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Williams–Beuren syndrome: genes and mechanisms

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Williams–Beuren syndrome (WBS; OMIM 194050) is caused by heterozygous deletions of ~1.6 Mb of chromosomal sub-band 7q11.23. The deletions are rather uniform in size as they arise spontaneously by inter- or intrachromosomal crossover events within misaligned duplicated regions of high sequence identity that flank the typical deletion. This review will discuss the status of the molecular characterization of the deletion and flanking regions, the genes identified in the deletion region and their possible roles in generating the complex multi-system clinical phenotype.

PHYSICAL AND NEUROBEHAVIORAL PHENOTYPES

Williams–Beuren syndrome (WBS; OMIM 194050) can be diagnosed clinically by a recognizable pattern of malformations, uneven cognitive disabilities—with relative preservation of linguistic abilities and gross deficiencies in visual-spatial processing—and characteristic behavior patterns. The clinical findings, including dysmorphologic, cardiovascular, ophthalmologic, renal and dental abnormalities, as well as neurological and cognitive features are variable (1–4). The diagnosis is confirmed by FISH analysis with cosmids containing part of the elastin (ELN) gene that is located in the center of the commonly deleted region (5). Findings that are either controversial or novel are discussed below.

Growth delay is multi-factorial with possible parent-of-origin effect

Short stature is a common feature. When data from all age groups are combined, 30–40% of WBS individuals fall below the third percentile of standard growth curves. We previously reported a significant difference in height, weight and head circumference depending on the parental origin of the deletion (3). Maternal origin was associated with lower growth parameters in 38 informative patients. In a series of 22 Danish WBS deletion cases, Brondum-Nielsen *et al.* (6) also found 'a tendency for milder growth retardation in patients with a paternal versus a maternal deletion', but the numbers of informative cases were too small to be conclusive. In contrast, Wu *et al.* (7) reported no correlation with parental origin in 49 informative cases of which 41% were below the third percentile for growth. The frequencies of vascular stenoses that could contribute to growth delay were similar in the two studies. In our series, 50% (24/48) had supravalvar aortic stenosis (SVAS) and 27% (13/48) had peripheral pulmonary stenosis, whereas Wu *et al.* (7) only provided a combined number of 70% (47/67) for both. From the available data, it is unclear whether the two patient populations are comparable with respect to ages at measurement, sex distribution and ethnic origin, all variables that contribute to the position of a measurement on the growth curve. A recent longitudinal study of mostly Caucasians revealed that short stature in WBS is due to a combination of factors: decreased growth rate in early childhood with delayed bone age, followed by a premature and abbreviated pubertal growth spurt with accelerated bone age, resulting in a final height of 152.4 ± 5.7 cm for girls and 165.2 ± 10.9 cm for boys (8). Bimodality in premature onset of puberty was observed for girls only, but no information for parental origin of the deletions in the two groups was reported. It remains to be seen whether there are imprinted genes in the deletion region which influence statural growth and/or onset of puberty.

Structural brain abnormalities are not consistent

Brain magnetic resonance imaging (MRI) data on 11 WBS subjects (five male, six female, aged 10–20 years) were subjected to quantitative morphometric analyses following an initial report of an unusual brain structure in WBS individuals (9). While the overall size of the WBS brains was diminished, the frontal lobes and the limbic regions of the temporal lobes appeared better preserved and the neocerebellum was said to be relatively enlarged (10,11). No consistent cerebral abnormalities were detected in a recent MRI study of 39 WBS cases (19 females, 20 males, aged 2 months–25years) (12).

Visual-spatial processing dysfunction contributes to apraxia

The gait abnormalities (e.g. gait apraxia) have sometimes been attributed to cerebellar dysfunction. In the absence of other cerebellar signs, it seems more likely that they are caused by problems with visual-spatial integration. Whereas strabismus is common, reduced stereoacuity and binocular vision were demonstrated even in the absence of strabismus (13,14). Withers (15) has called attention to the fact that WBS children are reluctant to change the surface on which they are walking or playing and dislike sandy or uneven surfaces. This 'new clinical sign' may not be limited to children. A recently diagnosed 48-year-old WBS individual stated: 'I don't like the ground and it doesn't like me'.

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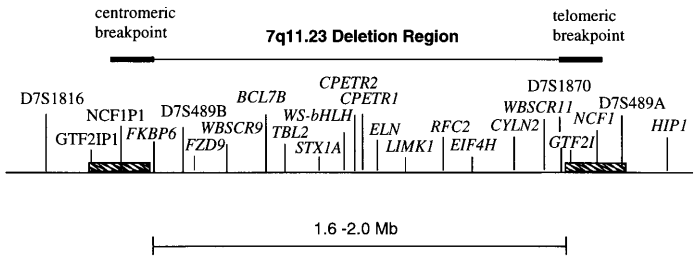

Figure 1. Map of genes in the WBS deletion region. Symbols for protein coding genes are in italics. Distances between the loci are not drawn to scale. Hatched boxes represent the two most closely flanking repeat clusters.

Personality and behavior change with age

Personality and behavioral characteristics have been well described for WBS children. Recent comparative studies of different age groups revealed significant changes over time (16). Adults are less restless, active, lively, outgoing and quarrelsome than children with WBS; they are better adjusted and more withdrawn with a tendency to depression. In a group of 70 adults with a mean IQ of 62, adaptive behavior scores and measures of independence were lower than for adults of similar age and intellectual abilities (17). The WBS group of adults exhibited high rates of emotional and behavioral problems, poor social relationships, disinhibitions, preoccupations, obsessions and high levels of distractibility and anxiety (18). Therefore, the typical WBS personality and neurobehavioral characteristics are detectable early in life and undergo changes with adjustments during lifetime. They are characteristic enough to set this population apart from others with similar overall intellectual impairment.

CYTOGENETICS

The typical WBS deletion affects only the Giemsa-light staining band 7q11.23 that contains ~8×10^6 bp. The deletion is visible on high-resolution chromosomes, but only if the relative position of the light-gray-staining sub-band 7q11.22 that subdivides band 7q11.2 is considered. Band 7q11.22 is normally placed closer to the centromere, but on a WBS deletion chromosome it assumes a central location. This observation led us to suggest that the deletion may be up to 2 Mb in size, consistent with the 2 cM genetic distance between the most proximal and most distal intra-deletion markers (3).

Larger deletions, visible by standard cytogenetic methods and extending telomerically by polymorphic marker analysis have been identified in patients with WBS features and seizure disorders such as infantile spasms with hypsarrhythmia (19–22).

