SHORT REPORT

Disruption of TCBA1 associated with a de novo t(1;6)(q32.2;q22.3) presenting in a child with developmental delay and recurrent infections

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A boy with developmental delay, particularly of speech, a distinct face, antineutrophil cytoplasmic antibodies, and recurrent infections was found to have an apparently balanced de novo t(1;6)(q32.3;q22.3) translocation. Fluorescent in situ hybridisation with BAC/PAC clones and long range polymerase chain reaction products assessed in the human genome sequence localised the chromosome 1 breakpoint to a 9.8 kb segment within a hypothetical gene, LOC388735, and the chromosome 6 breakpoint to a 12.8 kb segment in intron 4 of the *T-cell lymphoma breakpoint-associated target 1* (*TCBA1*) gene. Disruption and/or formation of *TCBA1* fusion genes in T cell lymphoma and leukaemia cell lines suggests a role for this gene in tumorigenesis. The isolated mouse *Tcba1* gene shows 91% amino acid sequence similarity with human *TCBA1*. It is expressed in fetal and adult brain and with lower levels in liver and testis. The human gene has been reported to be expressed exclusively in brain and thymus. Reduced *TCBA1* expression in brain and thymus may explain at least some of the symptoms in this patient. It is concluded that germline alterations of the *TCBA1* gene are associated with developmental delay and typical physical features.

De novo balanced chromosome rearrangements that disrupt or otherwise inactivate genes in the breakpoint regions can cause mental retardation or congenital malformations or both.[1–3] The cytogenetic and molecular characterisation of disease associated balanced chromosome rearrangements offers a successful instrument for identifying genes involved in inherited disease. Over the past decade, well over 100 genetic loci and genes of Mendelian disorders have been found by such positional cloning approaches.[4 5] Here we describe our analysis of a de novo t(1;6)(q32.3;q22.3) translocation in a dysmorphic and developmentally delayed boy with an early childhood history of recurrent chest infections. The constitutional chromosome breakpoint on chromosome 6q22 is of particular interest, because it coincides with a common breakpoint cluster region in T cell malignancies. The *TCBA1* gene is thought to be a target gene for the tumour specific chromosome rearrangements; however, its function remains to be elucidated.[6] Our patient provides important clues as to the phenotypic effects of constitutional *TCBA1* haploinsufficiency.

METHODS

Patient

The patient, a boy, was clinically assessed by one of us (DTP) and recruited to the study with parental consent. He was the oldest child of healthy non-consanguineous parents. There was no history of miscarriages. He was born at 38 weeks' gestation. His birth weight was on the 50th centile, and his occipito-frontal head circumference (OFC) was between the 75th and 91st centiles. He was found to have an atrioventricular septal defect, which closed spontaneously in the first few months of life, and a slightly dysplastic pulmonary valve. Macrocephaly was also noted and thought to be familial, with a paternal OFC of 5 cm above the 99.6th centile, and maternal OFC on the 91st centile. He had bilateral orchidopexies for undescended testes, and a fundoplication for marked gastro-oesophageal reflux. Other problems included recurrent chest infections, which had improved, asthma, and enuresis. His development was delayed, and formal assessment at 17 months showed a mental age of 12.3 months (GQ = 78). He had general learning difficulties, especially in the area of expressive speech.

When assessed at the age of five years, his height was on the 25th centile, weight on the 75th centile, and OFC between the 75th and 91st centiles. He was very fair skinned and had very blond, almost white hair, although both his parents had darkish hair. He was hyperteloric, and had blue eyes and small palpebral fissures. He also had prominent corneal nerves. His nose was prominent and he had a thin, tented upper lip. He had fetal finger pads, overlapping third and fifth toes, very hyperextensible joints, and a slightly hirsute back. On follow up at the age of eight years his facial features had coarsened and his hair had become darker (fig 1). He had also been extensively investigated for frequent loose stools, for which so far no cause had been found. He continued to have difficulties with his motor skills, and communication, particularly in the area of expressive speech.

Investigations have included cranial magnetic resonance imaging, which showed some enlargement of lateral and third ventricles with no evidence of aqueduct stenosis. A renal ultrasound, EEG, thyroid function test, sweat test, and lysosomal enzyme screen were normal. Immunological studies did not show any specific abnormalities of the lymphocyte subpopulations or of immunoglobulin and antibody production. However, he was found to have raised antineutrophil cytoplasmic antibodies of the cytoplasmic pattern (C-ANCA), which are often associated with vasculitides.

