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ARTICLE

Ulnar–mammary syndrome with dysmorphic facies and mental retardation caused by a novel 1.28 Mb deletion encompassing the *TBX3* gene

Eva Klopocki*[,1,4], Luitgard M Neumann[2,4], Holger Tönnies[2], Hans-Hilger Ropers[3], Stefan Mundlos[1] and Reinhard Ullmann[3]

[1]*Institute of Medical Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany;* [2]*Institute of Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany;* [3]*Max-Planck Institute for Molecular Genetics, Berlin, Germany*

Ulnar–mammary syndrome (UMS) is a rare autosomal-dominant disorder caused by mutations in *TBX3*. The condition is characterized by hypoplasia or aplasia of upper limbs on the ulnar side, mammary glands and nipples, and of apocrine glands in both sexes (MIM #181450). We report on a girl presenting with an UMS like phenotype, a dysmorphic facies, and mental retardation. Mutation analysis of *TBX3* and G-banded chromosome analysis from lymphocytes were performed. We used microarray-based comparative genomic hybridization (array CGH) to investigate the patient's genomic DNA for submicroscopic aberrations. No mutation of the *TBX3* gene was detected in our patient and chromosome analysis revealed a normal female karyotype (46,XX). Hybridization of a whole-genome tiling path array consisting of more than 36 000 BAC clones revealed an interstitial 1.28 Mb deletion within chromosomal band 12q24.21. The deleted region encompasses one known gene, *TBX3*. The deletion and haploinsufficiency of *TBX3* was confirmed by fluorescence *in situ* hybridization using BAC clones representing the deletion on the BAC array. To our knowledge, this is the first description of *TBX3* haploinsufficiency caused by a genomic deletion in a patient with UMS. We suggest that the UMS phenotype in conjunction with the characteristic facial changes and mental retardation observed in our patient is owing to the deletion of *TBX3* and the involvement of neighbouring genes.
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Introduction

Ulnar–mammary syndrome (UMS) is a rare autosomal-dominant disorder (UMS, MIM #181450) with a characteristic upper limb malformation phenotype ranging from hypoplasia of the terminal phalanx of the fifth digit to aplasia of hand and upper limbs on the ulnar side.[1] Abnormal development of mammary glands and nipples, teeth, genitalia, and of apocrine glands in both sexes are common clinical features in UMS patients.[2,3] Loss-of-function mutations in the *TBX3* gene have been described to cause UMS.[4,5] *TBX3* belongs to the T-box gene family, which encodes a large family of transcription factors sharing a DNA-binding domain called the T-box.[4,6,7] Missense mutations, and nonsense mutations that result in impaired DNA binding as well as splice site mutations have been detected in UMS patients.[4,8] Single-base deletion and insertions (frameshift mutations) lead to a truncated protein lacking the T-box domain, which is incapable of binding DNA.[9]

***Correspondence: Dr rer. nat. E Klopocki, Institute of Medical Genetics, Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Augustenburger Platz 1, Berlin 13353, Germany.**
Tel: +49 30 450569153; Fax: +49 30 450569996;
E-mail: eva.klopocki@charite.de
[4]These authors contributed equally to this work.
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Although our patient had typical features of UMS, no loss-of-function mutations in the *TBX3* gene were detected. We therefore investigated the patient's genomic DNA for submicroscopic aberrations. Microarray-based comparative genomic hybridization (array CGH; Solinas-Toldo *et al*[10]and Pinkel *et al*[11]) has recently been implemented in molecular cytogenetics as a novel method to detect submicroscopic genomic aberrations.[12–14] Higher resolution compared to conventional cytogenetic techniques makes array CGH a valuable tool in medical genetics. The use of tiling path arrays allows the detection of microdeletions or duplications as small as 100 kb.[15] Using high-resolution array CGH, we detected a novel microdeletion on chromosome 12q24.21 encompassing the *TBX3* gene.

