

## X-Linked Late-Onset Sensorineural Deafness Caused by a Deletion Involving *OA1* and a Novel Gene Containing WD-40 Repeats

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### Summary

We have identified a novel gene, transducin ( $\beta$ )-like 1 (*TBL1*), in the Xp22.3 genomic region, that shows high homology with members of the WD-40-repeat protein family. The gene contains 18 exons spanning ~150 kb of the genomic region adjacent to the ocular albinism gene (*OA1*) on the telomeric side. However, unlike *OA1*, *TBL1* is transcribed from telomere to centromere. Northern analysis indicates that *TBL1* is ubiquitously expressed, with two transcripts of ~2.1 kb and 6.0 kb. The open reading frame encodes a 526-amino acid protein, which shows the presence of six  $\beta$ -transducin repeats (WD-40 motif) in the C-terminal domain. The homology with known  $\beta$ -subunits of G proteins and other WD-40-repeat containing proteins is restricted to the WD-40 motif. Genomic analysis revealed that the gene is either partly or entirely deleted in patients carrying Xp22.3 terminal deletions. The complexity of the contiguous gene-syndrome phenotype shared by these patients depends on the number of known disease genes involved in the deletions. Interestingly, one patient carrying a microinterstitial deletion involving the 3' portion of both *TBL1* and *OA1* shows the *OA1* phenotype associated with X-linked late-onset sensorineural deafness. We postulate an involvement of *TBL1* in the pathogenesis of the ocular albinism with late-onset sensorineural deafness phenotype.

### Introduction

Ocular albinism with late-onset sensorineural deafness (OASD; MIM 300650) is an X-linked recessive disorder characterized by ocular albinism and progressive sensorineural hearing loss in the fourth and fifth decades of life. Men affected with OASD have translucent pale-blue irides and a reduction in the retinal pigment, with severe visual impairment. Findings from the otologic examination are normal; audiometry reveals high-tone sensorineural deafness in affected men in their third and fourth decades of life. The skin in patients with OASD is overtly normal but histologic examination reveals the presence of macromelanosomes in the epidermis, a feature consistent with ocular albinism of the Nettleship-Falls type (*OA1*) (O'Donnell et al. 1976). OASD differs from the X-linked albinism-deafness syndrome (MIM 30070) by the presence of patchy, cutaneous hypo- and hyperpigmentation and the absence of significant eye involvement in the latter disorder. The phenotypic distinctions between *OA1* and OASD suggest that the latter is an autonomous entity. Winship et al. (1993) reported a South African OASD kindred (for clinical description see Winship et al. 1984) showing tight linkage between OASD and DXS452, an Xp22.3 genetic marker previously linked to the *OA1* locus. Consequently, the gene associated with OASD is likely to be physically close to the *OA1* gene, raising the hypothesis that OASD and *OA1* are the result of a contiguous-gene syndrome. Alternatively, OASD may represent pleiotropic manifestations of *OA1* (Winship et al. 1993).

We have constructed a deletion map of the distal short arm of the human X chromosome, using DNA from individuals carrying deletions and translocations (Schaefer et al. 1993) involving this region of the genome and a highly detailed YAC-based physical map of the Xp22 region that spans ~40 Mb (Ferrero et al. 1995). The development of these maps was crucial for the identification of the gene responsible for Kallmann syndrome (*KAL1*; Franco et al. 1991), *OA1* (Bassi et al. 1995), X-linked recessive chondrodysplasia punctata (Franco et

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al. 1995), and Opitz G/BBB syndrome (Quaderi et al. 1997). Different strategies have been used in each case, leading to the identification of several additional genes (van Slegtenhorst et al. 1994; Schiaffino et al. 1995).

During the effort aimed at the identification of the *OA1* gene, cDNA selection experiments resulted in the isolation of a novel gene, *TBL1*, located outside the *OA1* critical region on the telomeric side. Here we report the characterization of this new gene whose sequence shares homology with members of the WD-repeat-protein family, identifying *TBL1* as new member of this large protein family in eukaryotes. Furthermore, deletion of *TBL1*, in an OASD family, suggests its involvement in the pathogenesis of the hearing phenotype of this disorder.

## Material and Methods

### cDNA Identification

Direct cDNA selection was done on a cosmid contig encompassing the entire *OA1* critical region and flanking sequences. The procedure was similar to that described by Lovett (1994), starting with primary cDNA reverse-transcribed from mRNA from several human tissues (brain, skeletal muscle, liver, and kidney).

After two rounds of selection, the enriched cDNA pool was radiolabeled with  $^{32}\text{P}$  and was used as a probe for a Southern blot containing *EcoRI*-digested cosmids. A 15-kb *EcoRI* genomic fragment from cosmid 248E4 was positive to the probe and was used to screen an oligo-dT-primed human adult retina cDNA library, in Lambda GT10 (a generous gift from Dr. J. Nathans, The Johns Hopkins University School of Medicine, Baltimore). Several cDNA clones were identified and used to screen cDNA libraries from different tissues.

Phage library plating and screening conditions were as described elsewhere (Sambrook et al. 1989). Recombinant phages recognized by the probes were isolated, and the insert recovered by *EcoRI* digestion and subcloned in pBluescript II SK- (Stratagene). The mouse cDNA clone R4 was isolated from a mouse retina cDNA library, in Lambda ZAP (a kind gift from Dr. Baher, Baylor College of Medicine, Houston), with use of a human cDNA clone (AR145, data not shown) as a probe, spanning portions of the 3' UTR and the coding region.

### DNA Sequencing

Both manual (with a Sequenase version 2.0 7-deaza-dGTP DNA sequencing kit from Amersham Life Science) and automated (with an Applied Biosystem ABI 377 fluorescent sequencer) sequencing were done on *TBL1* cDNA clones, with use of vector and gene-specific oligonucleotide primers. The nucleotide sequence of the

human *TBL1* cDNA has been submitted to GenBank under accession number Y12781.

### Computer Sequence Analysis

Sequence assembly and editing was done with both the AutoAssembler version 1.4 (Perkin Elmer-Applied Biosystems) and DNA Strider 1.2 programs (Marck 1988). We compared nucleotide and amino acid sequences with the nonredundant sequence databases present at the National Center for Biotechnology Information (NCBI) and at TIGEMNet, using the BLAST algorithm (Altschul et al. 1990). In particular, Blast-P searches were done by means of the 2.0 version of BLAST (Altschul et al. 1997). BLAST searches versus *Drosophila melanogaster* sequences were done by means of the Berkeley *Drosophila* genome project BLAST server (Rubin 1996).

Gene-prediction analysis was done with the Grail 1.3, GeneFinder, and GenView programs. We identified WD-40 repeats by using the hidden Markov models (HMM) search against the PFAM 2.1 database of the most common protein domains.

The global alignment program (gap; gap weight = 3.0, gap length weight = 0.1) and the local alignment program, Bestfit (gap weight = 5.0, gap-length weight = 0.3), were used to analyze identity and similarity among different nucleotide and amino acid sequences (Wisconsin Package version 10.0, Genetics Computer Group). Multiple sequence alignment was done by use of the Clustal-W algorithm (Thompson et al. 1994).