Fluorescence in situ hybridisation mapping

BAC and PAC clones were selected from the Wellcome Trust Sanger Institute ensembl contigs (http://www.ensembl.org) and obtained from the resource centre primary database of the German Human Genome Project (http://www.rzpd.de). In order to amplify larger (approximately 10 kb) BAC/PAC subfragments, the Expand Long Template polymerase chain

Abbreviations: FISH, fluorescence in situ hybridisation; OFC, occipito-frontal head circumference; RACE, rapid amplification of cDNA ends

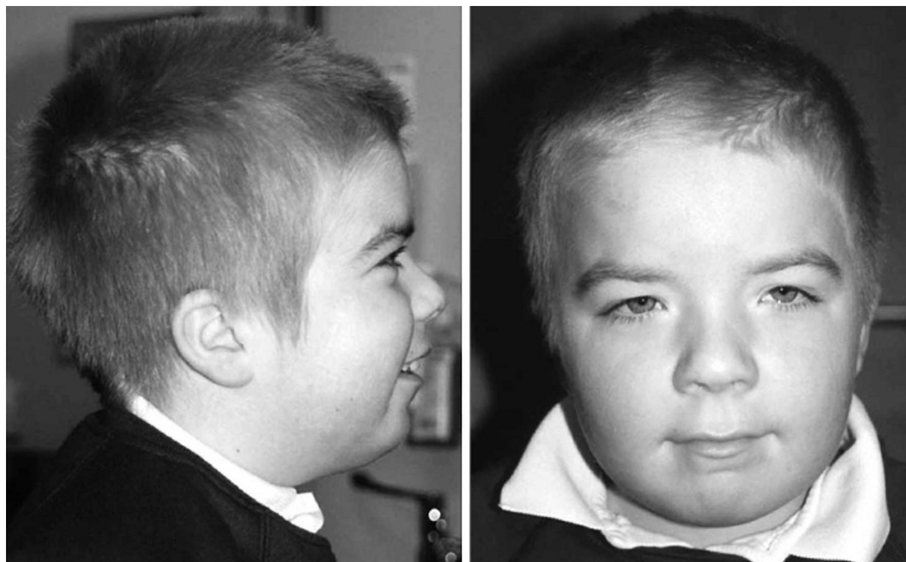

Figure 1 Profile and facial view of the t(1;6) translocation patient at the age of eight years. Written permission was obtained from the child's parents for these images to be reproduced.

reaction (PCR) system (Roche Products, Basel, Switzerland) was used according to the recommendations of the manufacturer, with a series of primer pairs (table 1) chosen from the genomic sequence of breakpoint spanning clones. Genomic BAC/PAC DNAs and their long range PCR products were labelled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche) by standard nick translation. Fluorescence in situ hybridisation (FISH) was carried out on metaphase spreads from EBV transformed lymphoblastoid cells using standard molecular cytogenetic techniques.[3]

Isolation of the mouse *Tcba1* gene

Total RNA was extracted from mouse tissues or whole embryos with Trizol reagent (Invitrogen, San Diego, California, USA) and reversely transcribed using Superscript III reverse transcriptase (Invitrogen). cDNA was synthesised from 3 μg of total RNA at 55℃ with mouse *Tcba1* reverse transcription primer (5′-cgattcgcagagacttaga-3′); 2 μl of this cDNA were then used as template in a PCR reaction with mouse *Tcba1* forward (5′-atgggttattgcagtggca-3′) and reverse primers. PCR was carried out with an initial denaturation at 94℃ for three minutes, 30 cycles of 94℃ for 45 seconds, 56℃ for 45 seconds, 72℃ for 1.5 minutes, and a final 10 minute extension at 72℃. To obtain the full length cDNA sequence, rapid amplification of cDNA ends (RACE) was done with the SMART RACE cDNA amplification kit (Clontech, Palo Alto, California, USA), according to the manufacturer's instructions. Briefly, 5′-RACE PCR was undertaken with a *Tcba1*-specific primer (5′-agcgatggtcttctggagcccagtctgg-3′) and Universal Primer A mix from the kit. 3′-RACE was done with another gene specific primer (5′-gtcccccagtcacatccttaggcgaacac-3′) and Universal Primer A mix. PCR was carried out with five cycles of 94℃ for 30 seconds, 72℃ for three minutes, five cycles of 94℃ for 30 seconds, 70℃ for 30 seconds, 72℃ for three minutes, and 25 cycles of 94℃ for 30 seconds, 68℃ for 30 seconds, 72℃ for three minutes, with a final 10 minute extension at 72℃. The PCR products were cloned into pCR2.1-TOPO vector (Invitrogen) and sequenced.