Materials and methods

Clinical report

The female patient was the second child of unrelated healthy parents. Pregnancy and delivery were uneventful (Apgar scores 9/10). Anal atresia was detected after birth and surgically corrected. When seen by us at the age of 6 weeks, she presented with severe bilateral limb abnormalities. In addition, a dysmorphic facies and a short neck were noted (Figure 1). Family history was negative for similar conditions. At age 3.5 length and weight were at P3, and occipitofrontal circumference at P25. Dysmorphic features included capillary naevus flammeus on the central forehead (which faded with age), large eyebrows, flat nose, big mouth, small lips, slight pectus carinatum, and bilateral hypoplastic nipples (Figure 1a and b). Tooth eruption was delayed. Premature dental decay was noted. The bilateral limb abnormalities were more precisely described as mesomelic dysplasia of both arms and oligodactyly with hypoplastic fingers one, two, and three and contractures of the existing fingers on both hands as well as partial cutaneous syndactyly between the second and third fingers (Figure 1c, d and e). She walked at 2.5 years. A moderate mental retardation was present and speech development was delayed. X-ray examinations revealed bilateral distal hypoplasia of humeri, absent ulnae, and complete absence of fourth and fifth digits including the corresponding metacarpals and carpals (Figure 2). Bone age was delayed by 6 months. Radiographs of the chest, thoracal and lumbal spine, right leg, and foot were normal. Abdominal ultrasound examination, echocardiography, long-time electrocardiogram, and ocular examination disclosed no abnormalities, and hearing was normal.

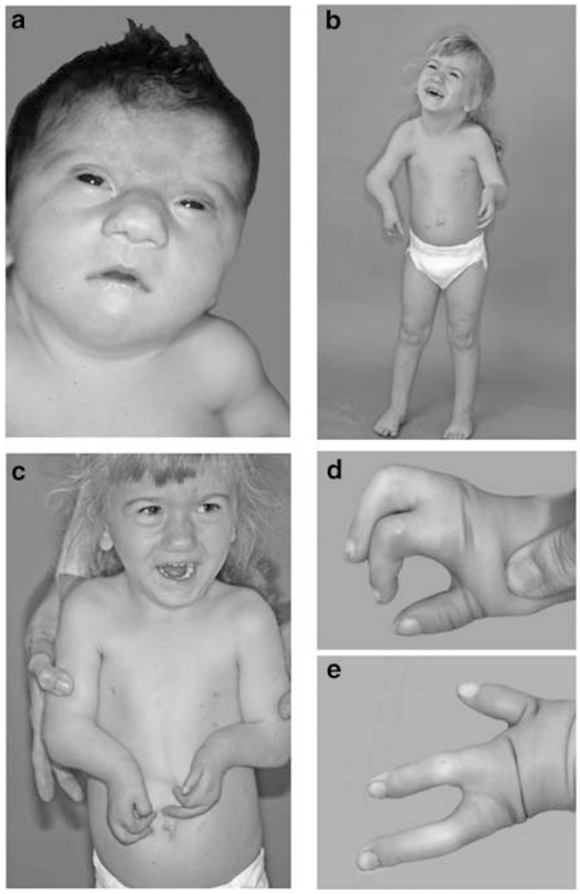

Figure 1 (**a**) Facial appearance of the patient at the age of 6 weeks. Note capillary naevus flammeus on the forehead, large eyebrows, bulbous nose, and small lips. (**b** and **c**) Phenotype at the age of $3\frac{1}{2}$ years. (**b**) Note inverted nipples, mesomelic dysplasia of the arms, bilateral absence of digit four and five; (**c**) mesomelic dysplasia of both arms, ulnar deviation of both hands; detailed picture of right (**d**) and left hand (**e**): absence of both fourth and fifth rays, hypoplastic fingers one, two, and three with flexion contractures of the middle interphalangeal joints of finger two and three on both hands, partial cutaneous syndactyly between second and third fingers.

Mutation analysis

Genomic DNA was prepared from peripheral lymphocytes as described previously. Exons 1–7 of *TBX3* were PCR-amplified by means of previously published exon-flanking primers.[4,5] Sequencing was carried out at the lab of Mike Bamshad (Department of Pediatrics, Eccles Institute of Human Genetics, University of Utah).