### Expression Analysis of *TBL1*

Northern blots of poly-(A)+ RNA, from various human adult and fetal tissues, were purchased from Clontech and hybridized, as described elsewhere by Sambrook et al. (1989). The pool of inserts of a mouse cochlea cDNA library, in Lambda ZAP II (L. Hampton and J. Battey, personal communication), was prepared by use of PCR with  $1 \times 10^6$  plaque-forming unit and T3 and T7 vector primers. A 1:20 pool dilution was then reamplified with *Tbl1*-specific oligonucleotides and synthesized on a mouse cDNA clone R4 (see cDNA Identification section): M176F1 5'-TCATGCAGCACAGACATGTG-3' and M176R1 5'-TCATGTCATCAGAGCAGGAGG-3'.

X-inactivation studies were done by means of RT-PCR, with a set of primers from the 3'UTR region (AR10F, 5' TGCCCTGCCTTCTTACATAAA 3' and AR10R, 5' GTCTGAGATGGAAGCTGTCTT 3'), by use of cDNA from somatic-cell hybrids retaining only the active or the inactive X chromosome (Agulnik et al. 1994). RT-PCR experiments were done according to the manufacturer's recommended conditions (Gibco BRL). *MIC2* and *PGK1* primers and PCR conditions were as

described elsewhere (Brown et al. 1990; Franco et al. 1991). Mouse-embryo sectioning and in situ hybridization were done as described elsewhere (Rugarli et al. 1993). A subclone of murine *Tbl1* (R4) into pBluescript KS– was linearized with an appropriated restriction enzyme, to transcribe either a sense or an antisense (<sup>35</sup>S)-labeled riboprobe, by use of the Stratagene RNA transcription kit. Slides were exposed for 2–3 wks.

**Results**

*Isolation of Human TBL1*

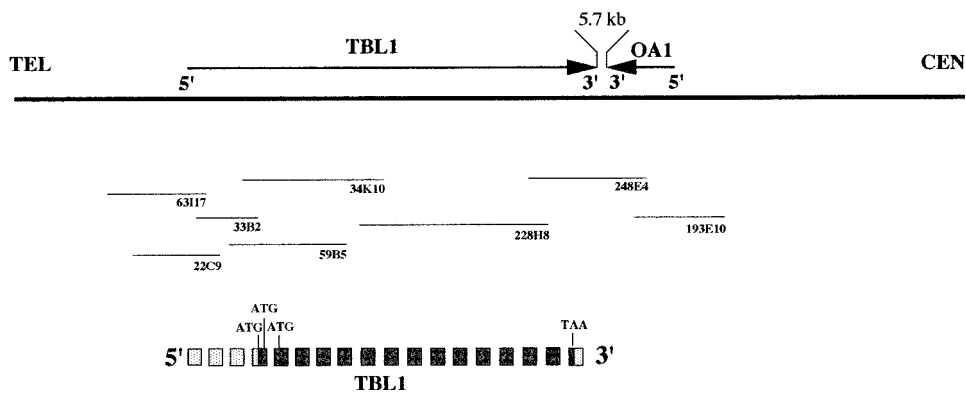
A cosmid contig covering the entire *OA1* critical region and flanking sequences in Xp22.3 (Wapenaar et al. 1993) was chosen for the isolation of transcribed sequences. cDNA selection was done with biotinylated cosmid DNA and double-strand cDNA derived from human total-embryo RNA. The cDNA, enriched after two rounds of selection, was used as a probe to hybridize an *EcoRI*-digested cosmid contig DNA. A 15-kb *EcoRI* fragment (from cosmid 248E4; fig. 1), located outside the *OA1* critical region on the telomeric side, was positive to the hybridization and was used as a probe to screen a human embryo cDNA library. Ten positive clones were obtained and the largest one, 176X (not shown), was chosen to rescreen an adult retina cDNA library. Screening of additional cDNA libraries, from different tissues, allowed us to build a cDNA contig of 5,718 nucleotides. This transcript corresponds to a novel human gene named “*TBL1*,” according to the Human Genome Organization nomenclature committee’s recommendation.

Two groups of cDNA with poly-A tails were isolated (the smallest of ~2.0 kb, and a larger one of 5.7 kb), consistent with the presence of two transcripts of 6.0 kb

and 2.1 kb as shown in the northern blot analysis (see Expression Analysis and X-Chromosome-Inactivation Studies section). A canonical polyadenylation signal was found only for the longest transcript, at nucleotide 5683. No canonical polyadenylation site was found in the vicinity of the poly-A tail in the shortest cDNA clones.

Three in-frame ATGs were found in the consensus cDNA sequence, whereas several in-frame stop codons were found upstream of the first ATG. The first two ATGs (nucleotide 478 and 538) were located in exon 4 (nucleotides 436–580 of the cDNA), an alternatively spliced exon that is not present in three cDNA clones (data not shown); the third ATG was located in exon 5 at nucleotide position 631. The TAA stop codon was located at nucleotide 2209 (exon 18).

We identified a portion of the mouse *Tbl1* cDNA by screening a mouse retina cDNA library, using an insert of a human cDNA clone as a probe spanning the 3’ portion of the coding region. Several positive clones have been identified and further characterized. One of the cDNA inserts was used to identify genomic clones, which allowed us to localize the mouse gene on chromosome X, band B-C, by FISH (Disteche et al. 1998). Preliminary sequence-analysis data on the murine *Tbl1* clones indicate a very high degree of identity, both at the nucleotide and protein levels, between the two species (data not shown). The homology starts in the human *TBL1* cDNA sequence at the third in-frame ATG (exon 5), whereas no homology can be detected upstream of this codon (data not shown). This suggests that the third in-frame ATG is the authentic initiation codon, resulting in a predicted human *TBL1* protein product of 526 amino acids. Sequences related to the mammalian *TBL1* gene have been identified in *Saccharomyces cerevisiae* and *Drosophila melanogaster* (see following section). A



**Figure 1** Genomic structure of *TBL1*. The orientation of *TBL1* with respect to *OA1* is shown (top). The number on the top indicates the distance between the two 3’ ends of the genes. The thin bars in the middle denote the cosmid, and are not drawn to scale. Shown at the bottom is the genomic structure of *TBL1*. Dot-filled squares denote untranslated exons, whereas blackened squares denote coding exons. TEL = telomere and CEN = centromere.

multiple sequence alignment indicates that significant homology is present along the entire length of the proteins, starting from the methionine corresponding to the third in-frame ATG in the human gene product (fig. 2A). On the basis of these observations, the numbering of the amino acid residues starts from the methionine corresponding to ATG, at nucleotide position 631.

#### Sequence Analysis and Homology with WD-40-Repeat Containing Genes

A sequence homology search against the nucleotide databases maintained at the NCBI revealed the presence of several human expressed-sequence tags (ESTs) corresponding to the query sequence. Analysis of the UniGene database (UniGene build #31, derived from GenBank release 105) identified a cluster of ESTs belonging to the *TBL1* transcript and derived from several human cDNA libraries. Six independent mapping assignments were done by different groups using radiation hybrid-analysis, allowing the mapping of the transcript between markers DXS1223–DXS1043, in agreement with our positional cloning data. Mouse ESTs have also been identified, which correspond to the mouse *Tbl1* transcript.

