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A t(4;6)(q12;p23) translocation disrupts a membrane-associated O-acetyl transferase gene (*MBOAT1*) in a patient with a novel brachydactyly–syndactyly syndrome

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Here, we report a patient with a novel brachydactyly–syndactyly syndrome and a *de novo* translocation 46,XY,t(4;6)(q12;p23). We mapped the breakpoint and identified genes in the breakpoint region. One of the genes on chromosome 6, the membrane-associated O-acetyl transferase gene 1 (*MBOAT1*), was disrupted by the breakpoint. This gene consists of 13 exons and encodes a protein of 495 amino acids. *MBOAT1* is predicted to be a transmembrane protein and belongs to the superfamily of membrane-bound O-acyltransferases. These proteins transfer organic compounds, usually fatty acids, onto hydroxyl groups of membrane-embedded targets. Identification of the transferred acyl group and the target may reveal the signaling pathways altered in this novel brachydactyly–syndactyly syndrome.

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Brachydactyly is characterized by shortening of the digits of the hands and feet because of abnormal development of the phalanges and/or metacarpals. It may occur as an isolated trait or as part of complex malformation syndromes. In 1951, Bell¹ categorized five types of inherited brachydactyly on the basis of malformation of the digits A, B, C, D and E. Here, we report a patient with short stature, mild facial dysmorphism, syndactyly and severe brachydactyly. The patient did not meet the criteria for acro-

dysostosis nor could the brachydactyly be categorized according to the Bell classification. The patient with this novel brachydactyly–syndactyly syndrome carried a *de novo* translocation 46,XY,t(4;6)(q12;p23). The breakpoints were mapped and genes were identified. One of the genes, the membrane-associated O-acetyl transferase gene (*MBOAT1*), was disrupted by the breakpoint.

Materials and methods

Subject

From the index patients' blood lymphocytes an Epstein–Barr virus (EBV)-transformed cell line was made and used as source for further investigations.

Conventional chromosome analysis was performed on phytohemagglutinin-stimulated lymphocytes from

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peripheral blood cultures using GTG-banding at a resolution level of 550 bands according to standard protocols.

FISH

One- and two-color fluorescence *in situ* hybridization (FISH) was performed on metaphase spreads made of EBV-transformed B cells of the patient according to standard techniques.² Fiber FISH was performed as described in Giles *et al.*³

Clones

Yeast artificial chromosome (YAC) clones were obtained from the CEPH (http://www.cephb.fr/ceph_yac.html) and the Leiden Genome Technology Center (LGTC) (<http://www.LGTC.nl>). Bacterial Artificial Chromosome (BAC) and P1 Artificial Chromosome (PAC) clones were obtained from the Sanger Institute (<http://www.sanger.ac.uk>), the BAC-PAC Resource Center (<http://gttf.uchc.edu/Info%20BACresources.html>) and the LGTC. The chromosome 4-specific cosmids were obtained from the LGTC. Expressed Sequence Tag (EST) clones were provided by the Resource Centre of the Human Genome Project, Berlin (<http://www.rzpd.de>).

YAC DNA was isolated using the MY kit (Amersham Biosciences Europe GmbH, Diegem, Belgium). DNA of the other clones was isolated using the standard Birnboim–Dole method or with the use of Nucleobond AX100 columns (Machery and Nagel GmbH, Düren, Germany).

Sequence analysis

Sequence analysis was performed by the LGTC, using the fluorescence-tagged dideoxy *Taq* cycle-sequencing procedure (Applied Biosystems bv, Nieuwerkerk ad IJssel, The Netherlands) and analyzed on an ABI 3700 sequencer.

Southern and Northern blots

Genomic DNA was isolated from lymphocytes using the standard salting method.

Southern blotting and hybridization was performed as described previously.⁴ For Northern analysis, commercially available Northern blots were used (Clontech Laboratories Inc., Mountain View, CA, USA). The human adult (total RNA) and fetal multiple tissue Northern blots were hybridized according to the manufacturer's instructions.

3' and 5' RACE

For 3' and 5' rapid amplification of cloned end (RACE) experiments, the marathon cDNA amplification kit (Clontech laboratories) was used together with marathon-ready cDNA from fetal kidney, testis and mammary gland. The kit was used according to the manufacturer's instructions. Several primer combinations were used.

Mutation analysis

For mutation analysis, the single stranded conformation polymorphism (SSCP) analysis was used.⁵ Primer pairs to generate polymerase chain reaction (PCR) products for GSH2 have been described in Dauwerse *et al.*⁷ For *MBOAT1* (= *ADYC2*) primers are presented in Supplementary Table 1. PCR was performed using RedTaq (Sigma–Aldrich, Zwijndrecht, The Netherlands) using 10 × RedTaq PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 11 mM MgCl₂ and 1% gelatin).