Cytogenetics

Standard karyotyping of GTG-banded chromosomes from lymphocytes at 450 bands resolution was performed according to standard procedures. To exclude Fanconi anaemia cytogenetic investigation of induced chromosomal breakage in response to mitomycin C was performed on patient's lymphocytes.[16]

Fluorescence *in situ* hybridization

BAC clones RP11-636B16, RP4-601P9, RP11-33I06, RP11-297J16, RP11-722K12, and RP11-8A1 were obtained from

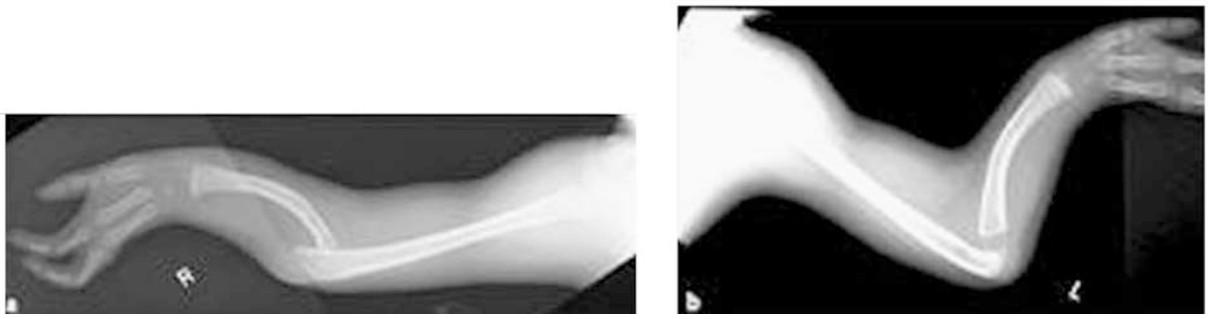

Figure 2 Radiographs of the arms showing bilateral aplasia of ulnae, and absence of fourth and fifth digit including, metacarpalia and carpalia, distal hypoplasia of humeri, bowing with shortening of radii, the only metacarpal bone is the os hamatum. (**a**) X-ray of the right arm and (**b**) X-ray of the left arm.

the RZPD (Deutsches Resourcenzentrum für Genomforschung, Berlin, Germany). BAC DNA was fluorescently labelled using nick translation, and hybridized to metaphase spreads of the patient's lymphocytes using standard procedures. BAC clones were labelled with spectrum orange. YAC clone 933e11 mapping to 12p and cep12 (Vysis, Downers Grove, IL) labelled with spectrum green were used as control probes. Chromosomes were counterstained with DAPI. All colour images of hybridized metaphase spreads were evaluated using an epifluorescence microscope (Axioscope, Zeiss, Germany) fitted with different single band-pass filter sets for DAPI, Spectrum Green and Spectrum Orange fluorescence. The microscope is equipped with a cooled CCD camera (Hamamatsu) for image acquisition. Image analysis was performed using the ISIS analysis system (Metasystems, Altlussheim, Germany).

Microarray-based CGH

Array CGH was carried out using a 36k whole human genome tiling path BAC array consisting of the 1 Mb Sanger Clone set (kindly provided by Nigel Carter, Wellcome Trust Sanger Centre),[17] a set of 390 subtelomeric clones (generated in the course of the EU initiative COSTB19: Molecular cytogenetics of solid tumours) and the human 32k Re-Array set (http://bacpac.chori.org/pHumanMinSet.htm; DNA kindly provided by Pieter de Jong).[18,19] Detailed protocols are available at the MPI webpage (http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/ProtocolsEntry.html). The patient's DNA and a pooled reference DNA were fluorescently labelled with Cy5 and Cy3, respectively, using the Bioprime Array CGH labelling kit (Invitrogen, Carlsbad, CA, USA). Hybridization on a SlideBooster Hybridization Station (Implen, Munich, Germany) was performed as described in the protocol (refer to MPI webpage), except that DNA was not sonicated before labelling and 3×300 ng DNA were used. Images were scanned using GenePix 4100A and analysed using GenePix Pro 6.0 software (Axon Instruments, Foster City, CA, USA). Further analyses and visualization were performed with CGHPRO.[20] 35882 BACs were included in the analysis. Raw data were normalized by 'Subgrid LOWESS'. The log 2 ratio of test to reference was calculated and plotted according to chromosomal position of the clones. Copy number gains and losses were determined by using a conservative threshold of 0.3 and −0.3, respectively. Aberrant signals including three or more neighbouring BAC clones were considered as genomic aberrations and were further evaluated by fluorescence *in situ* hybridization (FISH), unless they coincided with a published polymorphism as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/; version 13 December 2005).