DNA sequence analysis

DNA sequence analysis was done using the genome BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Amino acid sequence alignment and transmembrane helix prediction were performed using the TmHMM and Clustal programs of Heidelberg Unix Sequence Analysis Resources (HUSAR) (http://genius.embnet.dkfz-heidelberg.de).

RESULTS AND DISCUSSION

Chromosomal breakpoint mapping

Chromosome banding analysis of this patient revealed an apparently balanced reciprocal translocation involving the exchange of material between the long arms of chromosomes 1 and 6 (fig 2A). The parental karyotypes were normal. In order to map the de novo translocation breakpoints at higher DNA resolution, we have assembled BAC/PAC contigs of the critical chromosome regions 1q32.3 and 6q22.3 according to the database (fig 2D). Individual BAC/PACs from the breakpoint regions were co-hybridised with

Table 1 Long range polymerase chain reaction fragments of BAC/PACs for fluorescence in situ hybridisation mapping

Fragment	Forward primer (5′–3′)	Reverse primer (5′–3′)	Position in clone
157N22A	gag aat gcc act gag cta ct	agc cct cac aga tga gat tc	481–13590 bp
157N22B	gga tag ctt tcc agc acc ta	aac tgt agt gag cgt gct tc	14954–26020 bp
157N22C	tgc tgt ttc ctc tgt ctc ag	gtt ctg ctg gaa ctg cag at	51120–62350 bp
157N22D	ggt ctg ggt tct atg tca ct	gta gaa tct tgg agc agc tc	61421–73681 bp
157N22E	agc tgt ggc tca gta gaa ac	atc aag cta gtc aga ggc tg	26501–39350 bp
338C15A	acc agc ttc atg tgg cta tc	act gag ctt gtc tgc cat ct	7631–20200 bp
338C15B	cct gtc aag tgc aca gaa tg	tct cag atg cca gaa tcc ag	28441–42030 bp
338C15D	tgg tga cac tgg agc att ct	cgt act gac tac gga tgt tc	54011–63770 bp