A BLAST search versus the high-throughput genomic sequences (HTGS) database revealed that the Xp22.3 region, containing the *TBL1* and *OA1* genes, is being sequenced by the Human Genome Center at Baylor College of Medicine (Houston) in the context of the ongoing human genome project. A 320-kb unfinished nucleotide sequence containing 17 unordered pieces is available under the GenBank accession number AC003978.

A protein database search with use of TBL1 reveals a significant homology with all WD-40-repeat containing proteins. The highest homology was detected with a vegetable-incompatibility protein HET-E-1, from *Podospora anserina* (L28125); a *S. cerevisiae* hypothetical 59.1-kd protein of unknown function (S48268); a  $\beta$ -transducin-like protein from *Synechocystis* sp. (D63999); an AAC3 hypothetical protein from *Dicystostelium discoideum* (S05357); and murine, bovine, and human Lissencephaly-1 proteins (P43035, P43033, and P43034, respectively).

We identified six regions of high identity with  $\beta$ -transducin family WD-40 repeats, using the PFAM database of the most common protein domains and the HMM for each (Sonnhammer et al. 1997). The position of the WD-40 repeats in TBL1 and the alignment to the consensus sequence is shown in figure 2B. Repeats B and E are less conserved, with respect to the WD-40 consensus sequence, and show a lower score in the HMM analysis. Furthermore, repeat E is interrupted by an insertion of 8 amino acids in the middle of the repeat.

After masking all of the WD-40 repeats in the TBL1

polypeptide, we detected significant sequence identity only with the yeast hypothetical 59.1-kd protein of unknown function in the VPS15-YMC2 intergenic region of chromosome II (Mannhaupt et al. 1994). The overall amino acid sequence identity is 27%, with 36% sequence similarity.

Interestingly, a TBLASTN search, done with the Berkeley *D. melanogaster* genome project BLAST server (Rubin 1996), identified a number of *D. melanogaster* genomic sequences showing high identity to the human *TBL1* gene. The deduced partial amino acid sequence shows an extremely high level of sequence identity (81%) and similarity (84%) with human *TBL1*. A multiple sequence alignment among human *TBL1*, the *D. melanogaster*, and *S. cerevisiae* predicted gene products is shown in figure 2A.

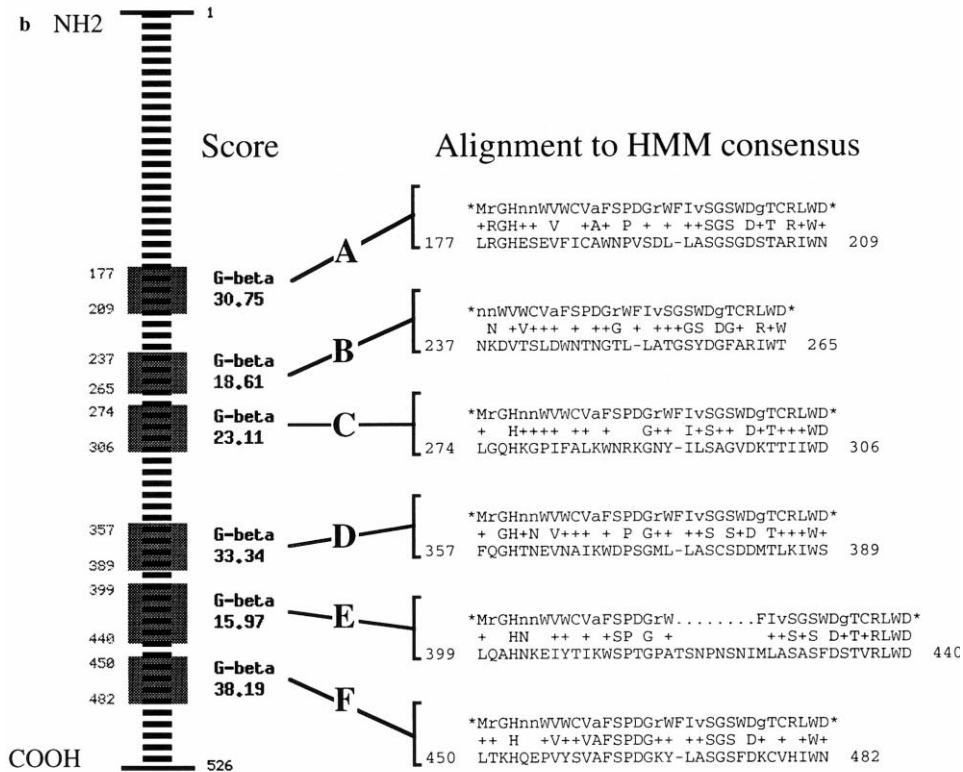
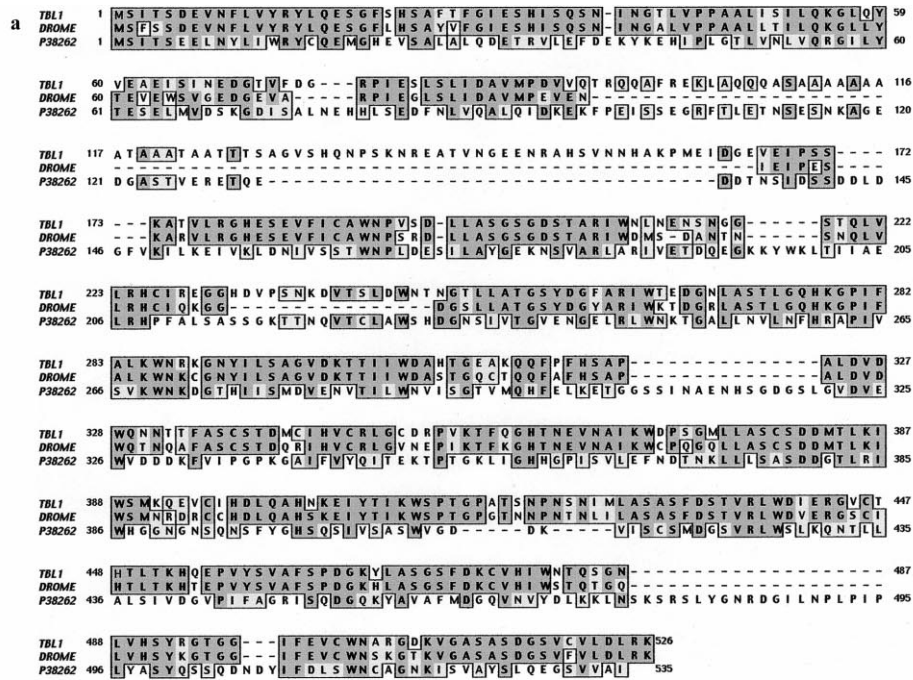
#### Expression Analysis and X-Chromosome-Inactivation Studies

Northern blot analysis, done with a *TBL1* cDNA probe derived from the coding region, showed the presence of two different transcripts of ~6.0 kb and 2.1 kb (fig. 3). The wide variety of adult and fetal tissues showing the presence of the transcripts indicates that the gene is ubiquitously expressed. Brain and heart samples show a strong expression only of the smaller transcript, whereas the upper-size transcript is barely detectable in these tissues with use of a 3' UTR probe (data not shown). The latter probe can detect only the 6.0-kb transcript (data not shown).