THE WBS DELETION IS FLANKED BY LARGE REPEATS CONTAINING GENES AND PSEUDOGENES

Our initial hope that the phenotypic variability may be due to variably sized overlapping deletions that would allow the creation of a 'phenotype map' was shattered by the finding of uniform deletion sizes (3). Polymorphic marker typing revealed the extent of the deletion to be ~1 cM in both directions from the ELN locus. In all informative WBS patients with ELN deletions detected by FISH, the proximal deletion breakpoints were between the polymorphic markers D7S1816 and D7S489B (Fig. 1). The distal deletion breakpoints were mapped between D7S1870 and D7S489A (3). This conclusion was confirmed in other patient populations (7,23–25). D7S489A and D7S489B are amplified with the same primer pair and are distinguished by the size ranges of the amplicons. At least one other locus (D7S489C) mapping in the proximal flanking region is also amplified with these primers. The notion that the deletion is flanked by highly identical repeated sequences, as suggested by Pérez-Jurado *et al.* (3), was further supported by the finding of sequences related to the human PMS2 mismatch repair gene at three sites flanking the WBS deletion (26). Human sequence homologs of yeast mismatch repair genes (PMS), such as the PMS2 gene on 7p22, may be mutated in hereditary non-polyposis colorectal cancer. Several loci related to PMS2 map to distinct regions of chromosome 7 including 7q22 and 7q11.23. High levels of sequence identity between the PMS2-like genes makes it difficult to map them individually; their association with the polymorphic dinucleotide repeat markers D7S489A and C, however, indicates that they are part of the cluster of repeated genes that flank the WBS deletion region (26). As none of the PMS2-like genes on 7q has been shown to encode a protein, they may be pseudogenes.

The most interesting finding is a partial duplication of the GTF2I gene that encodes the multifunctional transcription factor TFII-I (also called SPIN or BAP-135). GTF2I has a unique 5′ end that is consistently deleted in WBS and a duplicated 3′ portion that is almost identical to a partially truncated expressed pseudogene (GTF2IP1) which is located in the centromeric flanking region and is not deleted (27). Thus, the GTF2I gene demarcates the telomeric end of the unique sequence of the WBS deletion and the centromeric border of the distal duplicated region (Fig. 1). GTF2I and GTF2IP1 map to different genomic clone contigs that also contain other deleted and non-deleted loci.

Immediately telomeric to GTF2I, and in the same transcriptional orientation, is neutrophil cytosolic factor 1 (NCF1) which encodes the p47 cytosolic component of leukocyte NADPH oxidase (p47-phox). This protein complex is required for the oxidative burst in neutrophils. Deficiency of any one of the three subunits causes chronic granulomatous disease (CGD). The gene for NCF1 was mapped by us to 7q11.23 in 1990 (28). The signal intensity obtained with radioactive chromosomal *in situ* hybridization was much stronger than expected for a single copy locus, consistent with multiple copies at this site. Analysis of the BAC RG269P13 (GenBank accession no. AC005080) sequence revealed a nearly identical pseudogene (NCF1P1) downstream of GTF2IP1 within the centromeric flanking region duplication. Depending on where in the flanking duplication the recombination event leading to the WBS deletion occurs, the

NCF1 gene may or may not be deleted in WBS individuals. Hemizygosity at this locus is not expected to produce a phenotype as heterozygotes for the autosomal recessive form of CGD that is caused by NCF1 mutations are asymptomatic. About 90% of autosomal recessive CGD cases world-wide are caused by a recurrent mutation in NCF1, a GT dinucleotide deletion from a GTGT tetranucleotide located at the border of intron 1 and exon 2. As this GT deletion is present in NCF1P1, Görlach *et al.* (29) suggested that the frequently recurring mutation is caused by a homologous recombination or gene conversion event between NCF1 and an NCF1 pseudogene. Evidence for a second NCF1 pseudogene has been presented (29, 30). Together with the possibility of a third GTF2I-like gene, called IB291 by Osborne *et al.* (26), the existence of a third repeat cluster is a likely hypothesis. The location and organization of this third repeat and its possible involvement in WBS deletion formation have been presented (R. Peoples, Y. Franke, Y.-K. Wang, L. Pérez-Jurado, T. Paperna and U. Francke, manuscript in preparation).

Another duplicated gene in the 7q11.23 region is ZP3 which encodes a glycoprotein, located at the zona pellucida of the oocyte, that functions as a sperm receptor. Besides an intact copy of ZP3, there is a fusion gene, also at 7q11.23, between the 3′ portion of a duplicated ZP3 gene and a part of a duplicated POM121 gene. The fusion gene contains a polymorphic G nucleotide insertion in exon 8 which causes a frameshift and premature termination codon (31). The precise location of the ZP3-related genes is yet to be determined.

We estimate the size of the duplicated region in which the WBS deletion breakpoints cluster to be at least 300 kb, based on high-throughput genome sequence data from the Washington University Human Genome Sequencing Center database and our own pulsed-field gel electrophoresis map and BAC/PAC contig (R. Peoples, Y. Franke, Y.-K. Wang, L. Pérez-Jurado, T. Paperna and U. Francke, manuscript in preparation). Sequence comparisons reveal a very high degree of identity with only ~1 in 1000 nucleotides different.

These low-copy repeats predispose to unequal meiotic recombination between chromosome 7 homologs and/or to intrachromosomal rearrangements. Family studies of *de novo* deletion cases with genotyping of the grandparents revealed a high frequency (about two-thirds of cases) of interchromosomal rearrangements regardless of whether the deleted chromosome was maternally or paternally derived (32).

Gtf2i, *Ncf1* and *Zp3* are single copy genes in the mouse and have been mapped to mouse chromosome 5 near the *Eln*, *Limk1*, *Cpter1* loci which are homologs of human single copy genes within the WBS deletion (33). Comparative mapping of *Gtf2i*/*Ncf1* containing genomic clones by FISH revealed duplications within 7q11.23 and signals at 7p22 and 7q22 in chimpanzee and gorilla but single copy signals for mouse (34).

