Translocation between chromosomes 6 and 15 (45,XX,t(6;15)(q25;q11.2)) with further evidence for lack of imprinting of the insulin-like growth factor II/mannose-6-phosphate receptor in humans

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Abstract

We report a 24 year old female with growth retardation, microcephaly, and congenital abnormalities who has an unbalanced de novo translocation between chromosomes 16 and 6: 45,XX,t(6;15)(q25;q11.2). FISH analysis confirmed that the deletion on chromosome 15 is proximal to the Prader-Willi locus. Several genes have been assigned to the 6q25-qter region including the insulin-like growth factor II/mannose-6-phosphate (IGF-II/M6P) receptor. DNA analysis from our patient documented the loss of one IGF2R gene copy. These data confirm the localisation of the IGF2R receptor to distal 6q25.

We also showed reduced expression of the soluble and membrane bound IGF-II receptor, a gene dosage effect incompatible with imprinting. The IGF2R gene has been shown to be imprinted in the mouse but not in humans. Our data provide further evidence for lack of imprinting of this gene in humans.

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Key words: chromosome 6;15 translocation; mannose-6-phosphate receptor; imprinting.

The insulin-like growth factor II/mannose-6-phosphate (IGF-II/M6P) receptor gene has been mapped to 6q26-27.^{1,2} IGF2R is located in a region of other syntenic genes on human chromosome 6 and mouse chromosome 17.

This receptor is imprinted in the mouse.³ Mouse embryos inheriting a maternal *Igf2r* deleted chromosome totally fail to express *Igf2r* and are not viable in utero. A paternal deletion has no effect on expression, suggesting that only the maternal copy of the gene can be transcribed. In contrast, in humans, Ogawa *et al*⁴ and Kalscheuer *et al*⁵ have shown that the IGF-II receptor is expressed equally from maternal and paternal alleles in human tissues.

We report on a patient with a 6;15 de novo translocation including a deletion of the IGF2R gene. Cytogenetic studies indicate that the chromosome breakpoints are at 6q25 and 15q11.2. This case provides further confirmation by gene dosage studies on genomic

DNA, showing a heterozygous deletion, that the IGF2R gene is distal to 6q25. In addition biochemical gene dosage effect studies show half normal expression of the protein product confirming expression studies^{4,5} that this gene is not imprinted in humans.

Clinical data

The patient is a white female, the third child born to non-consanguineous parents. The mother was aged 39 at conception and the father was aged 49. The pregnancy and delivery were uncomplicated. Birth weight was 3070 g (5th centile) and head circumference 32 cm (5th centile). At birth she was noted to have bilateral choanal atresia, hypotonia, and congenital hip dislocation. Since birth she has shown global developmental delay with growth retardation, height, weight, and head circumference remaining below the 2nd centile.

On examination at the age of 24 her height was 1.49 m (<2nd centile), weight was 40 kg (<2nd centile), and head circumference was 51 cm (<2nd centile). The face was triangular with a long, narrow forehead, a high nasal bridge, and malar hypoplasia. Hypotelorism was present with mild left esotropia. The palate was high arched. The teeth were crowded with gingival hypertrophy. There was mild hirsutism and a low posterior hairline. The patient had small hands with proximally placed thumbs. Skeletal abnormalities included a right convex thoracic and left lumbar scoliosis with absence of the 12th ribs bilaterally. Sexual development was Tanner stage V.

Methods

CHROMOSOME ANALYSIS

CTG banding was performed on peripheral blood lymphocytes of the patient and her parents.

FLUORESCENT IN SITU HYBRIDISATION

Cells were synchronised overnight with thymidine, then washed three times, and processed for chromosome preparation after release of the block with bromodeoxyuridine (BrdU).

Probes D15S10, D15S11, and GABRB3, mapping to 15q11-q12 (control probe PML, mapping to 15q22), from Oncor were used and FISH was performed according to the manufacturer's instructions.

The digoxigenin labelled probe was tagged with FITC and the chromosomes were counterstained with propidium iodide.

The images were captured with a CCD camera, using an image analysis software (Applied Imaging). Ten cells were examined per probe.

GENE DOSAGE MEASUREMENT OF THE IGF-II/M6P RECEPTOR

Quantitative PCR was used: IGF2R: sense primer: GTCATTAATGTGTCCCCTGAGT; antisense primer: TTGCATGATATCT

TCAAGCCT. IGF1: sense primer: ACAAGCCCACAGGGTATGGCT; antisense primer: CTTCTGGGTCTTGGGCATGTC.

Both pairs of primers were added to the same PCR tube with 200 ng of DNA, 100 µmol/l of dCTP, dGTP, and dTTP, 50 µmol/l of dATP, 5 µCi of ³²P-dATP, 2.5 U of *Taq* polymerase (BRL), in a total volume of 50 µl of the buffer provided by the *Taq* supplier, the MgCl₂ concentration adjusted to 1.5 µmol/l.

