Published in final edited form as:
Exp Eye Res. 2006 September ; 83(3): 569–573. doi:10.1016/j.exer.2006.02.003.

Mutation screen of the *TUB* gene in patients with retinitis pigmentosa and Leber congenital amaurosis

Quansheng Xi, **Gayle J.T. Pauer**, **Elias I. Traboulsi**, and **Stephanie A. Hagstrom***
Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Abstract

TUB is the first identified member of the TULP family of four proteins with unknown function. A spontaneous mutation in murine *tub* causes retinal degeneration, obesity, and deafness. Mutations in another member of the TULP family, *TULP1*, are a cause of autosomal recessive retinitis pigmentosa (RP). These findings prompted us to investigate *TUB* as a candidate gene for RP and Leber congenital amaurosis (LCA). A mutation screen of the entire coding region of the TUB gene in 159 unrelated patients with autosomal recessive RP, 114 unrelated patients with simplex RP, and 21 unrelated patients with LCA uncovered 18 sequence variations. Of these, seven were missense mutations, six were isocoding changes, and five were intronic polymorphisms. All seven missense mutations were identified as heterozygous changes and no defect could be found in the other allele. None of the isocoding variants or intronic polymorphisms are predicted to create or destroy splice donor or acceptor sites based on splice-site prediction software. Although variant alleles of the *TUB* gene were found, none could be definitively associated with a specific retinal disease.

Keywords

Leber congenital amarousis; retinitis pigmentosa; tub; tulp; photoreceptor; mutation

1. Introduction

The TULP family of proteins consists of four members named TUB and TULPs 1–3 (Tubby-Like Proteins) (North et al., 1997). TULP proteins are localized primarily to nervous tissues with TUB and TULP3 widely distributed throughout the central nervous system and TULP1 and TULP2 restricted largely to the retina and testis, respectively (He et al., 2000; Ikeda et al., 1999a,b; Kleyn et al., 1996; Nishina et al., 1998; North et al., 1997; Sahly et al., 1998). An analysis of TULP proteins does not reveal significant homology with known proteins or functional motifs but are structurally characterized by strong C-terminal homology (60–90% amino acid identity) implying an important functional domain. Cell culture studies and the determination of the crystal structure of the conserved C-terminal domain of TUB have resulted in the proposal that TUB may respond to signals from G proteins to activate translocation from the plasma membrane to the nucleus where it might function as a novel transcription factor (Boggon et al., 1999; Santagata et al., 2001). Other proposed functions for TULP proteins include involvement in intracellular insulin signaling and vesicular trafficking (Hagstrom et al., 1999; Hagstrom et al., 2001; Kapeller et al.,

*Corresponding author. Ophthalmic Research - i31, Cole Eye Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA. Tel.: +1 216 445 4133; fax: +1 216 445 3670. hagstrs@ccf.org (S.A. Hagstrom).

1999). Although the precise function of TULP proteins is unknown, two of the proteins have been linked to photoreceptor degeneration, namely TUB and TULP1.

Tubby is the name for a strain of mice with a naturally arising loss of function mutation affecting the C-terminus in the *tub* gene (Kleyn et al., 1996; Noben-Trauth et al., 1996). *Tubby* mice develop obesity associated with insulin resistance that is likely attributable to defects in hypothalamic signaling. They also suffer progressive retinal degeneration and hearing loss as a result of photoreceptor and cochlear degeneration due to apoptosis of these sensory neurons (Ohlemiller et al., 1995). Electroretinograms of *tubby* mice are progressively reduced and are eventually extinguished by age 6 months (Heckenlively et al., 1995). The hearing loss is also progressive so that by age 5–6 months *tubby* mice appear to be deaf, although this feature can be modified by the genetic background (Ikeda et al., 1999a,b).