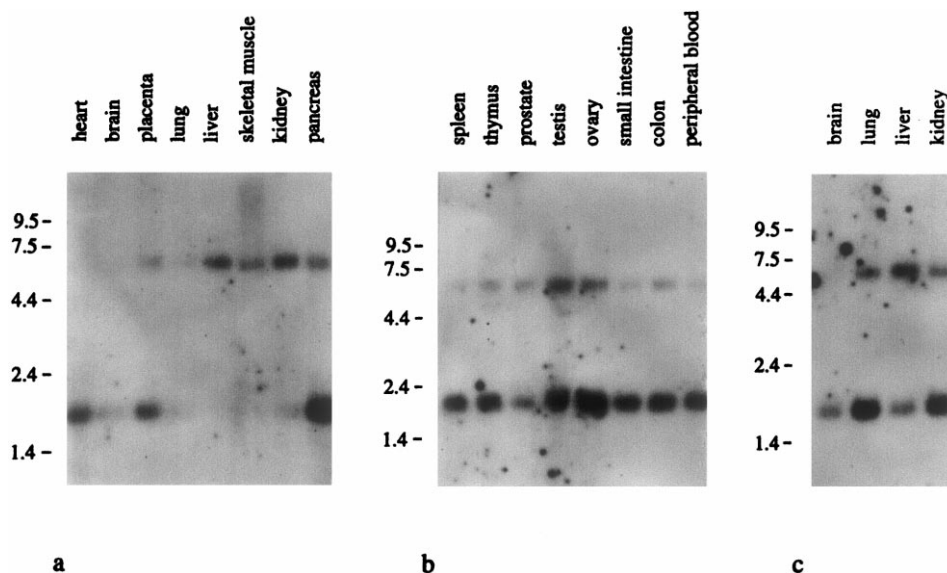
The mouse cDNA clone R4, encompassing the 3' portion of the coding region and part of the 3' UTR, was used for RNA in situ hybridization on 10.5- and 12.5-dpc mouse embryonic sections. Consistent with the northern blot experiment, expression is detectable, in all tissues, as a diffuse hybridization signal (data not shown).

Several sets of primers were designed on the nucleotide sequences of the mouse cDNA clone R4 and mouse EST clone (IMAGE clone 779831) containing the 5' region of the *Tbl1* transcript and were used to test the expression of the gene in a mouse cochlea cDNA library, by PCR. A clear signal was detected (fig. 4A), indicating that *Tbl1* is expressed in this tissue.

The ability to detect *TBL1* expression in cultured cell lines (lymphoblasts and fibroblasts; data not shown) allowed us to test the X-inactivation status of the gene. Reverse-transcribed RNA samples from hybrid cell lines that retain either the active or the inactive X chromosome were amplified by PCR, by use of oligonucleotides AR10F and AR10R from the cDNA sequence. As shown in figure 4B, specific PCR products were observed in samples from both the active and the inactive X hybrids, indicating that this gene escapes X-chromosome inac-



**Figure 2** *a*, Multiple sequence alignment obtained using the Clustal-W algorithm. TBL1 = human TBL1 polypeptide; DROME = predicted *D. melanogaster* protein encoded by P1 clone DS05054, P38262 = hypothetical yeast 59.1-kd protein (accession number P38262). Identical and conserved amino acid residues are boxed in dark and light gray, respectively. Numbering is not reported for the *D. melanogaster* protein, because the complete polypeptide could not be predicted from the currently available genomic sequences. The missing amino acid residues, as well as the gaps inserted by the Clustal-W program, are denoted by dashed lines. *b*, Position of WD-40 repeats in the *TBL1* gene, determined by the HMM search against PFAM. Scores are expressed in bits (LOD score, in base 2); a score >15 is almost always considered significant. The amino acid alignment to the consensus sequence of G- $\beta$ -transducin (WD-40) repeats is shown to the left for each domain.



**Figure 3** Northern blot analysis of *TBL1*. The cDNA probe AR145 was hybridized to three commercial northern blots (Clontech) containing 2  $\mu$ g poly-(A)+ RNA from various human adult (*a* and *b*) and fetal (*c*) tissues. The *TBL1* gene is ubiquitously expressed, with two transcripts of ~2.1 kb and 6.0 kb. In fetal tissues the smaller transcript seems to be more abundant than the larger one.

tivation. *MIC2* and *PGK1* control genes showed the expected amplification product from the hybrid cell lines.

#### Genomic Structure and Y-Chromosome Homology

Hybridization of cDNA clones to the cosmid contig in this region demonstrated that the *TBL1* gene spans ~150 kb of genomic DNA. Sequencing the 5' UTR of the gene revealed the presence of a CpG island containing *Bss*HII, *Eag*I, *Sma*I, and *Sac*I restriction sites. Unlike *OA1*, *TBL1* is transcribed from telomere to centromere, and the distance between the 3' ends of the two genes is <6 kb.

*Eco*RI cosmid fragments hybridizing to cDNA probes were subcloned in plasmid vectors and sequenced by means of oligonucleotide primers designed from the cDNA sequence. Eighteen exons were identified, and the sequence of all exon-intron boundaries was determined. Exon sizes and splice-junction sequences are shown in table 1. Only exon 9 does not show the canonical consensus sequences for splice sites.

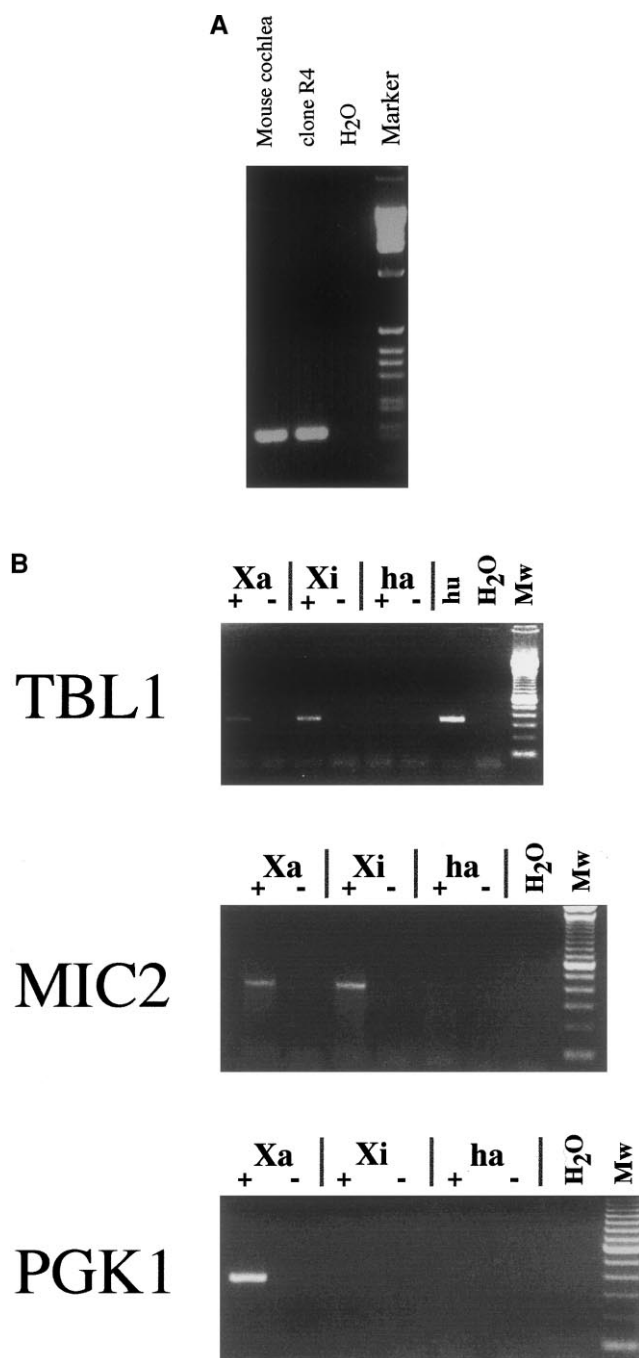
Southern blot analysis, done on human genomic DNA by means of *TBL1* cDNA clones as probes, identified Y-chromosome-specific bands in addition to the X-specific signals. Homology with the Y chromosome was then confirmed by fluorescence in situ hybridization, by means of both the X-specific cosmid 228H8 (encompassing part of the coding and the 3' UTR regions) and the 1.3-kb *Eco*RI cDNA fragment from the AR145 cDNA clone (data not shown).

