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Molecular Cloning, Expression Analysis, and Chromosome Mapping of WDR6, a Novel Human WD-Repeat Gene

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The WD-repeat proteins are found in all eukaryotes and play an important role in the regulation of a wide variety of cellular functions such as signal transduction, transcription, and proliferation. Here we report on the cloning and characterization of a novel human WD-repeat gene, *WDR6*, which encodes a protein of 1121 amino acids and contains 11 WD-repeat units. *WDR6* is unique since its 11 WD repeats are clustered into two distinct groups separated by a putative transmembrane domain. The *WDR6* gene was mapped to chromosome 15q21 by fluorescence *in situ* hybridization. Northern analysis demonstrated that *WDR6* is ubiquitously expressed in human adult and fetal tissues. *WDR6* is not homologous to any previously identified human WD-repeat genes including *WDR1* through *WDR5*. However, it was found to have significant sequence similarity with *Arabidopsis thaliana* hypothetical protein T7B11.12, yeast putative elongation factor G, and probable membrane protein YPL183c. All of them have been defined as WD-repeat proteins. Therefore, *WDR6* is a novel protein and probably belongs to a highly conserved subfamily of WD-repeat proteins in which T7B11.12 and YPL183c are its distantly related members.

Key Words: **WD-repeat protein; cDNA cloning; gene expression; developmental biology; chromosome mapping; membrane protein.**

WD repeats are minimally conserved domains of approximately 40 to 60 amino acids that are initiated by the glycine-histidine (GH) dipeptide 11 to 24 residues from their N-terminus and end with the tryptophan-aspartic acid (WD) dipeptide at their C-terminus. Between the GH and WD dipeptides is a conserved core sequence (1, 2). The repeating unit, first found in the subunit of the GTP-binding protein transducin, has been referred to as the transducin repeat, the GH-WD repeat, or the WD-40 repeat. Most WD-repeat proteins contain a cluster of 4–16 copies of the WD repeats. Studies have demonstrated that WD-repeat domains themselves are essential for protein-protein interactions (3, 4). WD-repeat domains are found in a diverse group of proteins including cell division control proteins, coatomer β subunits, transcription initiation factors, microtube associate proteins, actin interacting proteins, as well as enzymes like protein phosphatase 2A and myosin heavy chain kinase A (3–13). Recently, mutations in several novel WD-repeat proteins have been implicated in diseases such as X-linked sensorineural deafness, the Cockayne syndrome, and dactylaplasia (14–16).

Genes identified to be responsible for familial cardiac diseases are preferentially or exclusively expressed in the heart (17–20). Our laboratory is interested in identifying and elucidating the molecular mechanisms responsible for familial cardiomyopathies and supraventricular arrhythmias (21–23). We undertook a search for genes expressed in the heart and preferentially expressed in atria to identify candidate genes. We employed the technique of differential display-polymerase chain reaction (DD-PCR) (24). A novel gene that appeared to be preferentially expressed in the atria on DD-PCR was identified and cloned (GenBank Accession No. AF099100). Sequence analysis showed that this novel gene is predicted to encode a protein that contains 11 WD repeats. Our protein is different from previously identified WD-repeat proteins, so we named it WDR6 for WD-repeat protein 6 and the gene symbol has been approved by the HUGO/GDB Nomenclature Committee. We determined the human tissue

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expression of *WDR6* gene and mapped its chromosomal location.

MATERIAL AND METHODS

Isolation of the mRNA differentially expressed in the human cardiac atria. Total RNA was extracted from the atria and ventricles of a normal adult human heart with RNAzolB (Tel-TEST Inc.). The poly-A mRNA was further isolated from these total RNA by Oligotex mRNA kit (Qiagen). The DD-PCR was performed as reported (24). Briefly, the first step consists of reverse transcription of mRNA extracted from atria and ventricles of a normal adult human heart using a fully degenerated 6-mer-oligonucleotide as a primer. The second step consists of PCR amplification of internal regions of the cDNA with two or three longer primers with arbitrary but defined sequences according to certain criteria. DD-PCR products were separated by electrophoresis in 2% agarose gel and the products were extracted from the gel with Gel Extraction Kit (Qiagen) and cloned into PCR II vector (Topo TA cloning kit, Invitrogen). The positive clones were confirmed by release of the insert of the same size as the DD-PCR products and subsequently sequenced.

Expression analysis. Two human multiple tissue Northern (MTN) blots and multiple tissue expression (MTE) arrays (Clontech) were used in the Northern blot analysis to determine tissue distribution of *WDR6* transcripts. The probe, which was the insert isolated from the cloned DD-PCR product D31, was radioactively labeled with ^{32}P-dCTP by random priming with RanPrimer kit (Gibco BRL). Hybridization with Clontech's ExpressionHyb solution and stringent washes were performed following the manufacture's instruction.