SINGLE COPY GENES WITHIN THE WBS DELETION

Elastin (ELN)

Disruption of the ELN gene by translocation or partial deletion is associated with SVAS. This discovery provided the first clue to the location of the WBS microdeletion (35,36). The ELN gene encoding tropoelastin is composed of 34 exons distributed over ~50 kb in the middle of the 1.6 Mb WBS deletion region (Fig. 1). ELN mutations leading to premature stop codons also cause SVAS (37,38). The association between truncating or null mutations and arterial stenoses is further supported by studies of mutant mice hemizygous for elastin gene expression (39). Even though $Eln^{+/-}$ mice were reported to be clinically and hemodynamically normal, histological studies revealed increased numbers of elastic lamellae in their arterial vessel walls, similar to findings in the aortic wall (outside of the stenotic area) in patients with SVAS. It is intuitively contradictory that reduced *Eln* expression during intrauterine development should lead to increased thickness of the tunica elastica. In homozygous $Eln^{-/-}$ mice completely lacking tropoelastin synthesis, increased thickness of the aortic wall due to subendothelial accumulation of smooth muscle developed after embryonic day 17.5 and eventually led to obliteration of the vascular lumen and neonatal death (40).

Of all WBS individuals proven to be hemizygous for ELN, only 50–60% had documented SVAS (3). Narrowing of other arteries occurs, with peripheral pulmonary stenosis the most common, and can lead to serious complications; e.g. cerebral artery stenosis to stroke, renal artery stenosis to severe hypertension, coronary artery stenosis to myocardial infarction. While no extracardiac findings were reported in $Eln^{+/-}$ mice, some of the connective tissue abnormalities in WBS have been attributed to ELN hemizygosity: premature sagging of skin, hoarse voice, periorbital puffiness, herniae and diverticula of colon and bladder. More recent reports, however, present SVAS patients with ELN deletions or mutations with normal facial features (37,38). The consistency of the facial gestalt of WBS that is lacking in SVAS suggests that genes other than ELN are major contributors.

Mutations leading to structurally abnormal tropoelastin molecules rather than to a reduction of normal molecules are found in autosomal dominant cutis laxa (41,42). The fact that vascular wall abnormalities and intestinal diverticula may also be present in patients with cutis laxa suggests a continuum of disorders caused by ELN mutations (‘elastinopathies’, in analogy to ‘fibrillinopathies’) (43).

The other known genes in the deletion region are discussed as listed in Table 1, in the order of their discovery.

Replication factor complex subunit 2 (RFC2)

RFC2 encodes the second largest (40 kDa) subunit of this five protein complex that is essential for DNA strand elongation during replication. The gene had been mapped to the 7q11.2 region by Okumura *et al.* (44) and was shown to be within the deletion by Peoples *et al.* (45) and Osborne *et al.* (25). The RFC assembles proliferating cell nuclear antigen (PCNA) and DNA polymerase δ or ε on primed DNA templates in the presence of ATP. The RFC2 protein contains the ATP binding domain and interacts directly with PCNA and the polymerase. Loss of one copy of the RFC2 gene could affect the efficiency of DNA replication and contribute to growth and developmental defects.

LIM kinase-1 (LIMK1)

Located immediately telomeric to ELN (25), this gene is deleted together with ELN in some SVAS families. Encoding a non-receptor serine–threonine kinase containing LIM and PDZ domains, LIMK1 gained notoriety when it was implicated in the impaired visuo-spatial cognition that is characteristic for WBS (46). This claim was not confirmed, however, in recent studies of three SVAS individuals, two familial and one sporadic, with

Table 1. Genes in the WBS deletion (1999)

ELN	Elastin
RFC2	Replication factor C, subunit 2
LIMK1	LIM kinase-1
FZD9	Frizzled, *Drosophila* homolog, 9 (= FZD3)
STX1A	Syntaxin 1A
EIF4H	Eukaryotic translation initiation factor 4H (= WBSCR1)
GTF2I	General transcription factor II-I (= BAP135, SPIN)
CYLN2	Cytoplasmic linker protein (= WBSCR4, CLIP-115)
FKBP6	FK506 binding protein-6
WBSCR9	Bromodomain, PHD finger transcriptional regulator (= WSTF)
BCL7B	B-cell lymphoma 7 gene-related
WS-bHLH	Basic helix–loop–helix leucine zipper gene
TBL2	Beta transducin related gene-2 (WD40 motifs)
CPETR1	*Clostridium perfringens* enterotoxin receptor-1 (= CLDN4)
CPETR2	CPE receptor-2 (= RVP1, CLDN3)
GTF2IRD1	TFII-I repeat domain-containing transcriptional regulator 1 (=WBSCR11)

Genes within the WBS deletion region are listed in the approximate order of discovery. Alternative names and gene symbols are given in parentheses.

LIMK1 and ELN deletions, who were tested using the same psychometric test instruments (47). The visuo-spatial cognitive disabilities of WBS individuals are probably the result of haploinsufficiency for more than one gene in the deletion. LIMK1 may be a necessary, but insufficient, contributor. The physiological role of the LIMK1 protein has become clearer with the recent demonstration that it interacts with the cytoplasmic tail of the transmembrane receptor neuregulin with which it co-localizes at the neuromuscular synapse (48). A phosphorylation target of LIMK1 has been identified as cofilin, a regulator of the actin cytoskeleton (49), suggesting that LIMK1 may be involved in Rac-induced actin reorganization and cell movement.

Frizzled 9 (FZD9, previously called FZD3)

CpG island cloning applied to cosmids from the WBS deletion region led to the identification of a human gene with similarity to the *Drosophila* wnt receptor *frizzled* (50). The 591 amino acids include an N-terminal cysteine-rich ligand-binding domain and seven transmembrane domains. *Drosophila* or human cells, when transfected with a human FZD9 gene construct, bind *Drosophila wingless* (wg) protein. FZD9 is highly expressed in all areas of the brain, and also in eye, testis, skeletal muscle and kidney (50). Expression of the mouse homolog (*Fzd9)* was first detected in day 9 embryos in the entire neural tube encompassing the anlagen of the brain and spinal cord (51). At day 10, *Fzd9* expression was strongest in the telencephalon. *Fzd9* expression was also detected in derivatives of somites and in differentiating myocytes, the precursors of trunk skeletal muscle. Furthermore, *Fzd9* was highly expressed in all stages of spermatogenesis. Immunolocalization studies with an antibody against an FZD9 peptide showed that the protein is located at the cell membrane in unpermeabilized cells that are transfected with an Fzd9 cDNA clone (51).