Databases

Chromosome 4 YAC map: http://www-shgc.stanford.edu/Mapping/phys_map/Chr4YAC.html; <http://www.ncbi.nlm.nih.gov/BLAST/>; NIX at <http://www.hgmp.mrc.ac.uk/Registered/Webapp/nix/>; http://www.ensembl.org/Homo_sapiens/cytoview; <http://www.sbc.su.se/~miklos/DAS/maindas.html>; <http://smart.embl-heidelberg.de/>; subcellular localization at <http://bioinformatics.ustc.edu.cn/locsvmpsi/locsvmpsi.php>; CLUSTAL-W multiple sequence alignment program (<http://www.ebi.ac.uk/clustalw>) and Boxshade server (http://www.ch.embnet.org/software/BOX_form.html).

Results

Clinical report

The index patient was a mentally normal 35-year-old male of Indonesian descent with disproportionate short stature and infertility because of azoospermia (Figure 1a). He had a height of 1.40 m (–6SD) with an arm span of 1.26 m. The head circumference was normal and measured 54.5 cm (25th centile). He had a flat face with upward slanting of the palpebral fissures, a depressed nasal bridge, broad nasal root, mildly anteverted nostrils and low-set ears (Figure 2). The most striking clinical feature was the extreme brachydactyly of both hands, more pronounced in the right hand (Figure 1c–f) and, to a lesser degree, of the feet (Figure 1b). Interestingly, the right hand showed a partial cutaneous syndactyly between the second and third finger (Figure 1c). The aorta was sclerotic with insufficiency of the aortic valves. Radiographic examination at the age of 31 years revealed severe shortening of all tubular bones in both hands. In the left hand, all metacarpals and phalanges were short and broad. There was also fusion of capitate and hamate bones. A radiograph of the right hand showed shortening and broadening of the metacarpals and distal phalanges (Figure 3). With the exception of a roundly ossified middle phalanx in the index finger, the middle phalanges of the other digits were missing, probably the consequence of proximal symphalangism, which also resulted in a more slender appearance of the proximal phalanges III–V as compared with the left hand. There was no carpal fusion in the right hand. Both wrists showed hypoplasia of the distal ulnar end (ulna minus). The feet showed short and broad metatarsalia and phalanges with

Figure 1 Photographs of the patient. (a) Front view, note the disproportionate short stature. (b) Brachydactyly of the feet and the broad big toes. (c–f) Brachydactyly of the hands of the patient, more pronounced in the right hand (c and f), which also shows a partial syndactyly between the index- and the middle finger and to a lesser extent between the ring- and the little finger.

absence of the middle phalanx of both fifth toes (because of distal symphalangism). The spine films showed a lack of interpedicular widening of the lumbar vertebrae, mild scoliosis, indentation of the vertebral endplates and mild

posterior scalloping of the lumbar vertebral bodies. There was generalized shortening of the tubular bones in upper and lower limbs with mild bowing of the humeral diaphyses. The proximomedial part of the left tibia showed

Figure 2 Frontal (a) and lateral (b) view of the face of the patient showing a flat face with upward slanting of the palpebral fissures, a depressed nasal bridge, broad nasal root, anteverted nostrils and low set ears.

thinning of the cortex with expansion and broadening of the submetaphyseal region.

His parents were healthy like his five sibs and were not consanguineous. None of them had short stature. Conventional chromosome analysis revealed a *de novo* 46,XY,t(4;6)(q12;p23).

Mapping of the chromosome 4 translocation breakpoint

To find the gene(s) disrupted by the t(4;6), we mapped the chromosome 4 breakpoint more precisely. FISH with clones selected from the Stanford Human Genome Center chromosome 4 YAC map was performed on metaphase slides of the patient. Out of 40 YACs tested, five clones were found to span the breakpoint: 973B8 (1100 kb; Figure 4a), 967A3 (940 kb), 944C7 (1150 kb), 303B3 (330 kb) and 256B4 (600 kb). To obtain smaller clones in the breakpoint region, inter Alu-PCR products obtained from YAC 303B3 were hybridized to grids of the chromosome 4-specific cosmid library. Approximately 45 positive cosmids were obtained. Using metaphase FISH, fiber FISH and Southern blot analysis, these cosmids were analyzed and a map was constructed (Figure 5a). No cosmids could be found in the breakpoint region between cosmids 21E5 (centromeric of the breakpoint) and 124A9 (telomeric of the breakpoint), also after rescreening the grids of the chromosome 4-specific cosmid library with the ends of these cosmids. Next, by screening the pdj PAC library with cosmid 21E5, clones pdj194i7 and pdj200d9 were obtained. FISH experiments revealed that these PACs span the breakpoint and subsequent fiber FISH experiments with cosmids 21E5 and 124A9 in one color and one of the PACs, pdj194i7 or pdj200d9, in the other color confirmed that the cosmids fall into the PAC clones and flank the breakpoint region of approximately 40 kb (not shown).