#### Patient Analysis

We collected DNA from two carrier females and an affected male of an OASD family (previously described by Winship et al. 1993), who show the classic OA1 phenotype associated with OASD. Genomic DNA from this patient and from individuals carrying deletions spanning the genomic region containing *TBL1* (Schaefer et al. 1993) were analyzed by Southern blot, by means of several probes from *TBL1* cDNA and the flanking genomic region. As indicated in figure 5, *TBL1* is entirely deleted in patients BA199 (Bassi et al. 1994), BA127, BA163, and BA38 (Schaefer et al. 1993), whereas it is partly deleted in the patient with OASD and in patient BA126. A precise localization of the breakpoint in both patients was then done by PCR analysis, by means of primers designed on the exon and intron sequences of *TBL1*.

As already reported by Schaefer et al. (1993), patient BA126 has an interstitial deletion of ~6 Mb in which the distal breakpoint is localized between the arylsulfatase F and steroid sulfatase genes, whereas the proximal breakpoint had been localized distal to *OA1*. We found that the proximal breakpoint is located within the 3' portion of the coding region of *TBL1*, between exons 15 (deleted) and 16 (still present); therefore, the patient retains only the last three exons of the gene.

A preliminary characterization of a patient with OASD with a gene deletion, by means of Southern analysis with *TBL1* and flanking sequences including the *OA1* gene as probes, indicated that the interstitial de-



**Figure 4** A, *Tbl1* expression in a mouse cochlea cDNA library, as detected by PCR (see Material and Methods). The positive control R4 represents a clone obtained from a mouse retina cDNA library. B, Inactivation status of *TBL1*. Oligonucleotide primers from the 3' UTR region of *TBL1* and from the human *PGK1* and *MIC2* genes were used for the RT-PCR amplification of hamster/human cell hybrids retaining the human active (Xa) or inactive (Xi) X chromosome. Ha = parent hamster line (a plus sign [+]) and a minus sign [-] indicate the presence or absence of reverse transcriptase [RT] in the RT reaction, respectively); Hu = human genomic DNA; H<sub>2</sub>O = no DNA added; and Mw = molecular-weight marker. A 341-bp PCR product was observed in samples from both the active and the inactive X hybrids, indicating that *TBL1* escapes X-chromosome inactivation.

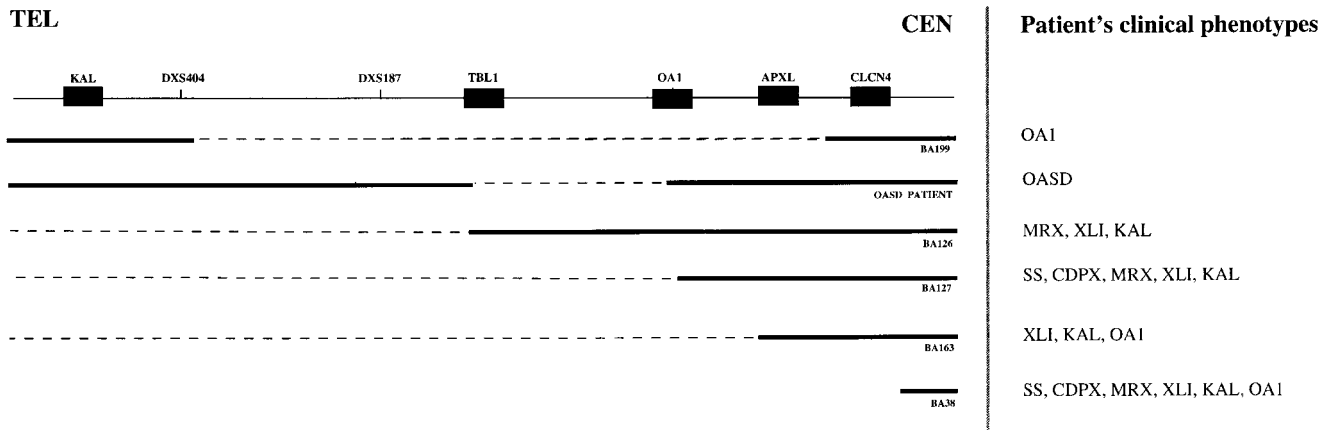
letion found in this patient extends from the most 3' region of *TBL1* into the *OA1* gene. PCR analysis with exon-specific primers shows that the patient is missing the last five exons (14–18) of *TBL1* and the last three exons (7–9) of *OA1* (data not shown). The availability of the nucleotide sequence AC003978, covering part of the *TBL1* and *OA1* genomic regions, allowed us to design a number of oligonucleotide primers in an attempt to amplify the breakpoint of the patient with OASD. Primers 12intfl (intron 12 of *TBL1*) and 6int (intron 6 of *OA1*), which are normally 39.8 kb apart on genomic DNA, successfully amplified a 630-bp PCR product in the patient with OASD and in the carrier mother. Furthermore, we tested the segregation of the PCR fragment containing the deletion breakpoint, on all the family members available, and confirmed that the deletion is present in all affected males and all carriers (fig. 6). No amplification was observed in several control DNA. Sequence analysis of the fragment shows that the breakpoint occurs within exon 13 at nucleotide 1630 of *TBL1* cDNA on the telomeric side, and 189 bp downstream of exon 6 (*OA1*) on the centromeric side. Therefore, the patient retains the first 12 exons and 40 bp of exon 13 of *TBL1* and the first 6 exons of *OA1*. On the *TBL1* side, the deletion breakpoint generates a hypothetical chimeric protein comprising the first 333 amino acids of *TBL1* plus 58 amino acids encoded by the adjacent intron 6 of the *OA1* gene. Overall, the deletion removes 38.3 kb of genomic DNA. Repeated elements, possibly predisposing the rearrangement, are present in *TBL1* intron 12 and in *OA1* intron 6 in the vicinity of the deletion breakpoints. Furthermore, a dinucleotide TT