Results

The patient described here presented with clinical features typical of UMS, that is, bilateral absent ulnae, complete absence of fourth and fifth digits (Figure 2), and anal atresia. In addition, facial dysmorphisms as well as mental retardation were noted, which have not been described as part of the UMS spectrum so far (Figure 1). The initially performed high-resolution karyotype was normal in the patient. Cytogenetic investigation of induced chromosomal breakage in response to mitomycin C yielded normal results. Mutation analysis by sequencing exons 1–7 of *TBX3* gene detected no mutations. The additional features present in this patient suggested a submicroscopic rearrangement as the cause of the patient's condition. To test for such microdeletions, we performed array CGH analysis with the patient's genomic DNA using a whole-genome tiling path array. An interstitial deletion on chromosome 12q24.21 was detected (Figure 3). According to the array CGH data, the two breakpoints were located between RP11-636B16 and RP11-297J16 (proximal breakpoint) and RP11-8A1 and RP11-115H15 (distal breakpoint). The array data were mapped on the human genome sequence using the Ensembl genome browser (www.ensembl.org) to determine the deletion size and to identify candidate genes. The size of the deletion was determined to be ranging from 113.6 to 114.9 Mb. *TBX3* is the only

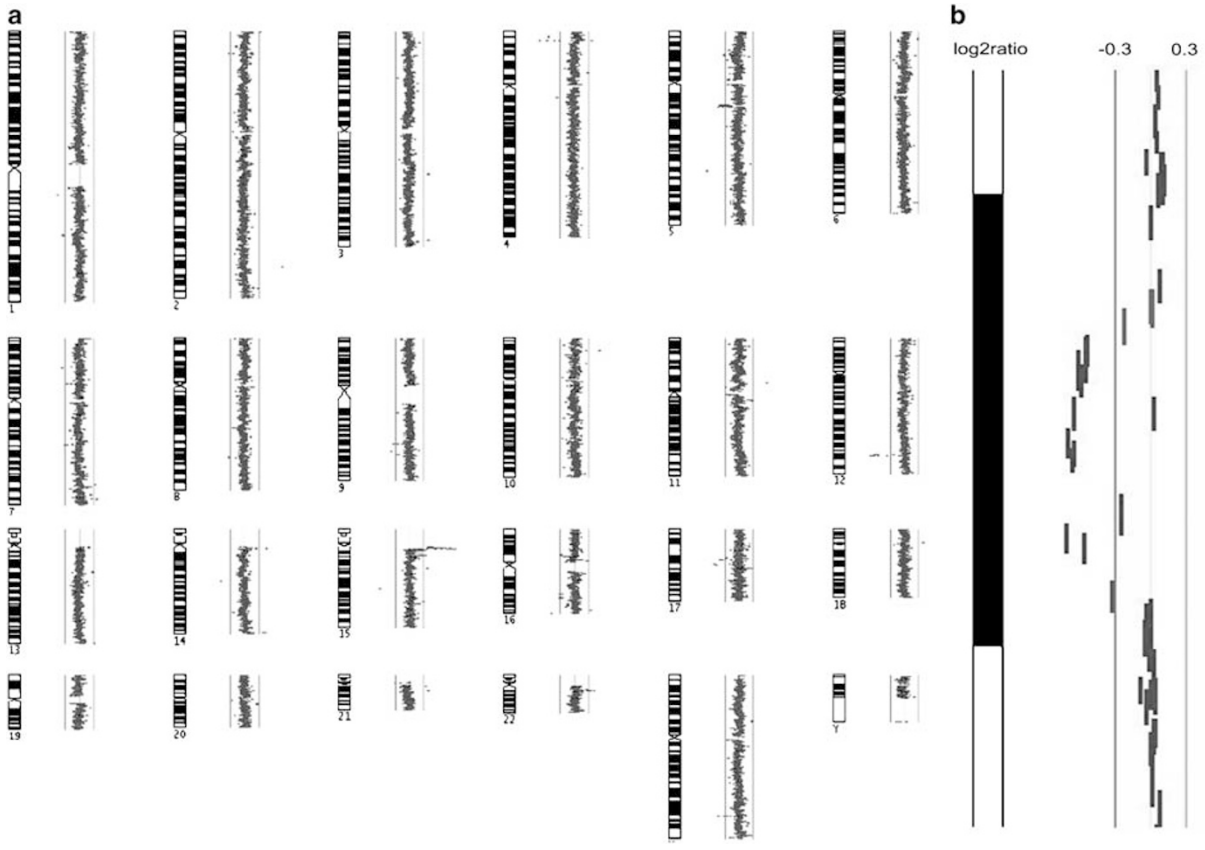

Figure 3 Array CGH profile. (**a**) Whole-genome view. Each spot in the profile represents a BAC clone. As BAC clones of the tiling path are overlapping, single clone aberrations were not considered true aberrations. For visualizing the content of low copy repeats in the ratio plots, each BAC clone was classified into one out of seven categories and colour-coded as described previously.[20] (**b**) Detailed view of the interstitial deletion on chromosome band 12q24.21. The red and green lines indicate the log 2 ratio thresholds −0.3 (loss) and 0.3 (gain), respectively.

known gene located within the deleted region (positions 113.57–113.58 Mb). A second gene, *THRAP2*, is located distal to the deletion (positions 114.85–115.17 Mb). *TBX5* is located proximal to the deletion (position 113.25–113.3 Mb).

To confirm the array CGH data, we conducted FISH analysis using a set of BAC clones located at 12q24.21. BAC clone RP11-636B16 located at the proximal deletion breakpoint had an intermediate log 2 ratio (−0.21) in the array CGH experiment and showed normal FISH signals on chromosome 12 of patient's metaphases. BAC clone RP11–115H15 located at the distal deletion breakpoint showed two signals in the FISH experiment. As expected from the array data (log 2 ratio $\leqslant$0.3), only one signal was detected