Mutations in the human *TULP1* gene are associated with retinitis pigmentosa (RP), an inherited form of progressive photoreceptor degeneration (Banerjee et al., 1998; Gu et al., 1998; Hagstrom et al., 1998; Paloma et al., 2000). RP patients with TULP1 mutations have a severe visual handicap in comparison with patients with RP due to defects in other genes. This disease is an early-onset, pan-retinal degeneration and the clinical findings document that *TULP1* mutations cause a severe photoreceptor degeneration involving both rods and cones (Hagstrom et al., 1998; Lewis et al., 1999). Genetic ablation of *tulp1* in mice also results in retinal degeneration but not the obesity phenotype or hearing deficits observed in *tubby* mice (Hagstrom et al., 1999; Ikeda et al., 2000). *Tulp1* −/− mice develop early-onset, progressive photoreceptor degeneration with involvement of both rods and cones.

For these reasons, we considered *TUB* a candidate gene for inherited retinal degeneration in humans. Here we report our mutation analysis of 294 unrelated patients with either RP or LCA.

2. Materials and methods

2.1. Ascertainment of patients

This study conformed to the tenets of the Declaration of Helsinki and was approved by the Internal Review Board of the Cleveland Clinic Foundation. All blood samples were obtained after informed consent was secured. All 294 index cases had retinal disease diagnosed through ophthalmologic examination. Among the patients, 159 were diagnosed with autosomal recessive retintis pigmentosa (ARRP), 114 were diagnosed with simplex RP (SRP), and 21 were diagnosed with Leber congenital amaurosis (LCA). The diagnosis of ARRP was given to patients with clinical findings compatible with RP that includes night blindness, constricted visual fields and a pigmentary retinopathy involving optic pallor, attenuated blood vessels and pigmentary changes. Patients with ARRP were from families with one or more affected siblings with parents who do not have RP or were the affected offspring of a consanguineous mating of parents without a history of RP. Patients with SRP had no affected relatives and had parents who had no known blood relationship. The diagnosis of LCA was given to patients with poor vision from birth accompanied by either clear-cut pigmentary retinopathy or with an electroretinogram that shows severely attenuated or extinguished photopic and scotopic responses. No patients were diagnosed with juvenile onset RP. Of the 294 patients, seven LCA families and no RP families were reported to be consanguineous. Unrelated individuals without symptoms or a family history of retinal disease were used as normal control subjects. Leukocyte nuclei were prepared from the blood samples followed by DNA purification using standard protocols. The majority of patients have been screened for mutations in 10 other known disease-causing

genes, RHO, CRX, RPGRIP1, GUCY2D, CRB1, RPE65, AIPL1, MFRP, PROML1, and ELOVL4. No mutations were identified in these genes in this patient cohort.

2.2. Mutation screening

For mutation detection, PCR products corresponding to the complete known TUB coding sequence (BC075031) were amplified from genomic DNA and analyzed by the single-strand conformation polymorphism (SSCP) technique. Fifteen primer pairs were designed to cover the 13 exons as well as the immediately flanking intron sequences and are listed in Table 1 along with the PCR conditions. The buffer pH, Mg^{++} concentration, annealing temperature, and presence or absence of 10% dimethyl sulfoxide were tailored to each primer pair to yield optimal amplification. The amplified DNA fragment encompassing exon 1 was digested with the restriction endonuclease HindIII and exons 4 and 6 were digested with DraII to yield smaller fragments for SSCP analysis (Table 1). PCR-amplified DNA was heat denatured and single-stranded fragments were separated by electrophoresis through two 6% polyacrylamide gels (one with 10% glycerol and one without). Gels were run at 10–20 W for 6–20 h at room temperature before drying and autoradiography. Variant bands detected by SSCP were analyzed by sequencing the corresponding PCR-amplified DNA segments using the Quick Start sequencing kit (Beckman-Coulter) following the manufacturer's protocol. A CEQ-2000 automated sequencer (Beckman-Coulter) was used to resolve sequences.