Syntaxin 1A (STX1A)

Syntaxin 1A, a member of the syntaxin family, is a 35 kDa brain-specific protein essential for neurotransmitter release from synaptic vesicles. It interacts with synaptotagmin and other synaptosomal proteins. STX1A was mapped centromeric to ELN within the WBS deletion (52). Its consistent deletion was confirmed by FISH analysis of 46 patients (53). When Yamaguchi *et al.* (54) discovered that suppression of syntaxin 1A enhances neurite sprouting, a process underlying neuronal plasticity, they speculated that hemizygosity for STX1A could contribute to the enhanced auditory memory documented for some WBS individuals.

Eukaryotic initiation factor 4H (EIF4H)

An unknown transcript (WSCR1, later renamed WBSCR1) predicted to encode 232 amino acids including an RNA-binding motif was identified in a 500 kb cosmid contig extending from ELN to the telomere (25). Subsequently, Richter-Cook *et al.* (55) isolated a new initiation factor from rabbit reticulocyte lysate that stimulates translation activities and RNA-dependent ATPase activities of other translation initiation factors *in vitro*. The amino acid sequence was identical to WBSCR1. The eIF4H protein serves as a positive regulator of protein synthesis at the level of translation initiation; it may also direct differential translation of mRNAs in different tissues.

Cytoplasmic linker 2 (CYLN2)

The cytoplasmic linker protein CLIP-170 (also called restin) attaches endosomes to microtubules. A related microtubule-binding protein, CLIP-115, is expressed predominantly in brain where it is found in the dendritic lamellar body, an organelle in bulbous dendritic appendages of neurons linked by dendrodendritic gap junctions (56). The human gene for this protein (CYLN2) was mapped to 7q11.23. It spans at least 140 kb within the telomeric end of the WBS deletion and includes two previously identified transcripts (25) WSCR 3 and 4 (same as WBSCR3 and 4) (57).

FK506-binding protein 6 (FKBP6)

FKBP6 encodes a protein with structural similarity to FKBP immunophilins that function as cellular receptors for the immunosuppressive drugs FK506 and rapamycin. All nine FKBP6 exons are contained within a cosmid that is deleted in WBS (58). FKBP6 is a single copy gene located near the common centromeric deletion breakpoint.

WBSCR9/WSTF

Southern hybridization with a YAC end sequence revealed cross-species conservation with Chinese hamster and led to the isolation of a gene for a novel putative transcriptional regulator (gene symbol WBSCR9) (59). The predicted 1483 amino acids, encoded by 20 exons extending over >60 kb, include numerous functional motifs: a bromodomain, a PHD (or C4HC3) finger domain, putative nuclear localization signals, four nuclear receptor binding motifs and two PEST sequences. Outside these motifs, the sequence is completely novel. The functional motifs suggest a nuclear protein undergoing rapid degradation that functions in steroid hormone receptor-modulated transcriptional

regulation. By northern analysis, an ~7 kb transcript was found in all tissues throughout development in humans and mice. In some human tissues, we also identified a smaller (4.5 kb) transcript generated by alternative splicing that deletes the large (1703 bp) internal exon 7. The same gene (called WSTF) was independently reported by Lu *et al.* (60) who did not describe any alternative splicing. Due to a sequencing error, WSTF predicts a prematurely truncated protein of 1425 amino acids.

BCL7B

A gene of unknown function, located between FZD9 and STX1A, was named BCL7B because of a 90% identity in the 51 N-terminal amino acids with a gene disrupted by a leukemia translocation called BCL7A (B cell CLL/lymphoma 7A) on 12q24 (61,62). Six exons spanning 14 kb of genomic DNA generate a 2.2 kb transcript (1.4 kb in cardiac muscle) that encodes a 202 amino acid protein without any recognizable motifs.

WS-bHLH

Still without an approved gene symbol, a partial transcript in the proximal part of the WBS deletion was reported as WS-bHLH (61). The predicted protein sequence contains highly conserved bipartite domains for DNA binding and a leucine zipper motif for protein–protein interaction suggesting a function as a transcription factor. The characterization of the complete gene is in progress (L. Pérez-Jurado, personal communication).

TBL2 (transducin b-like 2)

This single copy gene within the WBS deletion encodes a protein with four putative WD40 repeats of the type first described in the β-transducin family (63). Most of the >30 known proteins in the β-transducin family are involved in regulatory functions. Pérez-Jurado *et al.* (64) have recently characterized the complete human and mouse cDNAs and the human genomic structure of TBL2. Compared with the report of the same gene, named WS-βTRP by Meng *et al.* (61), TBL2 contains 21 additional amino acids at the N-terminus. The putative initiation methionine codon that was suggested for WS-βTRP is not conserved in the mouse sequence. Loss of one copy of TBL2 in WBS is likely to be significant for the phenotype because haploinsufficiency for another protein containing WD repeats, LIS1, causes Miller–Dieker lissencephaly syndrome (65). Another putative transcription factor containing four WD40 domains is the product of *TUPLE1* (TUP-like enhancer of split gene 1) in the DiGeorge/Velocardiofacial syndrome (VCFS) deletion at 22q11.

Claudin 4 (CLDN4/CPTER1) and claudin 3 (CLDN3/CPTER2)

A novel cDNA isolated from the WBS deletion was found to be related to the rat ventral prostrate protein (RVP1) (66). When the gene was independently identified as encoding the *Clostridium perfringens* enterotoxin receptor, it was named CPTER1. The true human homolog of RVP1 was also cloned (called CPTER2) and found to map near CPTER1 (66). The mouse homologs *Cpter1* and *Cpter2* were identified and assigned to the conserved syntenic region on mouse chromosome 5. Expression patterns of CPTER1 in both species are specific for lung, kidney, intestine, pancreas and thyroid, with absence of expression in brain, heart, muscle and spleen. Sequence analyses of the human and mouse genes revealed that they are members of the claudin family of four transmembrane proteins (66). Recently, the products of mouse *Cpter1* and *2* (reported as *claudin 4* and *3*, respectively) were located to tight-junctions of epithelial cells in kidney and liver (67). All claudin family genes are intronless. CPTER1 and 2 are the most closely related members and likely arose from a gene duplication event. A more distantly related relative, called TMVCF or *claudin 5*, maps within the VCFS deletion at 22q11 (68).