on patient's metaphases for BAC clones RP4–601P9 (113.56 Mb), RP11–33I06 (113.57 Mb), RP11–297J16 (113.58 Mb), RP11–722K12 (114.2 Mb), and RP11–8A1 (114.67–114.84 Mb). An example is shown in Figure 4 (Supplementary information). Thus, the FISH results confirmed an interstitial deletion of approximately 1.28 Mb on chromosome 12 extending from 113.56 to 114.84 Mb and haploinsufficiency of the *TBX3* gene. FISH on metaphase chromosomes of the patient's parents detected two signals on chromosome 12 for all probes investigated (data not shown), indicating that this deletion occurred *de novo*.

Discussion

The patient presented here shows a characteristic UMS phenotype, but has additional features that are not commonly considered to be part of the UMS phenotypic spectrum. To detect possible microdeletions, we analysed the genomic DNA by array CGH. Using a tiling path array, we were able to detect a *de novo* microdeletion of 1.28 Mb on chromosome 12q24.21 encompassing the *TBX3* gene.

TBX3 is a member of the T-box gene family, which is involved in the specification of fore and hind limb identity. At least five T-box genes are expressed in the developing vertebrate limb, that is, *TBX2*, *TBX3*, *TBX4*, *TBX5*, and *TBX15*.[21,22] Loss-of-function mutations in *TBX3* have been described to cause UMS, whereas *TBX5* mutations are known to cause Holt–Oram syndrome (HOS).[5,23,24] Bamshad *et al*[4] investigated the genotype–phenotype

correlation in the affected individuals of 10 families. No obvious clinical differences were detected between subgroups of patients with mutations in different TBX3 domains. Recently, Meneghini *et al*[25] described an association between mutations affecting the T-domain of TBX3 and higher frequency of severe limb phenotypes. *TBX3* is widely expressed in a variety of tissues and organs that are not affected in individuals with UMS or the *TBX3* knockout mice. This suggests that different tissues may require different critical levels of normal TBX3 protein for morphogenesis or a regulation of TBX3 by tissue-specific cofactors. Alternatively, other genes from the T-box family as a consequence of functional redundancy may be able to compensate for reduced levels of normal TBX3 protein in other tissues than the forelimb. So far, no homozygous mutations have been observed in humans. Compared to patients with *TBX3* point mutations, our patient presented with a rather severe manifestation of the syndrome. Bilateral symmetrical ulnar ray defects including the absence of the ulna as in the case presented here had previously been observed in *TBX3* inactivation studies in the mouse and the chick,[26] but have only been described in three out of 44 UMS patients from 10 families.[4,5]