**Table 1**

**Splice-Site Junctions of *TBL1***

Exon Number	3' Splice Site	5' Splice Site	Exon Size
1	...	GACGAGgtgagtcgc	...
2	tttctccagATTTCC	TGAAAAGgtacagaaaa	72
3	ttcagggtagGCTGGT	CATAAGgtaagcagcc	88
4	gttccaccagGAGCCC	GGAGAGgtactgcggt	144
5	ttttgaacagAGGGTG	AGTCAGgtaagaggct	107
6	gccacaccagGTTTTT	AACGAGgtacgtagct	145
7	ttgttccagGATGGC	CAGTCAGtgagtgtag	257
8	gctgtgacagATATAA	CTCCGGgtaaggactg	133
9	gaactagtggATCTGG	TGGAATgtaagcatct	141
10	tggccctcagACCAAT	AAGATGgtgagttctg	63
11	ttactaacagGTAACC	GACAAAgtgagtatta	97
12	cggaacacagACAACA	ATTCAGgtgagttttt	61
13	cctactcagCCCCTG	CACACAgtaagrgaga	121
14	tctcttcagAACGAG	TTGAAAGgtagagtcgg	74
15	tgttctgtagATCTGG	GGCAAGgtaagggcag	127
16	accttgctagTGCTTC	ACTCAGgtaagctccc	166
17	tgctttcagAGTGGA	GGCTCTgtaagcaaca	102
18	ttcctccagGTGTGT	...	...

NOTE.—Capital letters denote exonic sequences and lowercase letters denote intronic sequences.



**Figure 5** Schematic deletion map surrounding the *TBL1* locus. Heavy bars represent the portion of the X chromosome still present in the patient, whereas dashed lines indicate either X-Y translocation (patient BA127) or microinterstitial deletion (patients BA199, BA163, BA126). The entire genomic region is not drawn to scale. KAL = Kallmann syndrome 1 gene, *TBL1* = transducin  $\beta$ -like 1, OA1 = X-linked ocular albinism gene, APXL = apical protein from xenopus-like, and CLCN4 = voltage-gated chloride channel 4. Patients' clinical phenotypes are shown on the left, as follows: SS = short stature, CDPX = X-linked chondrodysplasia punctata, MRX = nonspecific mental retardation, XLI = X-linked ichthyosis, KAL = Kallmann syndrome; OASD = ocular albinism with late-onset sensorineural deafness, and OA1 = ocular albinism of the Nettleship-Falls type. TEL = telomere and CEN = centromere.

sequence is flanking the deletion on both sides, whereas in the rearranged sequence only two Ts are present (fig. 7). Comparison between the genomic sequence AC003978 and the 12intfl-6int amplification fragment in the OASD patient shows a 3-bp deletion in a stretch of As (in *TBL1* intron 12, located 300 bp upstream of the breakpoint) and a 2-bp substitution, which likely represent polymorphic variants.

Besides removing a portion of *TBL1* and *OA1*, the deletion identified in the patient with OASD removes a 5,700-bp region that lies between the 3' ends of the two genes. About 35% of this genomic region contains interspersed repeat elements, for a total of 1,991 bp. These include a MER2-type repeat (269 bp), a portion of a Mariner transposon (1,242 bp), and two ALU sequences (480 bp).

Extensive sequence analyses have been performed on the intragenic nucleotide sequence, after masking the repeat elements. These include BLAST homology searches versus both nucleotide (nonredundant nucleotide database, dbEST, HTGS, and genome survey sequence) and protein databases. No significant sequence matches have been identified, indicating that (1) no transcripts homologous to known sequences are present in the query sequence and (2) no evolutionary conserved sequences can be identified when comparing the query sequence with model organism sequences. Moreover, gene-prediction analysis failed to reveal any high-scoring exons that are commonly detected by more than one program (data not shown). Therefore, we can reasonably rule out the presence of other genes within the deleted region.

## Discussion

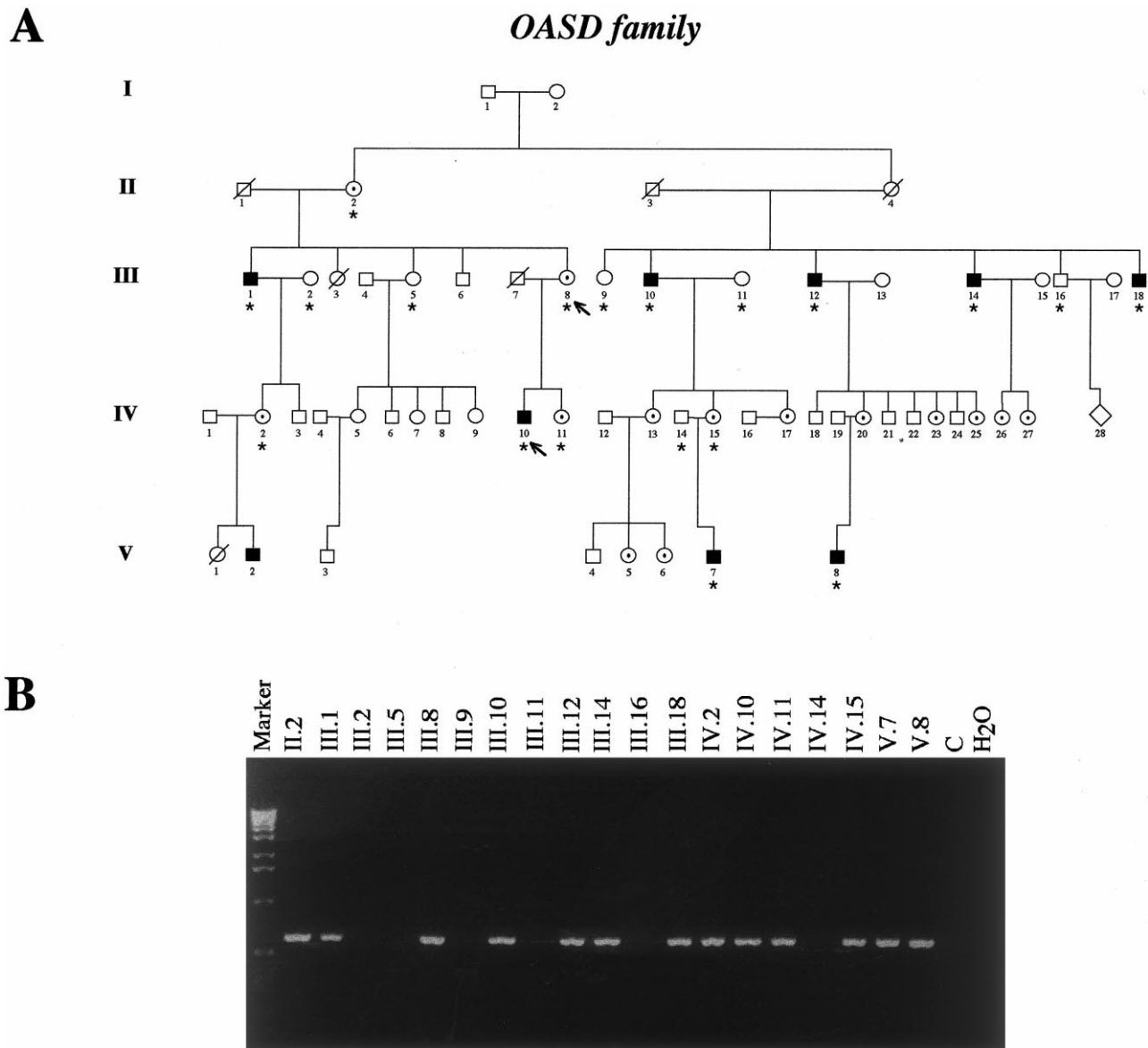
Progressive hearing loss is a very common disorder in humans. Sixteen percent of adults, and >30% of those aged  $\geq 60$  years, have a hearing loss of  $\geq 25$  decibels (Davis 1989). Currently, little is known about the causes for this progressive loss, other than it is highly heterogeneous and is a result of both environmental and genetic influences.