WBSCR11/GTF2IRD1

Another putative transcription factor gene was independently reported by Osborne *et al.* (69) as WBSCR11 and Franke *et al.* (70) as GTF2IRD1. The transcript and genomic structure data are in disagreement. We found that expression of GTF2IRD1 is ubiquitous with two transcripts of 3.6 and 5.0 kb that are generated by alternative splicing. The 944 amino acid predicted protein contains a region of high similarity to a unique motif with helix–loop–helix forming potential that also occurs within the transcription factor TFII-I (GTF2I). The gene is located just centromeric to GTF2I in the same transcriptional orientation. It appears to be single copy and falls entirely within the WBS deletion. GTF2IRD1was independently isolated as the gene encoding a novel troponin I upstream enhancer binding protein, called Mus TRD1 for muscle TFII-I-repeat domain-containing protein 1 (71). Although the reported cDNA sequence is almost identical to GTF2IRD1 and a similar 3.3 kb transcript was described, the predicted MusTRD1 protein is only 458 amino acids long due to an apparent sequencing error (70).

OTHER TRANSCRIPTS

Systematic searches for transcripts in the WBS deletion by cDNA selection methods have not revealed any novel genes apart from those summarized above. The status of the putative WSCR2 transcript (25) remains uncertain. The 'novel' transcript WS52 reported by Hockenhull *et al.* (30) is in fact the same as WSCR5 that lies within almost 100 kb of contiguous sequence near RFC2 (25). The gene has not been fully characterized. It is possible that the transcription of other genes in the deletion region is regulated in a developmental stage- or tissue-specific manner. Alternatively, stable transcript levels could be too low for detection by common cDNA selection strategies.

GENES OUTSIDE THE DELETION

The calcitonin receptor gene (CALCR) was an attractive candidate for involvement in infantile hypercalcemia, a manifestation of WBS. The gene was mapped outside the WBS deletion to band 7q21.3 (72). The huntingtin-interacting protein 1 (HIP1) gene appeared to be located near ELN by RH mapping. We excluded HIP1 from the common deletion region by PCR analysis of WBS-derived somatic cell hybrids and by dosage blotting (73). Large-scale restriction mapping by PFGE placed HIP1 in the telomeric flanking region (R. Peoples, Y. Franke, Y.-K. Wang, L. Pérez-Jurado, T. Paperna and U. Francke, manuscript in preparation). The CC chemokine MPIF-2 or eotaxin-2 (SCYA24) gene has been assigned to a YAC that contains the proximal breakpoint region repeat cluster. The gene does not seem to be deleted in WBS (74). Cysteine-rich intestinal

protein (CRIP) is the name for a group of LIM/double zinc finger containing proteins. Of the three known human CRIP genes, CRIP1 is expressed in heart, not in intestine. By RH mapping, CRIP1 was located between two markers in the telomeric flanking region of the WBS deletion (75). No studies of WBS-derived hybrid lines or dosage blots have been reported to definitely exclude this locus from the deletion interval.

GENOTYPE–PHENOTYPE CORRELATION

Various partial clone contigs and transcript maps of the WBS deletion region have recently been published (30,61,69). From these and our own clone contig and pulsed-field gel electrophoresis restriction mapping data (R. Peoples, Y. Franke, Y.-K. Wang, L. Pérez-Jurado, T. Paperna and U. Francke, manuscript in preparation) we conclude that the deletion is 1.6 Mb in size. Based on the genomic sizes of the known loci in the deletion and the abundance of SINE repeats in the sequenced BACs, we estimate that ~50% of all transcripts may be known. The completion of the transcript map will probably proceed by comparative sequencing of the conserved homologous regions in the human and mouse genomes (34).

The task of determining which of these genes are dosage-sensitive and contribute to which particular features of the phenotype appears daunting. The identification of rare individuals with smaller deletions might be helpful, but any conclusions derived from them need to be supported by exhaustive clinical and molecular studies. Tassabehji *et al.* (47) reported a girl (CS) with isolated SVAS and peripheral pulmonary stenosis who was otherwise normal. PCR analysis of an unspecified number of somatic cell hybrids derived from a lymphoblastoid cell line revealed a partial deletion. No data were presented that exclude the possibility of somatic mosaicism due to post-zygotic origin of this deletion. Therefore, to exclude all the genes in this patient's deletion from contributing to the WBS phenotype appears certainly premature.

When pondering the phenotypic consequences of chromosomal microdeletions, different models and mechanisms have to be considered. Besides an additive effect of individual haploinsufficient loci, possible interactions between genes that affect the same molecular pathways, sequence variants of the non-deleted alleles, parent-of-origin specific modulation of expression of the remaining alleles, alteration of gene expression in the flanking regions due to altered chromatin conformation caused by the deletion, and the effects of genes located elsewhere whose expression may be affected by the transcriptional regulators encoded by genes in the deletion already identified, could all play a role. To further dissect the pathogenetic mechanisms, single and multiple gene deletion models in animals need to be generated. Furthermore, small deletions or single gene mutations need to be identified in human patients who exhibit individual distinct features of the complex multi-system WBS phenotype.