3. Results

3.1. Sequence variations affecting the amino acid sequence

In the set of 273 recessive and sporadic RP and 21 LCA patients evaluated, seven missense changes (Arg49Gln, Lys96Gln, Ile318Val, Lys363Arg, Arg419Gly, Val431Ile, and Lys439Glu) were discovered (Table 2). Four of the missense changes (Lys96Gln, Ile318Val, Arg419Gly, and Lys439Glu) were found heterozygously in one index patient each. Lys439-Glu was found in one patient with LCA, Lys96Gln and Arg419Gly were found each in one patient with SRP, and Ile318Val was found in one patient with ARRP. Two patients, one with ARRP and the other with LCA, were heterozygotes for the fifth missense change, Arg49Gln; and two patients, one with ARRP and the other with SRP, were heterozygotes for the sixth missense change, Val431Ile. The seventh missense change, Lys363Arg, was identified heterozygously in 15 patients of all three diagnoses. None of these changes were found in the normal control individuals who were evaluated and the minor allele frequencies in patients for each sequence variant analyzed are listed in Table 2. In addition, the DNA from each patient that carried a missense change was directly sequenced in every exon and no second mutation was uncovered. Finally, none of the families of either the ARRP or LCA index patients with a missense change were available for segregation analysis.

3.2. Sequence variations not affecting the amino acid sequence

During our evaluation, 11 DNA sequence changes that did not obviously affect the sequence of the encoded protein were encountered (Table 2). Six of these were isocoding changes (Glu65, Lys81, Gly175, Ser441, Pro458, and Thr475) and five were intronic polymorphisms (c.203 + 29G → A, c.562 + 26G → C, c.730 + 16G → A, c.1380 + 18C → G, and c. 1552-18insGTCT). Four of the isocoding changes (Glu65, Lys81, Gly175, and Ser441) were identified in all three diagnoses screened. The rare variant Pro458 was found in one heterozygous patient with ARRP and the rare variant Thr475 was found in one heterozygous patient with SRP. Two of the intronic changes (c.562 + 26G → C and c.1552-18insGTCT) were found in all three diagnoses screened. One intronic change (c.1380 + 18C → G) was found in one patient with ARRP, c.730 + 16G → A was found in one patient with SRP, and c.203 + 29G → A was found in two patients, one with ARRP and one with SRP. The minor

allele frequencies in patients and in normal controls for each sequence variant analyzed are listed in Table 2. None of the isocoding or intronic changes is predicted to create or destroy splice donor or acceptor sites based on splice-site prediction software available at the neural network website (http://www.fruitfly.org/seq_tools/splice.html).

4. Discussion

In this study, 18 novel sequence changes were identified in *TUB* (Table 2). Of these, seven were missense mutations, six were isocoding changes, and five were intronic polymorphisms. Since family members were unavailable for segregation analysis, it is unclear whether any of the seven missense sequence abnormalities expected to alter the wild-type amino acid sequence are pathogenic. Five of these mutations (Ile318Val, Lys363Arg, Arg419Gly, Val431Ile and Lys439-Glu) affect the highly conserved C-terminal region of the TUB protein. Of these, three amino acid positions, Lys363, Arg419, and Val431, are invariant across the four human TULP proteins. However, the Lys363Arg sequence change is unlikely to cause retinal disease for several reasons. It was found in patients with both ARRP and SRP, it has an allele frequency of 2.5%, and the mutation changes one positively charged residue for another, both with similar basic side chains. Therefore, we consider this variant to be a polymorphism. The rare variants Arg419Gly and Val431Ile were each identified in a patient with sporadic RP and Val431Ile in a patient with ARRP. Although these residues are conserved in TULP proteins, we were unable to further analyze their pathogenicity since they either occurred in a simplex case or family members were not available for segregation analysis. Ile318 is one of the few amino acids in the C-terminal half of TULP proteins that is not conserved across the family members. It is unlikely that the missense change Ile318Val is pathogenic since the residue corresponding to position 318 in TULP3 is Val and both amino acids have similar nonpolar side chains. The heterozygous Lys439Glu mutation identified in a patient with LCA deserves consideration. Interestingly, amino acid position 439 in TUB corresponds to amino acid position 420 in TULP1. The heterozygous mutation Arg420Pro in TULP1, in conjunction with the heterozygous mutation Phe491Leu, causes ARRP (Hagstrom et al., 1998). In addition, the corresponding residue is conserved in TUB, TULP2 and TULP3 and codes for Lys. The Lys439Glu mutation changes a positively charged residue to a negatively charged residue. Unfortunately, the family members of this Egyptian patient with LCA were unavailable for segregation analysis and no second mutation was identified following sequence analysis of the entire coding region of the gene. Therefore there is no proof of Lys439Glu pathogenicity.