X-linked deafness accounts only for a small number of cases of hearing impairment. However, because of its characteristic pattern of inheritance, several large families have been found and subsequently studied.

In this paper we describe the isolation of a novel X-linked gene, *TBL1*, which is located 5.7 kb distal to the *OA1* gene in the Xp22.3 region. In addition, we demonstrate that a microinterstitial deletion removes the 3' portion of both the *OA1* and *TBL1* genes in a patient showing OA1 phenotype associated with X-linked OASD. These data support the possibility that alterations in *TBL1* are responsible for hearing impairment in this patient.

Unlike *OA1*, *TBL1* is transcribed from telomere to centromere. It is widely expressed in all tested tissues, with two transcripts of 2.1 and 6.0 Kb owing to the presence of an alternative 3' polyadenylation site. The smaller transcript seems to be more abundant than the large one, particularly in the fetal tissues. In contrast to the *OA1* gene, *TBL1* is ubiquitously expressed and escapes X-chromosome inactivation. Interestingly, all known genes in Xp22.3 telomeric to *TBL1* escape X-





**Figure 6** A, Pedigree of OASD family. Asterisks (\*) indicate the DNA samples analyzed in B. Arrows indicate the proband and the carrier mother whose PCR products were sequenced (see fig. 7). B, Segregation of the deletion breakpoint-containing fragment, as detected by PCR on genomic DNA in all affected males, carrier females, and normal controls (C). H<sub>2</sub>O = no DNA added. The numbers at the top of the lanes indicate the generation number and the individual number, respectively.

chromosome inactivation, whereas genes more centromeric are mostly expressed from the active X chromosome only. *TBL1* and *OA1* thus define a chromosomal boundary that might be relevant in the elucidation of the molecular mechanisms controlling the spreading of X-chromosome inactivation.

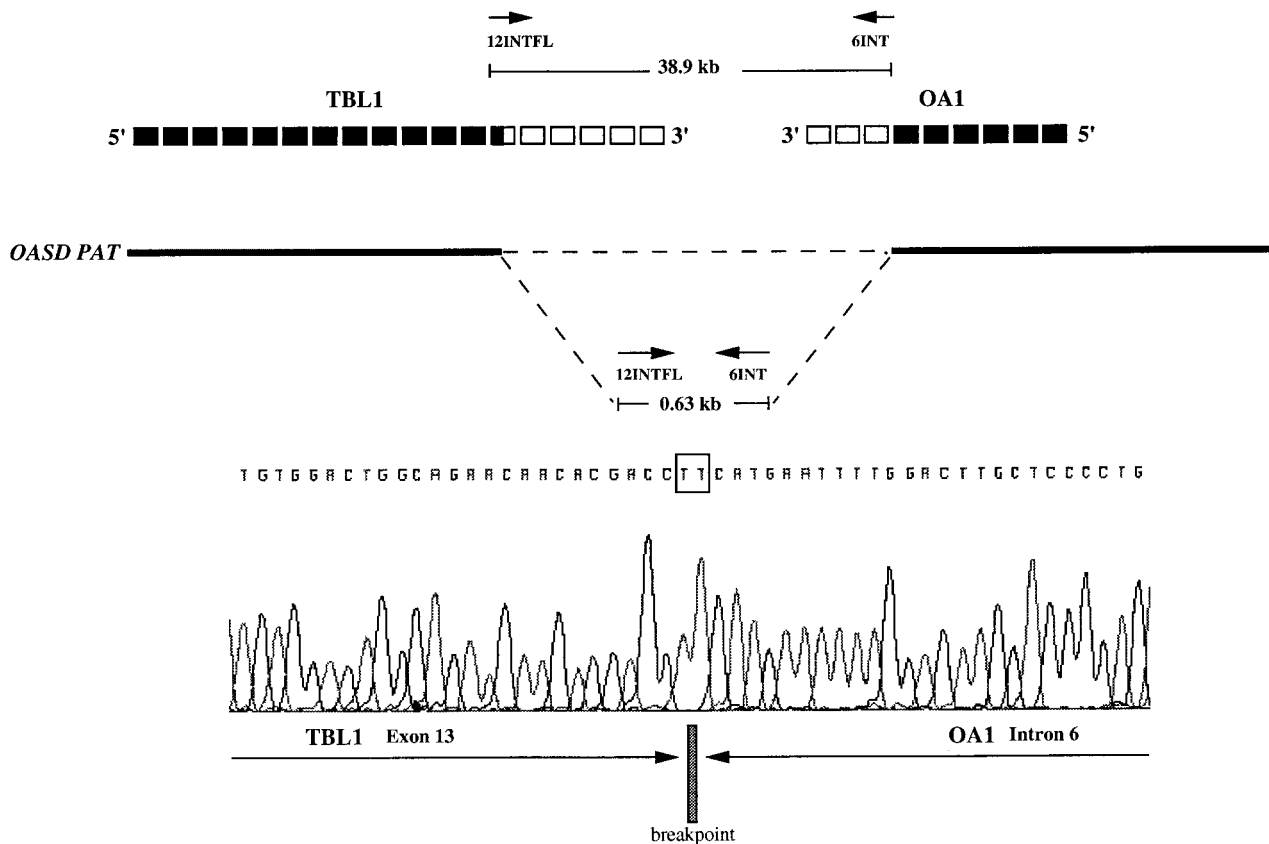
*TBL1* encodes a putative protein of 526 amino acids, showing a significant homology to the WD-40-repeat containing proteins, owing to the presence of six WD-40 repeats in the C-terminal portion of the predicted protein. Homology could be detected, outside the WD-

40 repeats, with both yeast and *D. melanogaster* putative proteins of unknown function. These amino acid sequences are deduced from genomic sequences, by use of gene-prediction programs. In particular, the sequence identity between *TBL1* and the fruit fly protein is >80%, indicating a high degree of conservation during evolution.

The WD-repeat motif, or  $\beta$ -transducin domain, is ~40 amino acids long and contains a number of conserved amino acids, including a Trp-Asp dipeptide, the “WD” that frequently terminates the repeat (Neer et al. 1994).