Screening of human atria cDNA library. A mixture of oligo(dT) 12–18 and random hexamer primed λ-ZAPII human atrial appendage cDNA library (kindly provided by Dr. Barbara Wible) was screened with the same probe as used in the Northern blotting. After conventional phage library screening by hybridization of the ^{32}P-dCTP labeled probe to the plaque lifts on membrane (NEN Life Science Products), 20 positive phage plaques were isolated. Excision of the pBluscript SK(−) plasmid from λ-ZAPII was performed according to Stragene's single-colony excision protocol. The final screen identified 26 positive colonies. The inserts of these clones were released and subjected to sequencing in both directions.

Rapid amplification of cDNA end (RACE). To obtain the 5′-end of the cDNA, a 5′-RACE PCR reaction was performed with Marathon amplification kit, human heart Marathon ready cDNA (Clontech). The primers used in this reaction were 5′-gene specific primer (5′-CCATCCTAATACGACTCACTAATGGGC-3′) and adaptor primer1 (5′-GCGGTCACCACACACCAGGAAGTCACC-3′). The amplification reaction was carried out according the manufacturer's instruction. A touch-down PCR, after initial denaturation for 1 min, was performed as 5 cycles of 30 s at 94°C and 4 min at 72°C, 5 cycles of 30 s at 94°C and 4 min at 70°C, and 20 cycles of 20 s at 94°C and 4 min at 68°C, and a final extension at 68°C for 10 min. RACE products were cloned into pCRII vector with TA cloning kit. Two clones containing an insert of 1.5 kb were sequenced and found to be the 5′-end of the cDNA. After both 5′- and 3′- ends of this cDNA were obtained, the full-length transcript was amplified with similar conditions to the RACE reaction except for 7 min of extension, and a different set of primers: *WDR6*-Forward (5′-CAGGTCTCGAGGACTACGTTTG-GCC-3′) and *WDR6*-Reverse (5′-AGAGCAAAGAGATGGGCCAG-TGACTC-3′). The amplified product was also cloned into pCRII vector as describe above.

DNA sequencing and data analysis. DNA sequencing was performed on ABI310 sequencer with BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer). Sequence fragments were assembled by Sequencher Program (Gene Codes Corporation). Sequences were analyzed with the use of the GCG package (Wisconsin Package Version 10.0, Madison, WI) (25). Homology search (26) was performed via BCM Search Launcher (http://www.hgsc.bcm.tmc.edu/SearchLauncher/). BLOCKS (27) and PROSITE (28) searches were performed to identify motifs. The detailed WD-repeat analysis was obtained from the PSA Sequence Analysis Server at the BioMolecular Engineering Research Center of Boston University (http://bmerc-www.bu.edu).

Chromosome mapping. Fluorescence *in situ* hybridization (FISH) was performed on metaphase chromosomes as previous described (29). In brief, a set of primers derived from 3′-untranslated region of the *WDR6* (Forward: 5′-TCCTGGTACAGCTCAGCAGCATG-3′, and Reverse: 5′-GCATAGCCAGGCCAGTATG-3′) was used to screen a BAC library (Research Genetics) by PCR. Two clones, 515F22 and 515O6, were confirmed to harbor the gene of interest. DNA extracted from clone 515O6 was labeled with digoxigenin-11-dUTP by nick-translation, used for FISH, and detected with anti-digoxigenin conjugated to rhodamine (fluorescent red). By DAPI staining, the *WDR6* was thought to hybridize to the long arm of chromosome 15. This was confirmed by two-color FISH using the labeled 515O6 clone and a classical satellite probe (D15Z1) which maps to the short arm of chromosome 15. The probe was labeled with biotin-16-dUTP (Oncor, Gaithersburg, MD) and detected with avidin conjugated to fluorescent isothiocyanate (FITC) (fluorescent green).

RESULTS

Using different combinations of 23 oligonucleotide primers, 125 differential display PCR reactions were performed. A total of 26 fragments, presented in the atria but not in the ventricles, were cloned and sequenced. Sequences from 21 different genes were obtained, of which 13 corresponded to known genes such as human KIAA 0181 gene, eotaxin gene, PHL-1 gene, protective protein, death associated protein 5, titin, and atrial natriuretic factor. The remaining eight fragments did not show any significant sequence homology to any known genes in the computerized database and, thus, represent novel genes. One of these fragments, D31, was selected for further analysis because it was the largest DD-PCR products we obtained and showed WD-repeat domains during initial analysis.

Cloning and sequence analysis of human WDR6. The cDNA fragment D31 obtained by DD-PCR was 1.4 kb. To obtain the full length transcript of this novel gene, we screened a human atrial cDNA library utilizing radioactively labeled D31 as a probe. A total of 26 positive colonies were isolated and sequenced. Nineteen of these clones have the same insert of 1.7 kb and were found to overlap with the 3′-end of D31 and contain poly-A signal. Cloning the 5′-end of the gene (1.5 kb) was achieved by RACE. The cDNA sequences from D31, positive library clones and RACE products were assembled and the consensus sequence was obtained. A full-length cDNA was amplified with primers derived from both ends of the consensus sequence, and subsequently cloned, and sequenced. The cDNA of 4079 base pairs showed an open reading frame of 3363 base pairs encoding a protein of 1121 amino acids with estimated molecular mass of 122 kDa (Fig. 1). The first start codon ATG is contained within a Kozak consensus sequence CCCA/GGC***AUG***G for translation initia-