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REFERENCES

1. Morris, C.A., Demsey, S.A., Leonard, C.O., Dilts, C. and Blackburn, B.L. (1988) Natural history of Williams syndrome: physical characteristics. *J. Pediatr.*, **113**, 318–326.
2. Jones, K.L. (1990) Williams Syndrome: An historical perspective of its evolution, natural history, and etiology. *Am. J. Med. Genet.*, **6** (suppl.), 89–96.
3. Pérez-Jurado, L.A., Peoples, R., Kaplan, P., Hamel, B.C. and Francke, U. (1996) Molecular definition of the chromosome 7 deletion in Williams syndrome and parent-of-origin effects on growth. *Am. J. Hum. Genet.*, **59**, 781–792.
4. Kaplan, P., Wang, P. and Francke, U. (1999) Williams Syndrome: A distinct neurobehavioral disorder. *J. Child Neurol.*, in press.
5. Lowery, M.C., Morris, C.A., Ewart, A., Brothman, L.J., Zhu, X.-L., Leonard, C.O., Carey, J.C., Keating, M. and Brothman, A.R. (1995) Strong correlation of elastin deletions, detected by FISH, with Williams Syndrome: Evaluation of 235 patients. *Am. J. Hum. Genet.*, **57**, 49–53.
6. Brondum-Nielsen, K., Beck, B., Gyftodimou, J., Horlyk, H., Liljenberg, U., Petersen, M.B., Pedersen, W., Petersen, N.B., Sand, A., Skovby, F., Stafanger, G., Zetterqvist, P. and Tommerup, N. (1997) Investigation of deletions at 7q11.23 in 44 patients referred for Williams–Beuren syndrome, using FISH and four DNA polymorphisms. *Hum. Genet.*, **99**, 56–61.
7. Wu, Y.Q., Sutton, V.R., Nickerson, E., Lupski, J.R., Potocki, L., Korenberg, J.R., Greenberg, F., Tassabehji, M. and Shaffer, L.G. (1998) Delineation of the common critical region in Williams syndrome and clinical correlation of growth, heart defects, ethnicity, and parental origin. *Am. J. Med. Genet.*, **78**, 82–89.
8. Partsch, C.J., Dreyer, G., Gosch, A., Winter, M., Schneppenheim, R., Wessel, A. and Pankau, R. (1999) Longitudinal evaluation of growth, puberty, and bone maturation in children with Williams syndrome. *J. Pediatr.*, **134**, 82–89.
9. Jernigan, T.L. and Bellugi, U. (1990) Anomalous brain morphology on magnetic resonance images in Williams syndrome and Down syndrome. *Arch. Neurol.*, **47**, 529–533.
10. Wang, P.P., Hesselink, J.R., Jernigan, T.L., Doherty, S. and Bellugi, U. (1992) Specific neurobehavioral profile of Williams' syndrome is associated with neocerebellar hemispheric preservation. *Neurology*, **42**, 1999–2002.
11. Jernigan, T.L., Bellugi, U., Sowell, E., Doherty, S. and Hesselink, J.R. (1993) Cerebral morphologic distinctions between Williams and Down syndromes. *Arch. Neurol.*, **50**, 186–191.
12. Brinkmann, G., Heller, M., Partsch, C.J., Gosch, A. and Pankau, R. (1997) Magnetic resonance imaging of the brain in Williams–Beuren syndrome [letter]. *Am. J. Med. Genet.*, **68**, 243.
13. Sadler, L.S., Olitsky, S.E. and Reynolds, J.D. (1996) Reduced stereoacuity in Williams syndrome. *Am. J. Med. Genet.*, **66**, 287–288.
14. Olitsky, S.E., Sadler, L.S. and Reynolds, J.D. (1997) Subnormal binocular vision in the Williams syndrome. *J. Pediatr. Ophthalmol. Strabismus*, **34**, 58–60.
15. Withers, S. (1996) A new clinical sign in Williams syndrome. *Arch. Dis. Child.*, **75**, 89.
16. Gosch, A. and Pankau, R. (1997) Personality characteristics and behaviour problems in individuals of different ages with Williams syndrome. *Dev. Med. Child Neurol.*, **39**, 527–533.
17. Davies, M., Howlin, P. and Udwin, O. (1997) Independence and adaptive behavior in adults with Williams syndrome. *Am. J. Med. Genet.*, **70**, 188–195.
18. Davies, M., Udwin, O. and Howlin, P. (1998) Adults with Williams syndrome—preliminary study of social, emotional and behavioural difficulties. *Br. J. Psychiatry*, **172**, 273–276.
19. Tsao, C.Y. and Westman, J.A. (1997) Brief clinical report: infantile spasms in two children with Williams syndrome. *Am. J. Med. Genet.*, **71**, 54–56.
20. Mizugishi, K., Yamanaka, K., Kuwajima, K. and Kondo, I. (1998) Interstitial deletion of chromosome 7q in a patient with Williams syndrome and infantile spasms. *J. Hum. Genet.*, **43**, 178–181.
21. Kahler, S.G., Adhvaryu, S.G., Helali, N. and Qumsiyeh, M.B. (1995) Microscopically visible deletion of chromosome 7 in a child with features of Williams syndrome. *Am. J. Hum. Genet.*, **57**, A117.
22. Zackowski, J.L., Raffel, L.J., Blank, C.A. and Schwartz, S. (1990) Proximal interstitial deletion of 7q: a case report and review of the literature. *Am. J. Med. Genet.*, **36**, 328–332.
23. Robinson, W.P., Waslynka, J., Bernasconi, F., Wang, M., Clark, S., Kotzot, D. and Schinzel, A. (1996) Delineation of 7q11.2 deletions associated with