Although TUB cannot be definitively ruled out as a cause for the retinal diseases we studied, our results suggest that disease-causing mutations in the regions of the gene screened are extremely rare. However, it is possible that pathogenic mutations might exist outside of the coding exons and the flanking intron splice sites evaluated. It is also possible that mutations in this gene may be so rare that not enough patients were screened to detect them, or that mutations in *TUB* may be present only in diseases not evaluated in this study. Nevertheless, our results suggest that *TUB* is not a major cause of the inherited retinal degenerative diseases evaluated in this study.

Acknowledgments

This research was supported by Cleveland Clinic Foundation, Cole Eye Institute, Karl Kirschgessner Foundation, American Health Assistance Foundation, and the Foundation Fighting Blindness.

References

Banerjee P, Kleyn PW, Knowles JA, Lewis CA, Ross BM, Parano E, Kovats SG, Lee JJ, Penchaszadeh GK, Ott J, Jacobson SG, Gilliam TC. TULP1 mutation in two extended Dominican kindreds with autosomal recessive retinitis pigmentosa. Nat. Genet 1998;18:177–179. [PubMed: 9462751]

Boggon TJ, Shan WS, Santagata S, Myers SC, Shapiro L. Implication of tubby proteins as transcription factors by structure-based functional analysis. Science 1999;286:2119–2125. [PubMed: 10591637]

Gu SM, Lennon A, Li Y, Lorenz B, Fossarello M, North M, Gal A, Wright A. Tubby-like protein-1 mutations in autosomal recessive retinitis pigmentosa. Lancet 1998;351:1103–1104. [PubMed: 9660588]

Hagstrom SA, North MA, Nishina PM, Berson EL, Dryja TP. Recessive mutations in the gene encoding the tubby-like protein TULP1 in patients with retinitis pigmentosa. Nat. Genet 1998;18:174–176. [PubMed: 9462750]

Hagstrom SA, Duyao M, North MA, Li T. Retinal degeneration in tulp1−/− mice: vesicular accumulation in the interphotoreceptor matrix. Invest. Ophthalmol. Vis. Sci 1999;40:2795–2802. [PubMed: 10549638]

Hagstrom SA, Adamian M, Scimeca M, Pawlyk BS, Yue G, Li T. A role for the Tubby-like protein 1 in rhodopsin transport. Invest. Ophthalmol. Vis. Sci 2001;42:1955–1962. [PubMed: 11481257]

He W, Ikeda S, Bronson RT, Yan G, Nishina PM, North MA, Naggert JK. GFP-tagged expression and immunohistochemical studies to determine the subcellular localization of the tubby gene family members. Brain Res. Mol. Brain Res 2000;81:109–117. [PubMed: 11000483]

Heckenlively JR, Chang B, Erway LC, Peng C, Hawes NL, Hageman GS, Rodercik TH. Mouse model for Usher syndrome: linkage mapping suggests homology to Usher type I reported at human chromosome 11p15. Proc. Natl Acad. Sci. USA 1995;92:11100–11104. [PubMed: 7479945]

Ikeda A, Zheng QY, Rosenstiel P, Maddatu T, Zuberi AR, Roopenian DC, North MA, Naggert JK, Johnson KR, Nishina PM. Genetic modification of hearing in tubby mice: evidence for the existence of a major gene (moth1) which protects tubby mice from hearing loss. Hum. Mol. Genet 1999;8:1761–1767. [PubMed: 10441341]

Ikeda S, He W, Ikeda A, Naggert JK, North MA, Nishina PM. Cell-specific expression of tubby gene family members (tub, Tulp1, 2, and 3) in the retina. Invest. Ophthalmol. Vis. Sci 1999;40:2706–2712. [PubMed: 10509669]

Ikeda S, Shiva N, Ikeda A, Smith RS, Nusinowitz S, Yan G, Lin TR, Chu S, Heckenlively JR, North MA, Naggert JK, Nishina PM, Duyao MP. Retinal degeneration but not obesity is observed in null mutants of the tubby-like protein 1 gene. Hum. Mol. Genet 2000;9:155–163. [PubMed: 10607826]