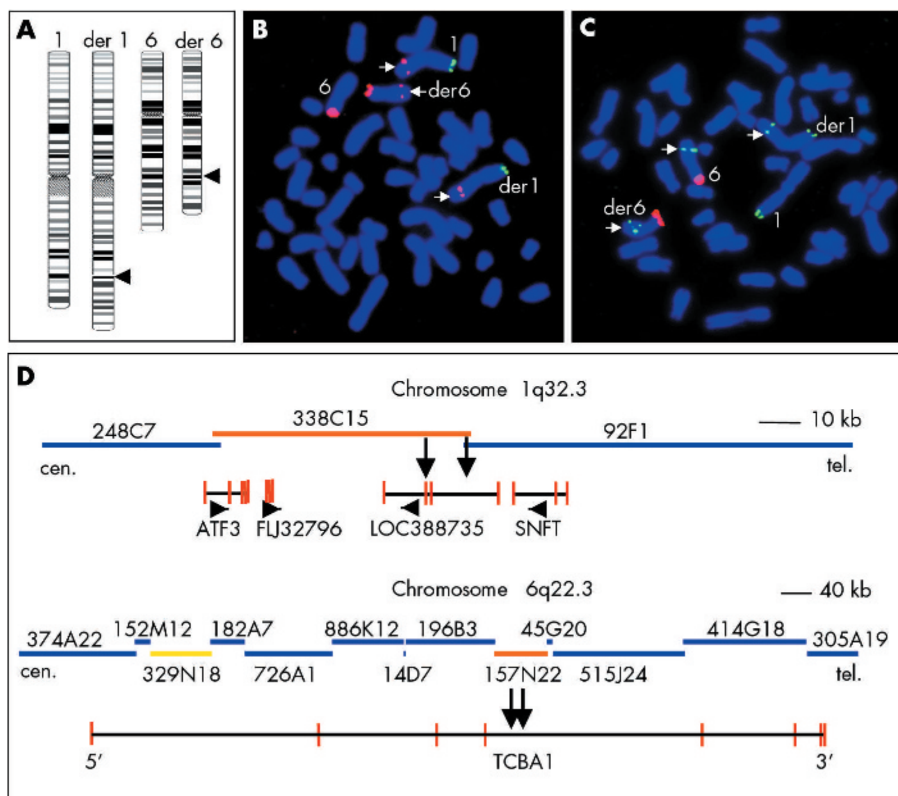

Figure 2 Molecular cytogenetic characterisation of the t(1;6)(q32.3;q22.3) translocation. (A) Ideograms of the patient's normal and derivative chromosomes 1 and 6. The breakpoints predicted by G band analysis are indicated by arrows. (B,C) High resolution fluorescence in situ hybridisation (FISH) mapping of BAC/PAC subfragments to patient's metaphase spreads. PACs CTB-14E10 (labelled by FITC in green) and CTB-62I11 (labelled by Cy3 in red) from the short arm ends of chromosome 1 and 6, respectively, were used for chromosome identification. The 1q32.3 breakpoint spanning polymerase chain reaction (PCR) fragment 335C15D (B; red signals indicated by arrows) produced signals on the normal chromosome 1, the der(1), and the der(6). The 6q22.3 breakpoint spanning PCR fragment 157N22E (C; green signals indicated by arrows) hybridised to the normal chromosome 6 and both derivative chromosomes. (D) BAC/PAC (blue, orange, and yellow bars) contigs of the breakpoint regions on chromosomes 1q32.3 and 6q22.3. The black lines below the contigs indicate the location of genes in the breakpoint regions, vertical red lines indicate exon sequences, arrowheads the direction of transcription. The breakpoint containing clones RP11-338C15 and RP1-157N22 are indicated by orange bars. The 1q32.3 and 6q22.3 breakpoint regions, respectively, are indicated by two vertical black arrows. The yellow clone RP3-329N18 is reported[7] to vary in copy number between individuals.

chromosome 1pter and 6pter identification probes to the patient's metaphase spreads. BAC RP11-338C15 at 209.4 Mb on chromosome 1 and PAC RP1-157N22 at 124.6 Mb on chromosome 6 produced FISH signals on the normal chromosome 1 and 6, respectively, and on both derivative chromosomes (data not shown), as expected for breakpoint spanning clones. To narrow down the breakpoint regions further, three long range PCR products—338C15A, 338C15B, 338C15D—were generated from the chromosome 1 breakpoint BAC and five PCR products—157N22A, B, C, D, and E—from the chromosome 6 breakpoint PAC (table 1). The 9.8 kb BAC fragment 338C15D produced a split hybridisation signal on the derivative chromosomes (fig 2B) and therefore must contain the 1q32.3 breakpoint. Similarly, the 6q22.3 breakpoint was localised to the 12.8 kb PAC fragment 157N22E, which hybridised to the normal chromosome 6 and both derivative chromosomes (fig 2C).

Disruption of the *TCBA1* gene on chromosome 6q22.3

According to our high resolution FISH mapping results the translocation breakpoint on chromosome 6 lies within intron 4 of the *TCBA1* gene (fig 2D), which encodes a transmembrane protein. The mouse *Tcba1* cDNA (GenBank accession number AY940478) was obtained from mouse brain RNA by reverse transcriptase polymerase chain reaction (RT-PCR) with primers directed against conserved regions of human *TCBA1* and subsequent 5′- and 3′-RACE PCRs. Human *TCBA1* has eight exons with the initiation codon located in exon 2, whereas the mouse gene has only seven exons and the initiation codon in exon 1 (fig 3A). Mouse *Tcba1* lacks the exon corresponding to human *TCBA1* exon 2. In both genes the stop codon is located in the last exon. Both the human and the mouse proteins are endowed with four transmembrane helices and share 91% amino acid sequence similarity (fig 3B). This evolutionary conservation is considered a good indicator of the gene's functional significance.