TEL

CEN



**Figure 7** Localization of the chromosomal breakpoint in a patient with OASD, and schematic representation of a submicroscopic deletion in the patient and nucleotide sequence of the junction fragment. The size of the deleted region and the position of the 12intfl (intron 12 of *TBL1*) and 6int (intron 6 of *OA1*) primers used for the amplification are shown (*top*). Primer sequences are as follows: 12intfl, 5'-GTGCAA-GAAGTGTACGGAG; and 6int, 5'-AGACTGTGCTAGGTGCCCTT. Unblackened squares denote the exons of both genes deleted in the patient, whereas blackened squares denote the deleted ones. The size of the amplification product in the patient (*middle*) and the sequence of the junction fragment at the breakpoint (*bottom*) are shown. The dinucleotide TT, present at the breakpoint, is denoted by an unblackened square. TEL = telomere and CEN = centromere.

Initially identified by sequence homology within transducin G-protein  $\beta$ -subunits and CDC4 (Fong et al. 1986), sets of 4–8 WD repeats have since been found in the sequences of >200 other eukaryotic proteins. Although proteins belonging to this family do not share any functional activity, all WD-repeat proteins seem to be regulatory and none are enzymes. They are grouped into two major classes: one in which the proteins are composed almost entirely of the repeat domain, and a second in which the repeats are restricted to the C-terminal domain (Spevak et al. 1993). Proteins in the first group are known to serve as  $\beta$ -subunits in the heterotrimeric G-protein complexes that transduce receptor-generated signals. Mammalian  $\beta$ -transducins and *S. cerevisiae* Ste4 fall into this class. Proteins in the second group are involved in activities as diverse as microtubule-

dependent processes (Cdc20), catabolite repression (Tup1), regulation of the RAS-cAMP pathway (Msi1), RNA splicing (Prp4), DNA replication (Cdc4), and neurogenesis (E-spl) (Spevak et al. 1993). There is no evidence that proteins of this group form part of the heterotrimeric G-protein complexes. The repeat is nevertheless expected to be involved in protein–protein interactions, as demonstrated for the transducin  $\beta$ - $\gamma$ -subunit complex (Sondek et al. 1996). On the basis of the homology data, the *TBL1* protein seems to be more closely related to members of the family other than the GTP-binding protein  $\beta$ -subunit subgroup. The presence of the WD-repeat suggests that *TBL1* might interact with other proteins in a manner that was described for the transducin  $\beta$ - $\gamma$ -subunit complex (Sondek et al. 1996). These studies revealed that

the structure of the  $\beta$ -subunit of transducin is a seven-bladed  $\beta$ -propeller, which is partially encircled by an extended  $\gamma$ -subunit. The  $\beta$ -propeller, which contains seven structurally similar WD repeats, defines the stereochemistry of the WD repeat and the probable architecture of all WD-repeat-containing proteins. It is not known whether any other WD protein requires the equivalent of a  $\gamma$ -subunit to fold correctly. Some amino- or carboxy-terminal extensions may act as built-in  $\gamma$ -subunit equivalents and promote the folding of the repeating units.

The finding of a patient affected by ocular albinism and late-onset sensorineural deafness, carrying a microinterstitial deletion involving the 3' portion of both *OA1* and *TBL1*, needs to be carefully evaluated. Patients with OASD manifest high-frequency hearing loss in their fourth or fifth decade of life, and only an accurate audiometry test performed in the fourth decade can reveal the onset of the disease.

As shown in Results and in figure 5, other patients available in our collection (Wapenaar et al. 1993) have either partial (patient BA126) or entire deletions (patients BA199, BA163, BA38, and BA127) of *TBL1* and apparently do not show any sign of deafness. Owing to the late onset of sensorineural deafness in patients with OASD, the age of these patients becomes crucial. As of this writing, only one patient has passed the third decade (patient BA127; aged >40 years). Unfortunately, he never underwent an audiometry test, and several attempts to have him reexamined have failed. Another patient, BA199, who is aged 38 years, has normal auditory function. Given the broad range of age at onset of deafness, even a negative result with the audiometry test does not represent reliable and definitive information. Furthermore, it might be difficult to establish whether the deletion of the *TBL1* gene in some of these patients results in recognizable phenotypic consequences. The phenotype of four patients (patients BA126, BA127, BA163, and BA38) is in fact quite complex, because of the deletion of from three to six disease genes, resulting in a contiguous-gene syndrome. The complexity of the phenotype observed in these patients (involving mental retardation in three cases) may hide the presence of other, more subtle, conditions and lead to an incorrect diagnosis. Therefore, at this stage, the data we have generated indicate that the different components of the phenotypic profile in patients with OASD (ocular albinism and late-onset sensorineural deafness) are the consequence of a contiguous microdeletion involving two genes in Xp22.3, *OA1* and *TBL1*.

It has been suggested in the past that *OA1* and sensorineural deafness may be allelic and thus result from the pleiotropic effect of mutations in the *OA1* gene. If this were true, a subset of patients affected with *OA1* would also show the clinical features of hearing loss

present in OASD. In a recent survey, Schnur et al. (1998) describe the deletion and mutation analysis in 29 unrelated North American and Australian patients affected with X-linked ocular albinism. Although a number of nonocular phenotypic abnormalities were reported, no hearing impairment was detected.

Therefore, the hypothesis of a contiguous-gene deletion involving *OA1* and *TBL1* in the pathogenesis of OASD appears plausible. Furthermore, the finding of *Tbl1* expressed in the mouse cochlea supports this conclusion.

Two nonsyndromic loci for X-linked deafness have been assigned to the short arm of the X chromosome: DFN4 (Xp21.2, between markers DXS997 and DXS992; Lalwani et al. 1994) and DFN6 (Xp22, between markers DXS7108 and DXS7105; del Castillo et al. 1996), both representing sensorineural-type deafness. The location of *TBL1*, outside the critical regions of both DFN4 and DFN6, makes the involvement of *TBL1* in these disorders extremely unlikely. Although additional evidence is needed to formally prove the involvement of *TBL1* in hearing loss, the data presented here indicate that this new gene represents a promising candidate for X-linked late-onset sensorineural deafness.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BLAST Sequence Similarity Searching, <http://www.ncbi.nlm.nih.gov/BLAST/> (for BLAST-P searches)  
*Drosophila* Genome Project BLAST server, <http://www.fruitfly.org/> (for *D. melanogaster* sequences)  
 GenBank, <http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html> (for nucleotide sequence of *TBL1* [Y12781])

GeneFinder, <http://www.cshl.org/genefinder/> (for gene prediction analyses)  
 GenView, <http://lita.itba.mi.cnr.it/~webgene/wwwgene.html> (for gene prediction analyses)  
 Grail 1.3, <http://compbio.ornl.gov/Grail-1.3/> (for gene prediction analyses)  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for OASD [300650] and X-linked albanism-deafness syndrome [30070])  
 PFAM, <http://genome.wustl.edu/Pfam/> (for protein domains)  
 UniGene, <http://www.ncbi.nlm.nih.gov/UniGene/index.html> (for UniGene build #31)

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