Kapeller R, Moriarty A, Strauss A, Stubdal H, Theriault K, Siebert E, Chickering T, Morgenstern JP, Tartaglia LA, Lillie J. Tyrosine phosphorylation of tub and its association with Src homology 2 domain-containing proteins implicate tub in intracellular signaling by insulin. J. Biol. Chem 1999;274:24980–24986. [PubMed: 10455176]

Kleyn PW, Fan W, Kovats SG, Lee JJ, Pulido JC, Wu Y, Berkemeier LR, Misumi DJ, Holmgren L, Charlat O, Woolf EA, Tayber O, Brody T, Shu P, Hawkins F, Kennedy B, Baldini L, Ebeling C, Alperin GD, Deeds J, Lakey ND, Culpepper J, Chen H, Glücksmann-Kuis MA. Identification and characterization of the mouse obesity gene *tubby*: a member of a novel gene family. Cell 1996;85:281–290. [PubMed: 8612280]

Lewis CA, Batlle IR, Batlle KGR, Banerjee P, Cideciyan AV, Huang J, Aleman TS, Huang Y, Ott J, Gilliam TC, Knowles JA, Jacobson SG. Tubby-like protein 1 homozygous splice-site mutation causes early-onset severe retinal degeneration. Invest. Ophthalmol. Vis. Sci 1999;40:2106–2114. [PubMed: 10440267]

Nishina PM, North MA, Ikeda A, Yan Y, Naggert JK. Molecular characterization of a novel tubby gene family member, *TULP3*, in mouse and humans. Genomics 1998;54:215–220. [PubMed: 9828123]

Noben-Trauth K, Naggert JK, North MA, Nishina PM. A candidate gene for the mouse mutation tubby. Nature 1996;380:534–538. [PubMed: 8606774]

North MA, Naggert JK, Yan Y, Noben-Trauth K, Nishina PM. Molecular characterization of TUB, TULP1, and TULP2, members of the novel tubby gene family and their possible relation to ocular diseases. Proc. Natl Acad. Sci. USA 1997;94:3128–3133. [PubMed: 9096357]

Ohlemiller KK, Hughes RM, Mosinger-Ogilvie J, Speck JD, Grosof DH, Silverman MS. Cochlear and retinal degeneration in the tubby mouse. Neuroreport 1995;6:845–849. [PubMed: 7612867]

Paloma E, Hjelmqvist L, Bayes M, Garcia-Sandoval B, Ayuso C, Balcells S, Gonzalez-Duarte R. Novel mutations in the TULP1 gene causing autosomal recessive retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci 2000;41:656–659. [PubMed: 10711677]

Sahly I, Gogat K, Kobetz A, Marchant D, Menasche M, Castel M-N, Revah F, Dufier J-L, Guerre-Millo M, Abitbol MM. Prominent neuronal-specific *tub* gene expression in cellular targets of *tubby* mice mutation. Hum. Mol. Genet 1998;7:1437–1447. [PubMed: 9700199]

Santagata S, Boggon TJ, Baird CL, Gomez CA, Zhao J, Shan WS, Myszka DG, Shapiro L. G-protein signaling through tubby proteins. Science 2001;292:2041–2050. [PubMed: 11375483]