The human *TCBA1* gene is reported to be expressed exclusively in fetal and adult brain and thymus.[6] Our RT-PCR experiments in the mouse showed a high level of *Tcba1* expression in fetal and adult mouse brain and lower expression levels in testis and liver (fig 3C). *TCBA1* is involved in human T cell lineage specific chromosome aberrations at 6q21–q22.[6] Similar to our patient, the T cell lymphoblastic lymphoma cell line, HT-1, has a breakpoint in PAC RP1-157N22 (within intron 4 of *TCBA1*), fusing *TCBA1* to the *SUMO-1-specific protease* (*SUSP1*) gene. In the adult T cell leukaemia cell line, ATN-1, a breakpoint is located in PAC RP1-196B3 (within intron 3 of the *TCBA1* gene). In this context, it is also interesting to note that PAC RP3-329N18, which is located in intron 1 of *TCBA1* (fig 2D, yellow bar), was reported to show copy number variation among normal individuals. In an array based comparative genomic hybridisation study, one of 39 unrelated healthy individuals showed a copy loss of the RP3-329N18-syntenic segment.[7] However,

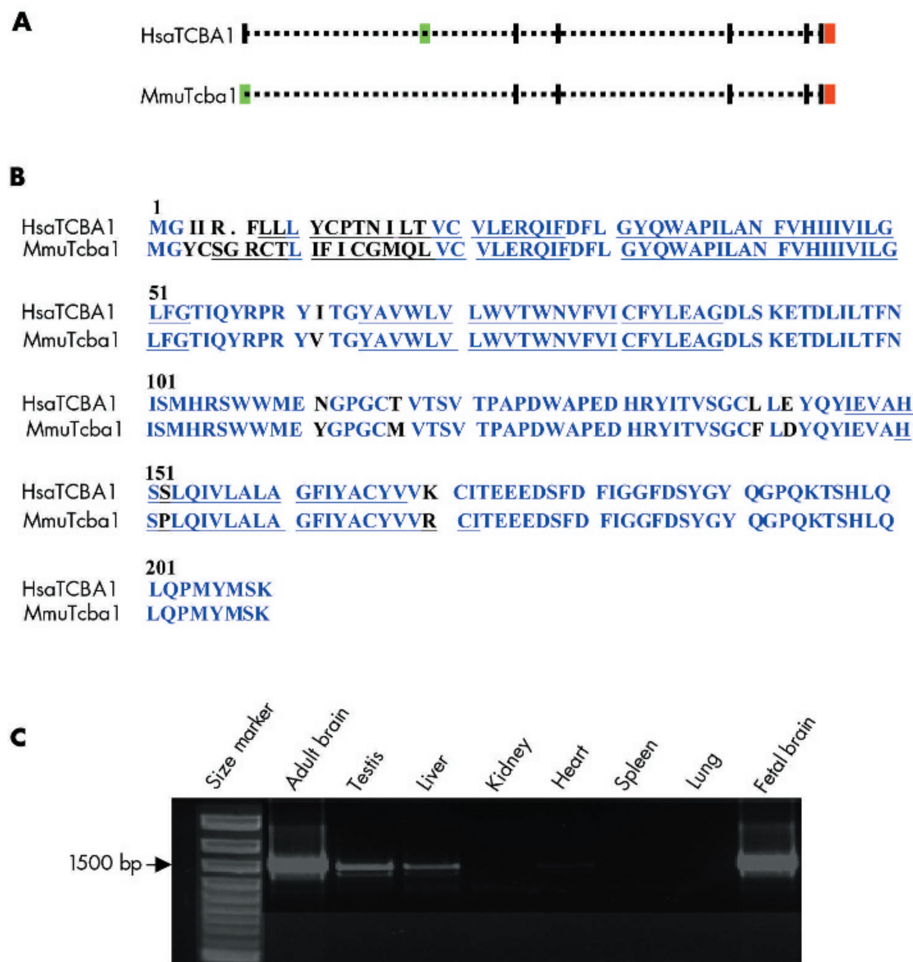

Figure 3 (A) Genomic structure of human (*Homo sapiens*, HSA) *TCBA1* and mouse (*Mus musculus*, MMU) *Tcba1*. Exons are indicated by vertical bars. The initiation codons (green bars) are located in exon 2 of human *TCBA1* and exon 1 of mouse *Tcba1*, respectively; the stop codons (red bars) are located in the last exon. (B) Amino acid sequence alignments of human TCBA1 and mouse Tcba1. Conserved amino acids are marked in blue. The four predicted transmembrane helix domains are underlined. (C) Reverse transcriptase polymerase chain reaction carried out with mouse RNA preparations from adult brain, testis, liver, kidney, heart, spleen, lung, and fetal brain. *Tcba1* is highly expressed in adult and fetal brain with lower expression in testis and liver.

by PAC FISH on metaphase spreads of 53 control individuals we always observed hybridisation signals of equal intensity on both chromosomes 6q22 (data not shown). Thus copy number gains or losses involving RP3-329N18-syntenic sequences appear to be relatively rare. Nevertheless, it is possible that loss of a large DNA segment from intron 1 affects *TCBA1* regulation and thus contributes to interindividual differences in the susceptibility to haematopoetic malignancies. The clustering of constitutional and tumour breakpoints within *TCBA1*, as well as large scale copy number variations involving intronic sequences, suggests that this genomic region is highly dynamic and predisposes to chromosome rearrangements.