The reaction was "hot started" by adding the *Taq* polymerase only after the rest of the reaction mixture had been heated to 94°C. Following three minutes of denaturation at 94°C, 22 cycles were performed, involving 30 seconds denaturation at 94°C, 30 seconds annealing at 60°C, 30 seconds ramp to 72°C, and 90 seconds extension at 72°C. A 10 minute final extension step was added after the last cycle.

The radiolabelled PCR products were resolved in 8% polyacrylamide and the total intensity of the bands quantitated by contact exposure to a phosphor plate using the Fuji Bio Imager. The ratio of the IGF2R band over IGF1 was calculated for the patient and each control. The patient's value was then expressed as a percentage of each control in the same experiment.

RELATIVE QUANTITATION OF THE SOLUBLE IGF-II/M6P RECEPTOR

We used the method described by Hardoin *et al.*⁶ One microlitre of serum was denatured in SDS and subjected to denaturing polyacrylamide gel electrophoresis (SDS-PAGE) in a BioRad mini apparatus, followed by electroblotting on nitrocellulose, and renaturing of the protein by extensive washing. The membrane was probed with radioiodinated IGF-II. The peptide was a gift of Eli Lilly (Indianapolis, IN) and was labelled by the chloramine T method to a specific activity of approximately 150 Ci/g. Autoradiographs of the blot were quantitated on an LKB laser densitometer.

RELATIVE QUANTITATION OF THE PHYSIOLOGICALLY ACTIVE MEMBRANE ASSOCIATED RECEPTOR

Cells were grown in 10% fetal bovine serum, harvested at confluence, and homogenised in 3M sucrose. Receptor bearing membranes (plasma membranes and Golgi endosomes) were obtained by high speed centrifugation (100 000 × *g* for one hour) following partial purification with a low speed run (15 000 × *g* for 15 minutes). The membranes were labelled with radioiodinated IGF-II by incubating overnight at 4°C in a shaking bath, in the presence or absence of excess unlabelled IGF-II.⁷ After washing, hormone receptor complexes were cross linked with 2% disuccinimidyl suberate, and the membranes were solubilised and subjected to SDS-PAGE on 7% polyacrylamide. The gel was dried and exposed to x ray film. The intensity of the bands was quantitated using an LKB laser densitometer.

Figure 1 Prophase banded karyotype of the patient.

Figure 2 FISH study of fibroblast chromosomes. This illustration is of probe D15S11 and control probe PML, mapping to 15q22 (Oncor). Two signals are seen on the normal chromosome 15 (A) as well as the translocated 15;6 (B).

Figure 3 (A) Linear relation between PCR template and product. The indicated amounts of purified DNA were amplified, as described in the text, in the presence of radioactive dATP. The sizes of the bands for IGF2R and IGF1 are 204 and 180 bp respectively. The gel separating the PCR products was subjected to quantitative phosphor imager analysis. The radioactivity, measured in arbitrary units, is shown below each band. (B) Comparison of patient with normal controls: 200 ng of DNA from the patient (P) and a normal control (C1, C2) were amplified for 22 cycles with pairs of primers for both the IGF2R and IGF1 fragments in the same tube in three different experiments. The intensity of the bands was quantitated with a Fuji Bio Imager and the ratio of the IGF2R fragment over the control IGF1 fragment was taken as an index of gene dosage.

Results

High resolution GTG banding analysis on cultures of peripheral blood lymphocytes from the proband and her parents was performed and showed an unbalanced translocation: 45,XX,t(6;15)(q25;q11.2) (fig 1). We were unable to identify the parental origin of the deleted chromosome as the mother had died and the father was lost to follow up. There were no informative heteromorphisms on the paternal chromosome 15.

The FISH studies indicated that the breakpoint is proximal to the Prader-Willi/Angelman region on chromosome 15. All cells showed the signal for each probe used, both on the normal chromosome 15 and the derivative

chromosome 6 as well as the control signal (fig 2).

IGF2R gene dosage was determined by quantitative PCR. The patient's DNA was used as template to amplify a 204 bp IGF2R cDNA fragment, corresponding to bases 8141-8345 of the published sequence.⁸ An 180 bp fragment of the third exon of the insulin-like growth factor I gene (IGF1) on chromosome 12 was also amplified as a reference sequence, since the patient had no detectable cytogenetic abnormality at that locus. The ratio of the intensities of the IGF2R and IGF1 PCR products was compared between the patient and four controls in three separate experiments.

Preliminary work showed, under these conditions, a linear relationship between the amount of DNA template and the intensity of each of the two PCR product bands over a 25-fold range of amount of template added (fig 3A).

The ratio of the IGF2R band over the IGF1 band in the DNA of four normal subjects in three separate amplifications ranged from 0.79 to 0.93. The corresponding ratio in the patient's DNA, amplified along side the controls in each experiment, was consistently lower. Expressed as a percentage of the corresponding control ratio, it was 56%, 37%, 42%, and 58%, giving a mean of 48.25% consistent with half the IGF2R DNA dosage in the patient.