Mapping of the chromosome 6 translocation breakpoint

Subsequently, we started analyzing the chromosome 6 breakpoint. Fourteen YACs from the CEPH YAC map and 21 BAC/PAC clones from the Sanger Center chromosome 6 map were mapped relatively to the breakpoint using FISH on metaphase chromosomes of the patient. Three YACs, 958a10, 927f7 and 934d11 (not shown) and one BAC, RP11-239H6 encompassed the chromosome 6 breakpoint (Figure 5b).

Identification of candidate genes on chromosome 4

Several techniques were used to search for genes in the breakpoint region. First, we searched for open reading frames in the 40 kb breakpoint region between cosmids 21E5 and 124A9 by exon trapping, Zoo blot analysis and by screening of a cDNA library, but no open reading frames could be detected.^{6,7} Second, a larger region was analyzed for open reading frames. Fragments of cosmids 21E5, 124A9, 232G12 and 199B11 were examined with the above-mentioned techniques and by sequencing. Sequence analysis of one of the fragments, E20N2, showed homology to the promoter region of the platelet-derived growth factor receptor α gene (*PDGFRA*). This fragment is present in cosmid 124A9, 20–60 kb from the translocation breakpoint. Basic local alignment search tool (BLAST) analysis of the sequence of E20N2 revealed homology with the partially sequenced BAC 56D20. NIX analysis of the sequence of 56D20 predicted the presence of two genes in this region; *PDGFRA* that we already picked up before and *GSH2*, the human homologue of the mouse gene *Gsh2*. *GSH2* is situated in cosmid 232G12, which is between 60 and 80 kb from the translocation breakpoint. In summary, on chromosome 4 no genes were found which are disrupted by the breakpoint (Figure 5a).

Figure 3 Radiographs of the patient taken at different ages ((a–c) at age 31 years; (d) at age 24 years). (a) Anteroposterior view of the left hand shows short and broad metacarpals and phalanges. Note fusion of capitate and hamate bones and hypoplasia of the distal part of the ulna. (b) Severe shortening of metacarpals and distal phalanges is also visible on the anteroposterior view of the right hand. In contrast to the left hand, the middle phalanges are absent with the exception of a round, hypoplastic bone in the index finger. The proximal phalanges III–V also have a more slender appearance, which is probably the consequence of proximal symphalangism. There is no evidence for carpal bone fusion but the distal part of the ulna is also hypoplastic (c) Lateral view of the lumbar spine reveals indentation of the endplates and mild posterior scalloping of the lower lumbar vertebrae (d) Anteroposterior view of the left knee shows cortical thinning and bulging of the proximomedial part of the tibia.

Identification of candidate genes on chromosome 6

The complete sequence of clone RP11-239H6 was available from the NCBI (sequenced by the Wellcome Trust Sanger Institute) and used in a NIX analysis. This revealed two

EST's, AA078851 and AW237409. AA078851 was fully sequenced and compared (BLAST) with other sequences in the NCBI database. From this, a cDNA sequence was constructed of 3233 bp containing an open reading frame

Figure 4 (a) FISH on metaphase chromosomes of the patient with chromosome 4 YAC 973B8, spanning the breakpoint, showing signals on the normal chromosome 4 (middle) and signals on the derivative chromosome 6 (left) and the derivative chromosome 4 (right). (b) Two-color FISH on metaphase chromosomes of the patient with chromosome 6 BAC RP11-239H6 (red) and the 50 kb *Bss*HII fragment from RP11-239H6 (green), both spanning the breakpoint. Red and green signals can be seen on the normal chromosome 6 (A), on the derivative chromosome 4 (B) and on the derivative chromosome 6 (C).

of 1485bp coding for a protein of 495 amino acids. Genomically, the gene spans approximately 112 kb including sequences from clones RP11-239H6, RP3-434O11 and RP1-130G2. From comparison of the cDNA with the genomic sequence, we concluded that the gene consists of 13 exons, with the start ATG in exon 1 and the

translation stop codon in exon 13. The gene was predicted to encode a protein of 495 amino acids and was highly homologous to a predicted gene in this region in the Ensembl database, *OACT1* (ENSG00000172197). Prediction of *OACT1* however, was based on sequence BC045695, which contains a sequencing error: 'deletion of a C' at position 1261 predicts another reading frame and therefore a shorter protein in translation. We sequenced this part of the gene in 12 individuals all containing a 'C' at the corresponding site.