Williams–Beuren syndrome and mapping of a repetitive sequence to within and to either side of the common deletion. *Genomics*, **34**, 17–23.
24. Urban, Z., Helms, C., Fekete, G., Csiszar, K., Bonnet, D., Munnich, A., Donis-Keller, H. and Boyd, C.D. (1996) 7q11.23 deletions in Williams syndrome arise as a consequence of unequal meiotic crossover. *Am. J. Hum. Genet.*, **59**, 958–962.
25. Osborne, L.R., Martindale, D., Scherer, S.W., Shi, X.M., Huizenga, J., Heng, H.H.Q., Costa, T., Pober, B., Lew, L., Brinkman, J., Rommens, J., Koop, B. and Tsui, L.C. (1996) Identification of genes from a 500-kb region at 7q11.23 that is commonly deleted in Williams syndrome patients. *Genomics*, **36**, 328–336.
26. Osborne, L.R., Herbrick, J.A., Greavette, T., Heng, H.H.Q., Tsui, L.C. and Scherer, S.W. (1997) PMS2-related genes flank the rearrangement breakpoints associated with Williams syndrome and other diseases on human chromosome 7. *Genomics*, **45**, 402–406.
27. Pérez-Jurado, L.A., Wang, Y.K., Peoples, R., Coloma, A., Cruces, J. and Francke, U. (1998) A duplicated gene in the breakpoint regions of the 7q11.23 Williams–Beuren syndrome deletion encodes the initiator binding protein TFII-I and BAP-135, a phosphorylation target of BTK. *Hum. Mol. Genet.*, **7**, 325–334.
28. Francke, U., Hsieh, C.L., Foellmer, B.E., Lomax, K.J., Malech, H.L. and Leto, T.L. (1990) Genes for two autosomal recessive forms of chronic granulomatous disease assigned to 1q25 (NCF2) and 7q11.23 (NCF1). *Am. J. Hum. Genet.*, **47**, 483–492.
29. Görlach, A., Lee, P.L., Roesler, J., Hopkins, P.J., Christensen, B., Green, E.D., Chanock, S.J. and Curnutte, J.T. (1997) A p47-phox pseudogene carries the most common mutation causing p47-phox-deficient chronic granulomatous disease. *J. Clin. Invest.*, **100**, 1907–1918.
30. Hockenhull, E.L., Carette, M.J., Metcalfe, K., Donnai, D., Read, A.P. and Tassabehji, M. (1999) A complete physical contig and partial transcript map of the Williams syndrome critical region. *Genomics*, **58**, 138–145.
31. Kipersztok, S., Osawa, G.A., Liang, L.F., Modi, W.S. and Dean, J. (1995) POM-ZP3, a bipartite transcript derived from human ZP3 and a POM121 homologue. *Genomics*, **25**, 354–359.
32. Baumer, A., Dutly, F., Balmer, D., Riegel, M., Tukel, T., Krajewska-Walasek, M. and Schinzel, A.A. (1998) High level of unequal meiotic crossovers at the origin of the 22q11.2 and 7q11.23 deletions. *Hum. Mol. Genet.*, **7**, 887–894.
33. Wang, Y.K., Pérez-Jurado, L.A. and Francke, U. (1998) A mouse single-copy gene, Gtf2i, the homolog of human GTF2I, that is duplicated in the Williams–Beuren syndrome deletion region. *Genomics*, **48**, 163–170.
34. DeSilva, U., Massa, H., Trask, B.J. and Green, E.D. (1999) Comparative mapping of the region of human chromosome 7 deleted in Williams syndrome. *Genome Res.*, **9**, 428–436.
35. Ewart, A.K., Morris, C.A., Atkinson, D., Jin, W., Sternes, K., Spallone, P., Stock, A.D., Leppert, M. and Keating, M.T. (1993) Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. *Nature Genet.*, **5**, 11–16.
36. Ewart, A.K., Jin, W., Atkinson, D., Morris, C.A. and Keating, M.T. (1994) Supravalvular aortic stenosis associated with a deletion disrupting the elastin gene. *J. Clin. Invest.*, **93**, 1071–1077.
37. Tassabehji, M., Metcalfe, K., Donnai, D., Hurst, J., Reardon, W., Burch, M. and Read, A.P. (1997) Elastin: genomic structure and point mutations in patients with supravalvular aortic stenosis. *Hum. Mol. Genet.*, **6**, 1029–1036.
38. Li, D.Y., Toland, A.E., Boak, B.B., Atkinson, D.L., Ensing, G.J., Morris, C.A. and Keating, M.T. (1997) Elastin point mutations cause an obstructive vascular disease, supravalvular aortic stenosis. *Hum. Mol. Genet.*, **6**, 1021–1028.
39. Li, D.Y., Faury, G., Taylor, D.G., Davis, E.C., Boyle, W.A., Mecham, R.P., Stenzel, P., Boak, B. and Keating, M.T. (1998) Novel arterial pathology in mice and humans hemizygous for elastin. *J. Clin. Invest.*, **102**, 1783–1787.
40. Li, D.Y., Brooke, B., Davis, E.C., Mecham, R.P., Sorensen, L.K., Boak, B.B., Eichwald, E. and Keating, M.T. (1998) Elastin is an essential determinant of arterial morphogenesis. *Nature*, **393**, 276–280.
41. Tassabehji, M., Metcalfe, K., Hurst, J., Ashcroft, G.S., Kielty, C., Wilmot, C., Donnai, D., Read, A.P. and Jones, C.J.P. (1998) An elastin gene mutation producing abnormal tropoelastin and abnormal elastic fibres in a patient with autosomal dominant cutis laxa. *Hum. Mol. Genet.*, **7**, 1021–1028.
42. Zhang, M.C., He L., Giro M., Yong S.L., Tiller G.E. and Davidson J.M. (1999) Cutis laxa arising from frameshift mutations in exon 30 of the elastin gene (ELN). *J. Biol. Chem.*, **274**, 981–986.
43. Furthmayr, H. and Francke, U. (1997) Ascending aortic aneurysm with or without features of Marfan syndrome and other fibrillinopathies: new insights. *Semin. Thorac. Cardiovasc. Surg.*, **9**, 191–205.
44. Okumura, K., Nogami, M., Taguchi, H., Dean, F.B., Chen, M., Pan, Z.Q., Hurwitz, J., Shiratori, A., Murakami, Y., Ozawa, K. *et al.* (1995) Assignment of the 36.5-kDa (RFC5), 37-kDa (RFC4), 38-kDa (RFC3), and 40-kDa (RFC2) subunit genes of human replication factor C to chromosome bands 12q24.2–q24.3, 3q27, 13q12.3–q13, and 7q11.23. *Genomics*, **25**, 274–278.
45. Peoples, R., Pérez-Jurado, L., Wang, Y.K., Kaplan, P. and Francke, U. (1996) The gene for replication factor C subunit 2 (RFC2) is within the 7q11.23 Williams syndrome deletion. *Am. J. Hum. Genet.*, **58**, 1370–1373.
46. Frangiskakis, J.M., Ewart, A.K., Morris, C.A., Mervis, C.B., Bertrand, J., Robinson, B.F., Klein, B.P., Ensing, G.J., Everett, L.A., Green, E.D., Proschel, C., Gutowski, N.J., Noble, M., Atkinson, D.L., Odelberg, S.J. and Keating, M.T. (1996) LIM-kinase1 hemizygosity implicated in impaired visuospatial constructive cognition. *Cell*, **86**, 59–69.
47. Tassabehji, M., Metcalfe, K., Karmiloff-Smith, A., Carette, M.J., Grant, J., Dennis, N., Reardon, W., Splitt, M., Read, A.P. and Donnai, D. (1999) Williams syndrome: use of chromosomal microdeletions as a tool to dissect cognitive and physical phenotypes. *Am. J. Hum. Genet.*, **64**, 118–125.
48. Wang, J.Y., Frenzel, K.E., Wen, D. and Falls, D.L. (1998) Transmembrane neuregulins interact with LIM kinase 1, a cytoplasmic protein kinase implicated in development of visuospatial cognition. *J. Biol. Chem.*, **273**, 20525–20534.
49. Yang, N., Higuchi, O., Ohashi, K., Nagata, K., Wada, A., Kangawa, K., Nishida, E. and Mizuno, K. (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature*, **393**, 809–812.
50. Wang, Y.K., Samos, C.H., Peoples, R., Pérez-Jurado, L.A., Nusse, R. and Francke, U. (1997) A novel human homologue of the *Drosophila frizzled* wnt receptor gene binds wingless protein and is in the Williams syndrome deletion at 7q11.23. *Hum. Mol. Genet.*, **6**, 465–472.
51. Wang, Y.K., Sporle, R., Paperna, T., Schughart, K. and Francke, U. (1999) Characterization and expression pattern of the frizzled gene Fzd9, the mouse homolog of FZD9 which is deleted in Williams–Beuren syndrome. *Genomics*, **57**, 235–248.
52. Osborne, L.R., Soder, S., Shi, X.M., Pober, B., Costa, T., Scherer, S.W. and Tsui, L.C. (1997) Hemizygous deletion of the syntaxin 1A gene in individuals with Williams syndrome. *Am. J. Hum. Genet.*, **61**, 449–452.
53. Nakayama, T., Matsuoka, R., Kimura, M., Hirota, H., Mikoshiba, K., Shimizu, Y., Shimizu, N. and Akagawa, K. (1998) Hemizygous deletion of the HPC-1/syntaxin 1A gene (STX1A) in patients with Williams syndrome. *Cytogenet. Cell Genet.*, **82**, 49–51.
54. Yamaguchi, K., Nakayama, T., Fujiwara, T. and Akagawa, K. (1996) Enhancement of neurite-sprouting by suppression of HPC-1/syntaxin 1A activity in cultured vertebrate nerve cells. *Brain Res.*, **740**, 185–192.
55. Richter-Cook, N.J., Dever, T.E., Hensold, J.O. and Merrick, W.C. (1998) Purification and characterization of a new eukaryotic protein translation factor. Eukaryotic initiation factor 4H. *J. Biol. Chem.*, **273**, 7579–7587.
56. De Zeeuw, C.I., Hoogenraad, C.C., Goedknegt, E., Hertzberg, E., Neubauer, A., Grosveld, F. and Galjart, N. (1997) CLIP-115, a novel brain-specific cytoplasmic linker protein, mediates the localization of dendritic lamellar bodies. *Neuron*, **19**, 1187–1199.
57. Hoogenraad, C.C., Eussen, B.H., Langeveld, A., van Haperen, R., Winterberg, S., Wouters, C.H., Grosveld, F., De Zeeuw, C.I. and Galjart, N. (1998) The murine CYLN2 gene: genomic organization, chromosome localization, and comparison to the human gene that is located within the 7q11.23 Williams syndrome critical region. *Genomics*, **53**, 348–358.
58. Meng, X., Lu, X., Morris, C.A. and Keating, M.T. (1998) A novel human gene FKBP6 is deleted in Williams syndrome. *Genomics*, **52**, 130–137.
59. Peoples, R.J., Cisco, M.J., Kaplan, P. and Francke, U. (1998) Identification of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams–Beuren syndrome deletion at 7q11.23. *Cytogenet. Cell Genet.*, **82**, 238–246.
60. Lu, X., Meng, X., Morris, C.A. and Keating, M.T. (1998) A novel human gene, WSTF, is deleted in Williams syndrome. *Genomics*, **54**, 241–249.
61. Meng, X., Lu, X., Li, Z., Green, E.D., Massa, H., Trask, B.J., Morris, C.A. and Keating, M.T. (1998) Complete physical map of the common deletion region in Williams syndrome and identification and characterization of three novel genes. *Hum. Genet.*, **103**, 590–599.
62. Jadayel, D.M., Osborne, L.R., Coignet, L.J.A., Zani, V.J., Tsui, L.C., Scherer, S.W. and Dyer, M.J. (1998) The BCL7 gene family: deletion of BCL7B in Williams syndrome. *Gene*, **224**, 35–44.