Table 1

TUB amplification primers and conditions

Amplicon	Sense primer	Antisense primer	Anneal temp. (°C)	Buffer (pH)	$MgCl_2$ (mM)	Fragment (bp)
1	GAAGTGGGACCATCCCTTAAACCC	AATGGTTGGGCCCATGGGCTAAAG	60	8.4	1.5	242
2	GAGGCTGCAAGGATGTGGAGAGTC	TCTCCTGTGGTTGGCCCTTTCCCA	62	8.4	1.5	149
3	TGTATCCTGGCACCTCACGGGCAA	CCAGCCCAGACTCTTCCCTTCTAC	62	8.6	1.5	149
4	TTCCTGGTGGAGGCAGTGGGTCAG	TCCCCGAAGCAGTGACGGGATGTG	68	8.4	1.5	252
5 (5′)	CCCACTGCCTGTTTCTCTCTCTCC	TCCCTTCTTCTCCTTCCTAGCGGC	64	8.4	1.0	181
5 (3′)	CCAACAGCACCAGCTTCAGCCAAG	AAACCTTCCCCAGCCCTACAGTGG	64	8.4	1.0	164
6	CTCCTCCTCCTGGATGTAACTCAG	GGGACTGAGTTTTGGGGGTATCAC	62	8.4	0.5	255
7	GACTTCTCCTCATTCATCCCTTCT	AGTCCTCCAAAAACTGCTGTGGAT	58	8.4	0.5	207
8	ATAGGAGGCAGCCTCAGCCCCTAG	ACCTTCTTCCCATCCTCACGGTCC	66	8.4	1.5	202
9	GTGCAGACCAGAGGCTGAGTCTGG	TCTACAGACAGCTTGCCCCTGCCC	66	8.6	1.5	231
10	CAGAGGGTGCATGACTCTATACTG	TCTACCTTGGAAATCAGAGACCCC	60	8.6	1.5	200
11	GTAAATAGACGCCTCAGGTGGCCA	CTAGGATGATGGGGAAAGAGGGGA	62	8.6	1.5	187
12 (5′)	TCATGGTGCCAAAGGCCTGGGCCT	CCTGTGTGACGCGCCCATGGAAGT	66	8.6	0.25	182
12 (3′)	AAGAACACGGAGAGTATCATCGAG	TTGGGTATCCTACGGACCATAATG	60	8.6	1.5	184
13	GGCTCTACCATTCCTGTCCTGTCC	AAGCTCCGCTCCAGGCTGGGCAAC	64	8.4	1.0	232

Table 2

Sequence alterations identified in *TUB*

Exon	Sequence	Amino acid	Allele frequency		Patient diagnoses
			Patients	Controls	
1	CGA → C*A*A	Arg49Gln	2/588 = 0.3%	0/648 = 0.0%	ARRP, LCA
2	GA*G* → GA*A*	Glu65Glu	37/588 = 6.3%	ND	ARRP, SRP, LCA
2	CCG*G*CCC → CCG*A*CCC	c.203 + 29G → A	2/588 = 0.3%	ND	ARRP, SRP
3	AA*G* → AA*A*	Lys81Lys	3/588 = 0.5%	ND	ARRP, SRP, LCA
4	*A*AG → *C*AG	Lys96Gln	1/588 = 0.2%	0/622 = 0.0%	SRP
5	GG*C* → GG*T*	Gly175Gly	80/588 = 1.1–%	ND	ARRP, SRP, LCA
5	CCT*G*CCC → CCT*C*CCC	c.562 + 26G → C	43/588 = 7.3%	ND	ARRP, SRP, LCA
6	GGG*G*CCC → GGG*A*CCC	c.730 + 16G → A	1/588 = 0.2%	ND	SRP
8	*ATC* → *G*TC	Ile318Val	1/588 = 0.2%	0/650 = 0.0%	ARRP
9	AAA → AGA	Lys363Arg	15/588= 2.5%	ND	ARRP, SRP
10	*C*GT → *G*GT	Arg419Gly	1/588 = 0.2%	0/730 = 0.0%	SRP
11	*G*TC → *A*TC	Val431Ile	2/588 = 0.3%	1/730 = 0.0%	ARRP, SRP
11	*A*AG → *G*AG	Lys439Glu	1/588 = 0.2%	0/730 = 0.0%	LCA
11	AG*C* → AG*T*	Ser441Ser	14/588 = 2.4%	18/730= 2.7%	ARRP, SRP, LCA
11	CC*C* → CC*T*	Pro458Pro	1/588 = 0.2%	0/730 = 0.0%	ARRP
11	CTT*C*CTC → CTT*G*CTC	c.1380+ 18C → G	1/588 = 0.2%	0/730 = 0.0%	ARRP
12	AC*G* → AC*A*	Thr475Thr	1/588 = 0.2%	ND	SRP
13	TGTGCC → TGT*GTCT*GCC	c.1552-18InsGTCT	93/588 = 1.6%	ND	ARRP, SRP, LCA

ND – not determined.

Exp Eye Res. Author manuscript; available in PMC 2011 January 20.