Alterations of *TCBA1* have so far not been connected with C-ANCA associated vasculitides such as Wegner's granulomastosis, microscopic polyangiitis, or Churg-Strauss syndrome. However, the finding of raised C-ANCA levels in our patient in association with his history of recurrent respiratory problems, and also his gastrointestinal symptoms, could be compatible with a vasculitis, and investigations are ongoing.

Disruption of a hypothetical gene on the chromosome 1q32.2

The breakpoint region on chromosome 1q32.3 contains two hypothetical and two known genes (fig 2D). The *activating transcription factor 3* (*ATF3*) gene lies approximately 50 kb proximal and the *small nuclear factor isolated from T-cells* (*SNFT*) gene approximately 30 kb distal to the breakpoint. The 1q32.3 breakpoint spanning fragment 338C15D is contained in the hypothetical gene LOC388735. However, so far the only evidence for the existence of this gene is a single EST derived from anaplastic oligodendroglioma. Neither RT-PCR of RNAs from fetal and adult brain and multiple other tissues, nor PCR screening of a human brain cDNA library (RZPD No 588) with LOC38873 specific primers (forward 5′-agtatgtgcatcaccagca-3′; reverse 5′-tgagactccaccattacag-3′), nor northern blots have revealed any transcript(s), supporting this gene (data not shown).

Conclusions

Our patient with de novo translocation t(1;6)(q32.3;q22.3) provides evidence that constitutional inactivation (haploinsufficiency) of the *TCBA1* gene causes developmental delay and a distinct phenotype. The expression patterns of the human and mouse genes in fetal and adult brain, thymus, liver, and testis are consistent with some features of our patient, in particular developmental delay and recurrent infections. Although we cannot exclude the formal possibility that a *TCBA1* fusion gene is formed by the translocation, our data argue in favour of a loss of function mutation producing

a truncated protein. Isolation and partial characterisation of the orthologous mouse *Tcba1* gene is a first step towards functional studies in knock-out mice.

ELECTRONIC DATABASE INFORMATION

BLAST program: http://www.ncbi.nlm.nih.gov/BLAST/; Heidelberg Unix Sequence Analysis Resources: http://genius.embnet.dkfz-heidelberg.de; Ensembl Genome Browser: http://www.ensembl.org; Resource Center Primary Database of the German Human Genome Project: http://www.rzpd.de

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Conflicts of interest: none declared

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ECHO

Carriers of mild *CFTR* mutations risk chronic pancreatitis

Please visit the *Journal of Medical Genetics* website [www.jmedgenet.com] for a link to the full text of this article.

A sequencing study has settled the question whether mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene predispose to idiopathic chronic pancreatitis (ICP). They do—not just when occurring in compound heterozygotes but in cystic fibrosis carrier states with rare/mild *CFTR* mutations so the risk of ICP in white populations is higher than previously thought.

Patients with ICP at a tertiary referral clinic in north west Germany had twice the rate of mutant *CFTR* alleles as geographically and ethnically matched controls. Apart from serious heterozygous *CFTR* mutations there were 19 rare/mild *CFTR* mutations, some comprising 5T mutations and 5T mutations with 12TG repeats, compared with only five in the controls. In fact, 5T-12TG allele variations were about five times commoner in patients and were significantly commoner combined with other *CFTR* mutations too. Subjects with one mild *CFTR* mutation or 5T-12TG combination were also considered at increased risk of ICP.

Sequencing of all 27 CFTR exons from amplified DNA was performed for 62 patients with ICP and 60 unrelated healthy controls.

ICP is caused by mutations in *PRSS1*, *SPINK1*, and *CFTR* genes. *CFTR* specifies a widespread chloride channel and severe mutations cause classic CF. Different types of mutation causing different types of channel malfunction probably result in different diseases. *CFTR* is so large that only complete sequencing can tell which mutations may cause ICP. Also geographically and ethnically matched controls are needed as no reliable data on frequency of uncommon *CFTR* mutations in healthy control populations are available.

▲ Weiss FU, *et al. Gut* 2005;**54**:1456–1460.