The characteristic facial appearance and the mental retardation observed in the patient described here are not typical for UMS. The large deletion is the most likely explanation for this broader phenotype. Although *TBX3* is the only known gene within the deleted region, positional effects or other non-coding sequences of the deleted interval may have a regulatory role and may influence the expression of nearby genes such as *TBX5*, which is located at the proximal side of the deleted segment. Mutations in *TBX5* cause HOS (OMIM 142900[23,24]), a condition characterized by severe cardiac and skeletal abnormalities, in particular defects of the radial ray including triphalangeal thumb, hypoplastic/absent thumb/radius. Bilateral hypoplastic thumbs, hypoplastic fingers II and III, and hypoplastic bowed radii observed in our patient are radial ray defects, which might argue for a combined gene defect of *TBX3* and *TBX5*. It is possible that the deletion of *TBX3* and the genomic region upstream has a regulatory effect on the *TBX5* gene. The *THRAP2* (thyroid hormone receptor-associated protein 2, *PROSIT240*) gene is located distal to the deletion on 12q24.21. *THRAP2* has been identified by two different groups while studying patients with balanced translocations.[27,28] The authors suggest that THRAP2 is a novel component of the thyroid hormone receptor-associated complex, which is involved in transcriptional regulation. The first case described by Muncke *et al*[28] displays a congenital heart defect (transposition of the great arteries), delayed motor development, nearly absent speech, and mental retardation. The chromosomal breakpoint was located between the first and second exon of *THRAP2*. The second case was described to have a Noonan-like phenotype with normal intelligence and normal development (O Bartsch, personal communication). The breakpoint in this case is located 28 kb downstream of exon 1. The father had the same balanced translocation, but without clinical symptoms. The case presented here shares clinical features with the case described by Muncke *et al*,[28] that is, mental retardation, delayed motor, and speech development. Analysis of *THRAP2* expression detected high expression in skeletal muscle, heart, all subregions of the brain, and abundant expression in the foetal brain.[28] The authors propose a possible role for *THRAP2* in early heart and brain development. A disrupted or deregulated *THRAP2* could therefore be a cause for developmental defects resulting in a retardation phenotype. This hypothesis is supported by three cases with a contiguous deletion of *TBX5* and *TBX3* with telomeric breakpoint positioned between 114.75 and 114.78 Mb (J Kohlhase, personal communication). All three carriers are of normal intelligence (J Kohlhase, personal communication), suggesting that the critical region for the retardation phenotype on 12q24.21 is between 114.78 and 114.84 Mb.

UMS associated with known *TBX3* mutations has been interpreted as the result of haploinsufficiency for this gene. Our finding that a heterozygous deletion of the *TBX3* gene also gives rise to UMS provides direct evidence for *TBX3* haploinsufficiency as the mechanism causing this syndrome. The additional features observed in our patient clearly distinguish this phenotype from UMS caused by *TBX3* mutations and argue for a positional effect and/or the involvement of other neighbouring genes.

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References

1 Schinzel A: Ulnar–mammary syndrome. *J Med Genet* 1987; **24**: 778–781.
2 Franceschini P, Vardeu M, Dalforno L *et al*: Possible relationship between ulnar–mammary syndrome and split hand with aplasia of the ulna syndrome. *Am J Med Genet* 1992; **44**: 807–812.
3 Pallister P, Herrmann J, Opitz J: Studies of the malformation syndromes in man XXXXII: a pleiotropic dominant mutation affecting skeletal, sexual and apocrine-mammary development. *Birth Defects Orig Artic Ser* 1976; **12**: 247–254.