With the 495 amino-acid sequence, a position-specific iterated-BLAST multiple sequence alignment was performed. Homology was found to a group of acyltransferases, the super family of membrane-bound *O*-acyltransferases (MBOAT).⁸ Therefore, we named this novel gene *MBOAT1*. The presumed enzymatic role of the proteins is supported by the conservation of polar residues within and adjacent to the membrane. Particularly, the invariant histidine that we also identified at position 381 in the *MBOAT1* protein is suggested to be an active-site residue.⁸ In mice, the homologous gene is located on chromosome 13 (ENSMUSG00000038732) that shows 81% identities and 91% similarities to the human gene (Supplementary Figure 1). MBOAT proteins usually have eight to 10 membrane-spanning regions. Using the DAS transmembrane prediction server, nine transmembrane-spanning domains were predicted for MBOAT1, with an extracellular N-terminus and an intracellular C-terminus. The protein has a predicted subcellular localization in the plasma membrane according to LOCSVMpsi (reliability index: 10; 99% accuracy). In the N-terminus of the murine protein, a signal sequence of 47 amino acids was predicted that had not been found in MBOAT1, despite the high sequence similarity.

Northern blots were hybridized with a probe from exon 1. Two transcripts, of 3.2 and 5.0 kb, which are probably because of alternative splicing, were seen in all fetal and adult tissues (Figure 6). Therefore, we conclude that the *MBOAT1* gene is ubiquitously expressed.

The other EST in the region, AW237409 named *ADY3* (Acro DYsostosis Candidate 3 gene), is a unique EST that has no overlap with any known human EST. However, it does have an overlap with 43 bp of exon 1 of the *MBOAT1* gene. The 432 bp of this EST consists of 3 exons spanning 110 kb genomically. RACE experiments only extended the 3' end of the gene with an alternative third exon. However, *ADY3* is encoded by the other strand and is not disrupted by the translocation breakpoint.

Fine mapping of the chromosome 6 breakpoint

To further pinpoint the location of the breakpoint within RP11-239H6, DNA of this BAC was digested with restriction endonuclease *Bss*HII. From the sequence, we determined that this would give rise to a fragment of 50 kb, which was subsequently isolated from an agarose gel and used in a

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Figure 6 Human fetal total RNA Northern blot hybridized with an ADYC2 exon1 probe showing two transcripts of 3.2 and 5.0 kb. Lanes 1–4 correspond to RNA from brain, lung, liver and kidney, respectively.

The facial features of our proband are to some extent reminiscent for acrodysostosis but the radiographic anomalies in the hands are different. Especially the symphalangism makes the diagnosis of acrodysostosis and other acromelic dysplasias, such as acromesomelic dysplasia Maroteaux type, unlikely. We are not aware of a similar phenotype reported in the literature. The finding of a *de novo* reciprocal translocation between chromosomal band 4q12 and chromosomal band 6p23 prompted us to map the breakpoints of this chromosomal abnormality in order to better understand the pathogenesis of this disorder.

We identified *MBOAT1* as the gene disrupted by the translocation in our patient. *MBOAT1* encodes a novel protein with unknown function. It is a multimembrane-spanning protein with homology to a subgroup of acyltransferases and therefore classified to the superfamily of MBOAT. These proteins transfer organic compounds, usually fatty acids (eg, cholesterol, diacylglycerol, palmitoyl) onto hydroxyl groups of membrane-embedded targets.⁸

Interestingly, at least two other well-known members of the MBOAT super family, *Skinny hedgehog* and *Porcupine*, are

involved in developmental processes as well.^{9–11} *Skinny hedgehog* encodes an apparent acyltransferase critically required for Hedgehog (Hh) palmitoylation, a modification vital for Hh signaling activity. In another Hh gene, *indian hedgehog*, heterozygous missense mutations have been found in the amino-terminal signaling domain in all affected members of three large, unrelated Chinese brachydactyly type A1 families.¹² Indian hedgehog (Ihh) is a morphogen produced by hypertrophic chondrocytes in the growth plate. It regulates proliferation of chondrocytes and can also regulate the rate of differentiation of chondrocytes indirectly through stimulation of parathyroid hormone-related protein and is therefore essential for bone formation.^{13,14} Although the exact functions of most of the MBOAT proteins have not been clearly established yet, it is evident that some of these acylating enzymes appear to be highly specific and may prove to be of general importance in regulating the function of secreted proteins during development.¹⁰ Identification of the target for MBOAT1 and the transferred acyl group may reveal the signaling pathways altered in this novel brachydactyly-syndactyly syndrome and provide us with more insights in the growth and development of hands and feet.

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