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Maternally inherited duplication of the possible imprinted 14q31 region

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The existence of parent of origin differences in the expression of some genes, a process known as genomic imprinting, has been recognised and documented over the past several years. This epigenetic marking process results in the differential expression of normal genes, depending on whether they are of maternal or paternal origin. A number of human disorders have been identified as resulting from alterations in genomic imprinting.¹ One type of genetic abnormality which can unmask genomic imprinting is uniparental disomy (UPD), in which both chromosomes of one pair are inherited from one parent, with no contribution from the other.² Distinct phenotypes exhibited by patients with maternal and paternal UPD(14) strongly suggest that at least some genes on human chromosome 14 are subject to imprinting effects.³⁻⁶ This finding is supported by studies in the mouse indicating that the distal portion of chromosome 12, recognised as a candidate imprinted region, is syntenic with human chromosome 14q.⁷

Nevertheless, no imprinted genes have yet been identified on chromosome 14, and the location of the imprinted region on human chromosome 14 remains unclear.

Analysis of parental origin effects in human trisomy for chromosome 14q⁸ or monosomy for the same chromosome⁹ makes the region 14q23-q32 a candidate region for containing imprinted genes.¹⁰ Moreover, the observation of a partial duplication of 14q in a developmentally delayed girl with minor abnormalities and her phenotypically normal father led Robin *et al*¹¹ to propose that the 14q24.3-q31 region may be imprinted. As genotype-phenotype correlations in patients with aneuploidy for this region may help the identification of imprinted genes on human chromosome 14, we report here a direct 14q31 duplication observed in a child with mild developmental delay and his phenotypically normal mother. This duplication was also detected in five relatives with a normal phenotype and maternal inheritance through three generations.

Figure 1 Partial R and G banded karyotype from the proband showing the normal (left) and the duplicated (right) chromosomes 14.

Case report

Our patient, a 2 month old boy, was referred following a cyanotic episode. He was the first child of healthy, unrelated parents. The family history was uneventful and he was born by caesarean section. Birth weight, length, and head circumference were 3515 g, 54 cm, and 33 cm, respectively. At the age of 2 months, clinical examination showed dysmorphic features (macrosomia, metopic suture, neck hyperextension without opisthotonos, large nose, prominent philtrum, gingival hyperplasia, and bilateral palmar simian crease) and axial hypotonia with moderate distal hypertonia. EEG and MRI scan of the brain, medullary ultrasound, abdominal ultrasound, pulmonary and skeletal x ray, ECG and retinal examination were all normal. The patient walked at 20 months. By the age of 27 months, clinical evaluation of the patient showed very slight dysmorphism, slight overgrowth (+2 SD),

moderate psychomotor delay with specific speech delay, hypotonia, and behavioural and sleep disturbance.

Routine R and G banding techniques, performed on metaphases from PHA stimulated lymphocytes of the proband, showed an elongated chromosome 14 with an extra G positive band on the long arm (fig 1). High resolution banding suggested that the patient had a duplication of band 14q31. The same 14q duplication was found in the proband's mother and four other members of his family, all of whom had a normal phenotype (fig 2A).

Fluorescence in situ hybridisation (FISH) was performed with various probes, in order to characterise the rearrangement. A chromosome 14 specific paint, used as recommended by the manufacturer (ONCOR), confirmed that the extra material originated from chromosome 14 (data not shown). FISH experiments using YAC probes estimated to cover the 14q31 band were performed, in order precisely to define the duplicated chromosomal region in the proband. The YAC clones 813e6, 930c12, 749g4, 815d5, 872e3, 756a4, 945d2, 856g8, and 965b9, obtained from the CEPH (Centre d'Etude du Polymorphisme Humain), were labelled with biotin-14dCTP or digoxigenin-11dUTP by random priming (Bioprime DNA labelling system, Life Technologies and Dig high prime, Roche Diagnostics), then hybridised on metaphase chromosomes or interphase nuclei. The hybridised biotin signals were made visible with fluorescein labelled avidin, and the digoxigenin signals were visualised with rhodamine labelled anti-digoxigenin antibody following standard protocols.¹² The results are summarised in fig 2B. The more proximal YACs (813e6 and 930c12), as well as the more distal ones (945d2, 856g8, and 965b9), gave signals of equal intensity on the normal and the duplicated chromosomes 14, indicating the presence of only two copies in the patient's genome. In contrast, YACs 749g4, 815d5, 872e3, and 756a4 showed two signals

Figure 2 (A) Pedigree of the proband (indicated by an arrow). (B) The different YACs used for delineating the extent of the duplication are listed on the right of the chromosome 14 diagram, with their normal (-) or duplicated (+) status.