4 Bamshad M, Le T, Watkins W *et al*: The spectrum of mutations in TBX3: genotype/phenotype relationship in ulnar–mammary syndrome. *Am J Hum Genet* 1999; **64**: 1550–1562.
5 Bamshad M, Lin R, Law D *et al*: Mutations in human TBX3 alter limb, apocrine and genital development in ulnar–mammary syndrome. *Nat Genet* 1997; **16**: 311–315.
6 Bollag R, Siegfried Z, Cebra-Thomas J, Garvey N, Davison E, Silver L: An ancient family of embryonically expressed mouse genes sharing a conserved protein with the T locus. *Nat Genet* 1994; **7**: 383–389.
7 Herrmann B, Labeit S, Poustka A, King T, Lehrach H: Cloning of the T gene required in mesoderm formation in the mouse. *Nature* 1990; **343**: 617–622.
8 Sasaki G, Ogata T, Ishii T, Hasegawa T, Sato S, Matsuo N: Novel mutation of TBX3 in a Japanese family with ulnar–mammary syndrome: implication for impaired sex development. *Am J Med Genet* 2002; **110**: 365–369.
9 Wollnick B, Kayserili H, Uyguner O, Tukel T, Yuksel-Apak M: Haploinsufficiency of TBX3 causes ulnar–mammary syndrome in a large Turkish family. *Ann Genet* 2002; **45**: 213–217.
10 Solinas-Toldo A, Lampel S, Stilgenbauer S *et al*: Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes, Chromosomes Cancer* 1997; **20**: 399–407.
11 Pinkel D, Segraves R, Sudar D *et al*: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998; **20**: 207–211.
12 Shaw-Smith C, Redon R, Rickman L *et al*: Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J Med Genet* 2004; **41**: 241–248.
13 Schoumans J, Anderlid B, Blennow E, Teh B, Nordenskjöld M: The performance of CGH array for the detection of cryptic constitutional chromosome imbalances. *J Med Genet* 2004; **41**: 198–202.
14 Vissers L, Vries Bd, Osoegawa K *et al*: Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003; **73**: 1261–1270.
15 Ishkanian A, Malloff C, Watson S *et al*: A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet* 2004; **36**: 299–303.
16 Tischkowitz MD, Hodgson SV: Fanconi anaemia. *J Med Genet* 2003; **40**: 1–10.
17 Fiegler H, Carr P, Douglas EJ *et al*: DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer* 2003; **36**: 361–374.
18 Krzywinski M, Bosdet I, Smailus D *et al*: A set of BAC clones spanning the human genome. *Nucleic Acids Res* 2004; **32**: 3651–3660.
19 Osoegawa K, Mammoser AG, Wu C *et al*: A bacterial artificial chromosome library for sequencing the complete human genome. *Genome Res* 2001; **11**: 483–496.
20 Chen W, Erdogan F, Ropers H, Lenzner S, Ullmann R: CGHPRO – a comprehensive data analysis tool for array CGH. *BMC Bioinformatics* 2005; **6**: 85.
21 Gibson-Brown J, Agulnik S, Silver L, Niswander L, Papaioannou V: Involvement of T-box genes Tbx2–Tbx5 in vertebrate limb specification and development. *Development* 1998; **125**: 2499–2509.
22 Agulnik S, Papaioannou V, Silver L: Cloning, mapping, and expression analysis of TBX15, a new member of the T-box family. *Genomics* 1998; **51**: 68–75.
23 Basson C, Bachinsky D, Lin R *et al*: Mutations in human TBX5 [corrected] cause limb and cardiac malformations in Holt–Oram syndrome. *Nat Genet* 1997; **15**: 30–35.
24 Li Q, Newbury-Ecob R, Terrett J *et al*: Holt–Oram syndrome is caused by mutations in TBX5, a member of the *Brachyury* (T) gene family. *Nat Genet* 1997; **15**: 21–29.
25 Meneghini V, Odent S, Platonova N, Egeo A, Merlo G: Novel TBX3 mutation data in families with ulnar–mammary syndrome indicate a genotype–phenotype relationship: mutations that do not disrupt the T-domain are associated with less severe limb defects. *Eur J Med Genet* 2006; **49**: 151–158.
26 Davenport T, Jerome-Majewska L, Papaioannou V: Mammary gland, limb and yolk sac defects in mice lacking Tbx3, the gene mutated in human ulnar–mammary syndrome. *Development* 2003; **130**: 2263–2273.
27 Musante L, Bartsch O, Ropers H-H, Kalscheuer V: cDNA cloning and characterization of the human THRAP2 gene which maps to chromosome 12q24, and its mouse ortholog Thrap2. *Gene* 2004; **332**: 119–127.
28 Muncke N, Jung C, Rüdiger H *et al*: Missense mutations and gene interruption in PROSIT240, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). *Circulation* 2003; **108**: 2843.

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