A truncated form of the IGF-II/M6P receptor has been shown to circulate in human plasma. This 230 kDa proteolytic cleavage form comprises practically all of the extracellular domain, retains full binding activity for both IGF-II and M6P, and appears to correlate with the general abundance of the receptor molecule in tissues.^{9,10}

Relative quantitation of this receptor form was performed in the patient's plasma using the technique of ligand blotting.⁹

The 230 kDa bands, representing the soluble IGF-II/M6P receptor in the serum of the patient, were compared with those of seven healthy young adult women (fig 4). The bands in the two lanes representing the patient were clearly the lowest of the group. By densitometry, the intensities of the bands were 0.5 and 0.32 densitometric units for the patient and 1.20 (SD 0.4) for the controls.

Relative quantitation of the physiologically active membrane associated receptor was also performed, using 100 µg of purified microsomal membrane protein from early passage cultured fibroblasts from the patient and an age matched normal female control, by affinity cross linking.

Again, the intensity of the 250 kDa IGF-II/M6P-R band of the patient was approximately half that of the control (fig 5).

Discussion

We describe an additional case of terminal deletion of 6q. This deletion was first reported by Mikkelsen and Dyggve¹¹ in 1973. At least 20 cases of terminal deletion of 6q have been reported to date.¹²⁻¹⁴ As described, distal deletion of 6q23 or 6q25 may cause various major

Figure 4 Quantitation of the soluble form of the IGF-II/M6P receptor in serum by western blotting. Serum from the patient (P) and seven sex and age matched controls (C1–C7) was electrophoresed, blotted on nitrocellulose, and probed with radiolabelled IGF-II. The bands were quantitated by densitometry. Similar results were obtained in one replicate experiment using the same samples.

Figure 5 Low passage fibroblasts from the patient and an age and sex matched control were homogenised and a membrane fraction was isolated by sucrose gradient centrifugation. One hundred micrograms of membrane protein was affinity cross linked with radiolabelled IGF-II in the presence or absence of an excess unlabelled peptide. The membranes were then solubilised and subjected to SDS-PAGE and autoradiography.

malformations, such as malformations of the eye, internal organs, and brain. Our patient showed the common features of distal chromosome 6 deletion including mental retardation and hypotonia, growth deficiency, microcephaly, epicanthus, and hand abnormalities. Specifically as a feature of deletion at 6q25, this patient, in common with the 6q25 deletion case of Liberfarb *et al.*,¹⁵ has choanal atresia indicating that this may be specific to deletions distal to this band.¹⁵ In common with the 6q25.3 deletion case of Meng *et al.*,¹² our patient manifested a long upper lip, scoliosis, and hypertrichosis. We did not detect internal malformations such as urological, cardiovascular, or structural brain anomalies.

As IGF2R has a role in the internalisation of IGF2 at the cell surface and leads to degradation of IGF2, it has been suggested that IGF2R regulates IGF2 levels in the circulation, hence influencing growth, which may explain prenatal onset of the growth retardation evident in 6q deletion.¹⁶

This case provided us with the opportunity to study further the effects of deletion of one copy of the IGF2R mapped to 6q26–27. It is believed that IGF-II exerts its mitogenic actions by binding to the type I IGF receptor, homologous to the insulin receptor. In addition, IGF-II binds to the IGF-II/M6P receptor, a membrane glycoprotein that does not recognise insulin or IGF-1 but binds lysosomal enzymes and is essential for their targeting to the lysosomes.^{8,10} The biological actions, if any, of IGF-II via this receptor remain to be defined. A soluble, truncated form of IGF-II/M6P-R can be measured in human serum.⁹ It is believed to represent a degradation pathway for, and a reflection of the abundance of, the membrane associated receptor.¹⁶

Interestingly, the mouse IGF-II gene (*Igf2*) is imprinted, as transgenic mice carrying an *Igf2* disruption totally fail to express IGF-II when they inherit the disruption from their father but have normal transcript levels when the same disruption is inherited from the mother.¹⁷ The intrauterine growth failure phenotype of these mice with normal postnatal growth has confirmed, in the mouse, the role of IGF-II as a fetal growth factor.¹⁷ In contrast, the gene encoding the IGF-II receptor undergoes paternal repression.³ This unique situation of imprinting, in opposite parental genders, of the genes coding for a ligand receptor pair provides a rare opportunity to gain insight into the process of parental imprinting.

The measurement of the soluble and membrane associated forms of IGF-II/M6P-R in our patient shows it to be significantly lower than in age matched controls. In addition, quantitative DNA studies indicated that one gene copy is deleted.

This locus has been shown to be parentally imprinted in the mouse. If this were indeed an imprinted locus in the human, then viability of a 6q deletion would be dependent on inheritance of the deleted chromosome from the father. In the presence of parental imprinting and the coexistence of a parental deletion one would expect to observe an all or none phenomenon, measurable in terms of the protein product. Thus, the protein product would be completely absent or 100% present. We have observed a gene dosage effect at this locus in our patient in that 50% of the normal amount of the protein product was present. This is compatible with expression from both gene copies in this patient and in accordance with previous expression studies.^{4,5} The results presented here provide additional evidence, in the adult, for a discrepancy of imprinting between the murine and human homologue for the IGF2 receptor gene.

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