are given in the table, and examples for three markers, D14S67, D14S48, and D14S73, are shown in fig 3.

Patients are defined as heterozygous (+ +) or hemizygous (+ -) depending on the inheritance of alleles from their parents. Where the parents share a common allele and the patient has inherited only this allele, it is impossible to determine whether the patient has one or two copies and the result is defined as uninformative (0). The maternal grandparents of patient 3 were also studied.

Patient 1 is deleted at the loci D14S53, D14S55, D14S73, and D14S67, as determined from PCR analysis of the flow sorted chromosomes (fig 3). She is deleted for the paternal allele at D14S140, D14S68, and D14S67, and is uninformative for intervening loci (D14S74 and D14S48). This is consistent with a deletion of paternal origin extending from D14S53 to D14S67. Biochemical analysis showed that her serum concentration of  $\alpha_1$ -antitrypsin was 96% of normal, whereas that of  $\alpha_1$ -antichymotrypsin was 43% of normal. The  $\alpha_1$ -antitrypsin (PI) and  $\alpha_1$ -antichymotrypsin (AACT) genes are both localised to chromosome 14q32.1.<sup>21</sup> The observations led the authors of the initial report to speculate that the AACT locus might be deleted on one of her chromosomes; however, the DNA analysis shows that she is heterozygous at this locus, and so her low serum level of  $\alpha_1$ -antichymotrypsin is unrelated.

Patient 2 was initially described as having a terminal deletion of chromosome 14. A retrospective examination of her karyotype analysis can alternatively be interpreted as an interstitial deletion. The DNA analysis shows that she is heterozygous, and therefore not deleted, for PI, AACT, and D14S81 and for a number of more telomeric markers (D14S13, D14S16, and D14S20; not shown).<sup>22</sup> She has a deletion of paternal origin at the D14S74, D14S48 (fig 3B), D14S68, and D14S67 loci, and is uninformative for the intervening markers D14S55, D14S140, and D14S73, consistent with an interstitial deletion extending from D14S74 to D14S67.

Patient 3 and his mother are deleted only at the D14S73 locus (fig 3C); the origin of the deletion is paternal in the mother and maternal in the patient, consistent with the cytogenetic findings. Both are heterozygous for D14S67, the locus flanking D14S73 on the telomeric side. Although both are uninformative for the locus centromeric to D14S73, D14S68, the grandfather is heterozygous at this locus. As he also carries the deletion, this result suggests that D14S68 does not lie in the deleted region.

The provisional order of markers in the region and the extent of the deletion in each patient is shown in fig 4. The deletions in patients 1 and 2 provide information on gene order as follows: locus D14S53 lies distal to D14S61 and D14S76, and proximal to D14S74 and D14S55; loci D14S68, D14S73, and D14S67 must lie proximal to D14S81.

### Discussion

There is evidence for a distinct clinical phenotype associated with interstitial deletions of distal chromosome 14 in the 14q31 region, which includes a characteristic facies, skeletal

abnormalities, dental abnormalities, and developmental delay. Here we present three patients with interstitial deletions of chromosome 14q31. One of the three patients, patient 1, is deleted for all of band 14q31 (deletion 14q24.3→14q32.1) and has the largest deletion defined by molecular analysis (D14S53 to D14S67). The physical size of band 14q31 can be estimated to be 8.8 Mb.<sup>23,24</sup> Patient 2, initially described as having a terminal deletion of chromosome 14, in reality has a smaller deletion of band 14q31 which overlaps with that of patient 1 (D14S74 to D14S67). The phenotypes of the two patients also overlap, although the abnormalities are non-specific; both have epicanthic folds, dental abnormalities, protuberant ears, narrowed palate, delayed speech, and mild developmental delay.

Patient 3 and his mother were defined by karyotype analysis as having an interstitial deletion of part of band 14q31 inherited from the maternal grandfather. They have the smallest deletion detected by molecular analysis, consisting of a single anonymous DNA marker, D14S73. Patient 3 also has the most normal phenotype, characterised by cupped ears and mild developmental and speech delay. It is difficult to estimate the amount of DNA deleted in the absence of a physical map of this region. However, the genetic distance between D14S48 and D14S81 has been estimated on the CEPH Consortium linkage map to be 10 cM, so the physical distance may also be small. The deletion in patient 3 lies within that of patient 1, and presumably within that of patient 2, who is uninformative at D14S73. The order of markers D14S68, D14S73, and D14S67 could not be confirmed by these deletions. The very small deletion seen in patient 3 and his mother is consistent with the mild phenotype; a number of small deletions or duplications of chromosome bands have been seen in phenotypically normal subjects.<sup>25</sup>

These results localise a number of genetic markers to the chromosome band 14q31, providing a link between the genetic and physical maps of chromosome 14. The order of markers derived from the deletions is consistent with the linkage maps<sup>11,16</sup> and the data from analysis of somatic cell hybrids.<sup>17</sup> Deletions in patients 1 and 2, but not likely in patient 3, may overlap with the region to which the gene for Machado-Joseph disease has been localised by linkage analysis, just proximal to D14S81 (fig 4).<sup>7,8</sup> Following the initial submission of this report, the MJD gene was cloned and found to contain an unstable repeat in the coding region. At present, it is not known whether MJD results from loss or gain of function. If the former is true, patients 1 and 2 may be at risk for the development of MJD and further analysis is required.

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