63. Neer, E.J., Schmidt, C.J., Nambudripad, R. and Smith, T.F. (1994) The ancient regulatory-protein family of WD-repeat proteins [Erratum. *Nature* (1994) **371**, 812]. *Nature*, **371**, 297–300.
64. Pérez-Jurado, L.A., Wang, Y.-K., Francke, U. and Cruces, J. (1999) TBL2, a beta transducin family member, mapped to the Williams–Beuren syndrome deletion and the homologous region on mouse chromosome. *Cytogenet. Cell Genet.*, in press.
65. Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W.B., Caskey, C.T. and Ledbetter, D.H. (1993) Isolation of a Miller–Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature*, **364**, 717–721.
66. Paperna, T., Peoples, R., Wang, Y.K., Kaplan, P. and Francke, U. (1998) Genes for the CPE receptor (CPETR1) and the human homolog of RVP1 (CPETR2) are localized within the Williams–Beuren syndrome deletion. *Genomics*, **54**, 453–459.
67. Morita, K., Furuse, M., Fujimoto, K. and Tsukita, S. (1999) Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc. Natl Acad. Sci. USA*, **96**, 511–516.
68. Sirotkin, H., Morrow, B., SaintJore, B., Puech, A., DasGupta, R., Patanjali, S.R., Skoultchi, A., Weissman, S.M. and Kucherlapati, R. (1997) Identification, characterization, and precise mapping of a human gene encoding a novel membrane-spanning protein from the 22q11 region deleted in velo-cardio-facial syndrome. *Genomics*, **42**, 245–251.
69. Osborne, L.R., Campbell, T., Daradich, A., Scherer, S.W. and Tsui, L.C. (1999) Identification of a putative transcription factor gene (WBSCR11) that is commonly deleted in Williams–Beuren syndrome. *Genomics*, **57**, 279–284.
70. Franke, Y., Peoples, R.J. and Francke, U. (1999) Identification of GTF2IRD1, a putative transcription factor within the Williams–Beuren syndrome deletion at 7q11.23. *Cytogenet. Cell Genet.*, in press.
71. O'Mahoney, J.V., Guven, K.L., Lin, J., Joya, J.E., Robinson, C.S., Wade, R.P. and Hardeman, E.C. (1998) Identification of a novel slow-muscle-fiber enhancer binding protein, MusTRD1. *Mol. Cell. Biol.*, **18**, 6641–6652.
72. Pérez-Jurado, L.A., Li, X. and Francke, U. (1995) The human calcitonin receptor gene (CALCR) at 7q21.3 is outside the deletion associated with the Williams syndrome. *Cytogenet. Cell Genet.*, **70**, 246–249.
73. Wedemeyer, N., Peoples, R., Himmelbauer, H., Lehrach, H., Francke, U. and Wanker, E.E. (1997) Localization of the human HIP1 gene close to the elastin (ELN) locus on 7q11.23. *Genomics*, **46**, 313–315.
74. Nomiyama, H., Osborne, L.R., Imai, T., Kusuda, J., Miura, R. and Tsui, L.C. (1998) Assignment of the human CC chemokine MPIF-2/eotaxin-2 (SCYA24) to chromosome 7q11.23. *Genomics*, **49**, 339–340.
75. Garcia Barcelo, M., Tsui, S.K.W., Chim, S.S., Fung, K.P., Lee, C.Y. and Waye, M.M.Y. (1998) Mapping of the human cysteine-rich intestinal protein gene CRIP1 to the human chromosomal segment 7q11.23. *Genomics*, **47**, 419–422.