Figure 3 Double FISH experiment both with a YAC included in the duplication (YAC 815d5, green signal) and a YAC located outside the duplication (YAC 965b9, red signal). Chromosomes and nuclei were counterstained in blue with DAPI. (A) Metaphase of the proband showing the superimposition of the green and red signals, resulting from the condensation of the metaphase chromosomes. (B) Partial karyotype from the same metaphase, showing the normal (left) and the duplicated (right) chromosomes 14. Both green and red signals have been dissociated in order to evaluate their size better. The green signal (YAC 815d5) located on the duplicated chromosome 14 is larger than the one located on the normal chromosome 14. It is also larger than each red signal (YAC 965b9) on both chromosomes. (C) In interphase nuclei, two red (YAC 965b9) and three green (YAC 815d5) signals are visible. The three green signals correspond to one copy on the normal and two copies on the duplicated chromosome 14.

of different sizes, the larger one being located on the duplicated chromosome 14. In order to confirm these results, two YACs with different FISH patterns were cohybridised on the same

Figure 4 The orientation of the duplication was studied by dual colour FISH on interphase nuclei, using YACs located at the borders of the duplicated segment: YAC 749g4 (green signal) at the proximal edge and YAC 756a4 (red signal) at the distal edge. Alternating of red and green signals shows the direct orientation of the duplication on the rearranged chromosome.

metaphase preparations: YAC 815d5 (green signal) presumed to be included in the duplication and YAC 965b9 (red signal) presumed to map outside the duplication. As expected, the green signal (815d5) on the duplicated chromosome 14 was larger than the red signal (965b9) on the same chromosome (fig 3A, B). The same experiment was performed on interphase nuclei, where the undercondensation of the chromatin allows increased resolution. This allowed the detection of two red signals (YAC 965b9) and three green signals (YAC 815d5), clearly showing a duplication of the region covered by YAC 815d5 (fig 3C). Different YAC probes were hybridised with metaphase spreads from subjects III.2 and III.3, confirming the presence of the same 14q duplication in the proband and his relatives.

In order to determine whether the duplication is direct or inverted, the proximal and distal duplicated YACs (749g4 and 756a4 respectively) were labelled differently, hybridised to interphase nuclei, and visualised with specific fluorochromes. Alternate green and red signals observed in nuclei show that the two copies of the duplication are organised in direct orientation on chromosome 14 (fig 4).

In order to gain insight into the mechanism that gave rise to this duplication, we used mic-

Figure 5 Polymorphism analysis with two informative markers included in the 14q31 duplication, D14S1052 and D14S67. For the proband (IV.1), his mother (III.2), and his uncle (III.3), all carrying the duplication, only two distinct alleles are identifiable, suggesting the monochromosomal origin of the rearrangement. The arrows indicate the allele shared by the three subjects, corresponding to the abnormal chromosome.

rosatellite analysis to determine whether the duplication originated from one or two homologous chromosomes 14. Genomic DNA from the proband and other family members was extracted from whole blood samples using standard methods. DNA polymorphisms were analysed, as previously described,¹³ using polymerase chain reaction (PCR) amplification of short sequence repeats from the following markers: D14S68, D14S67, D14S1052, and D14S1033 (Genethon). For three informative markers located in the duplicated region (D14S68, D14S67, and D14S1052), only two different alleles were observed in the patient (IV.1), his mother (III.2), and his uncle (III.3) (fig 5). One of these alleles is shared by these three members of the family and probably represents the abnormal chromosome 14. This result suggests a monochromosomal origin of the duplicated segment, probably resulting from unequal sister chromatid exchange.

Finally, on the basis of the genetic map of chromosome 14,¹⁴ we were able to estimate the approximate length of the duplicated segment to be between 3 and 9 cM. These data are in agreement with our cytogenetic observations, as the duplication corresponds approximately to cytogenetic band 14q31, whose physical size is estimated to be 8.8 megabases.^{15 16}

Discussion

In this study, we describe a phenotypically abnormal patient carrying a duplication of the 14q31 band, which occurs in the potentially imprinted region on this chromosome.^{8 9 10 11} The duplication, which was also found in five other family members, was maternally inherited through three generations. Apart from the proband (IV.1), who has minor dysmorphic features and psychomotor delay associated with behavioural and sleep disturbance, all family members carrying the abnormal chromosome are phenotypically normal. This suggests that the abnormal phenotype of the proband is probably unrelated to the dupli-

cated segment. Moreover, duplication of the 14q31 band, when maternally inherited, does not seem to be associated with an abnormal phenotype.

It has been suggested by Robin *et al*¹¹ that a similarly duplicated chromosome 14 (duplication 14q24.3-q31), if paternally transmitted, does induce an abnormal phenotype. This parent of origin dependent phenotypic disparity associated with similar duplications suggests a possible imprinting effect. The fact, however, that the duplications are not identical allows three different hypotheses to be formulated with respect to imprinting effects.

Firstly, the imprinted genes could be located outside the 14q24.3-q31 interval. This region may contain genes that do not have a deleterious effect if overexpressed, and duplication will therefore produce no phenotypic effect. In that case, the association of the 14q duplication with an abnormal phenotype, both in the proband described here and in that described by Robin *et al*,¹¹ would be merely coincidental.

Secondly, the imprinted genes could be located in band 14q24.3 and not in band 14q31. In that case they would be duplicated in the patient described by Robin *et al*¹¹ but not in the family described here. In such a situation, no parent of origin phenotypic effect would be associated with a 14q31 duplication. Then, a 14q31 duplication of paternal origin would be associated with a normal phenotype, as we show to be the case here for a 14q31 duplication of maternal origin. This hypothesis is supported by the fact that G positive bands, such as the 14q31 band, contain very few genes.¹⁷

Thirdly, imprinted genes could be located in band 14q31 and duplicated both in the family reported here and in that of Robin *et al*.¹¹ If this were the case, both observations are in agreement and suggest that imprinted genes from this region are paternally expressed. However, the presence of paternally expressed genes only is not consistent with the observation that the maternal UPD(14) phenotype is moderate compared to the paternal UPD(14) phenotype. This would suggest that maternally expressed genes are located elsewhere in the 14q23-q32 region. Unfortunately, it is not possible to choose between these three hypotheses because no case of paternally inherited 14q31 duplication has yet been described.

So far, only a few genes have been mapped to the human 14q31 band (NCBI <http://www.ncbi.nlm.nih.gov>), and none of them has been shown to be imprinted. Those that have been mapped include the genes encoding galactosylceramidase (*GALC*), the thyroid stimulating hormone receptor (*TSHR*), the suppressor of lin-12 (*C elegans*)-like (*SEL1L*), neurexin III (*NRXN3*), and a member of the G protein coupled receptor superfamily (*GPR68/OGRI*).

Further studies of chromosome 14 aneuploidy are needed in order to define the imprinted region better and identify the corresponding genes.

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A novel missense mutation in the GTPase activating protein homology region of *TSC2* in two large families with tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder (OMIM 191092) characterised by autism, seizures, mental retardation, benign tumours of the brain, heart, kidney, lung, and skin, and malignant tumours of the kidney.¹ TSC has a wide range of phenotypic variability, with some subjects severely affected and others only mildly affected. There are two TSC genes, *TSC1* on chromosome 9q34² and *TSC2* on chromosome 16p13.³ Approximately two thirds of cases of TSC appear to result from de novo germline mutations.

In 1994, an extended four generation family with 19 affected members was reported in which 34 members (17 affected with TSC and 17 unaffected) underwent both physical and psychiatric assessments.⁴ The majority of the affected subjects had mild physical expression of TSC, but there was significant clustering of neuropsychiatric disorders among affected subjects compared with their unaffected relatives. The disorders that were over-represented included mood disorder, anxiety disorder, and autism. The largest difference was observed in anxiety disorder, which was seen in 10 of the affected subjects and in two of the unaffected subjects ($p=0.016$). One affected child had

pervasive developmental disorder and one had autism. Analysis of this family suggested that TSC could present phenotypically with mild physical signs and symptoms, but with significant neuropsychiatric disease. Linkage to the *TSC2* gene locus on chromosome 16p13.3 was shown with a lod score of over 3.⁴

We report here the identification of a missense mutation in exon 34 of the *TSC2* gene in affected members of this family. We also examined a second four generation family⁵ from the same geographical area as the first family but not known to be related to them. The same exon 34 mutation was found in affected members of the second family.

Methods

To search for mutations in the coding regions of the *TSC2* gene we used single strand conformation analysis (SSCP). The primers amplifying each of the 41 exons of *TSC2* and the PCR conditions have been described previously.⁶ The PCR products were run on MDE gels (AT Biochem). To maximise the detection of variant bands, each PCR product was run on two gels, one without glycerol and one with 5% glycerol. Samples in which variant bands were detected were reamplified and sequenced. This