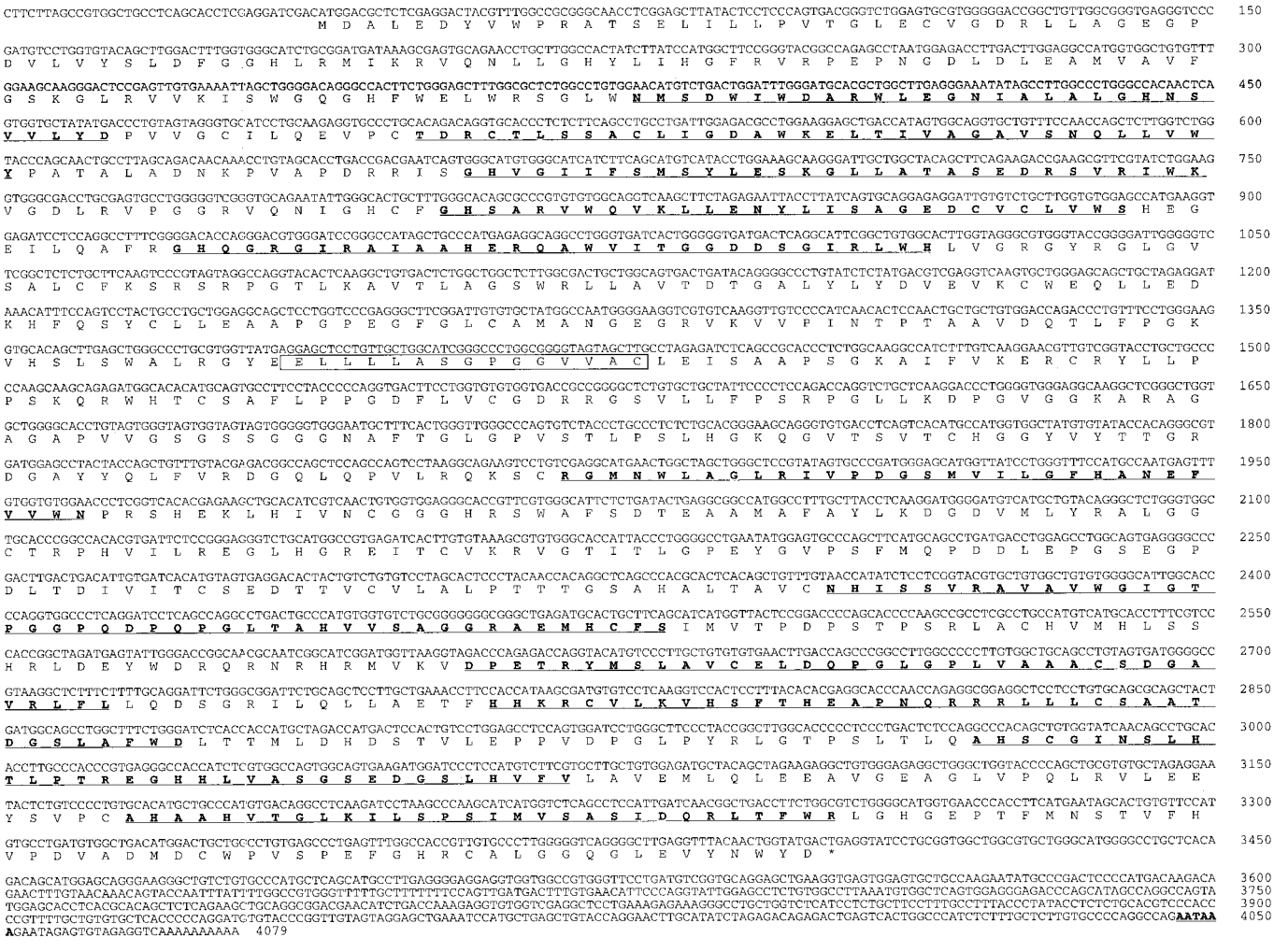


FIG. 1. Nucleotide sequence of human *WDR6* cDNA and its deduced amino acid sequence. WD repeats and poly A addition signal were in boldface and underlined. A predicated transmembrane domain was indicated with a box. The nucleotide sequence has been deposited with GenBank under Accession No. AF099100.

tion (30, 31). The poly (A) tail is preceded by a polyadenylation signal (AATAAA) of 18 bp.

BLOCKS and PROSITE analysis of amino acid sequences of the predicted 122 kDa protein identified four regions with high sequence homology to the β-transducin WD repeat. Using WD-repeat consensus sequence (1, 2) and BMERC search engine, seven additional WD repeats were identified in this protein. All of the 11 WD repeats found in our novel protein have been compared with the WD-repeat consensus sequence and showed significant similarity between them (Fig. 2), suggesting our novel gene encodes a WD-repeat protein. Interestingly, the distribution of these repeat units in our novel protein is unique in that five of them are close to its N-terminus and the remaining six are close to the C-terminus (Fig. 1). These two clusters of WD repeats are separated by a region of 285 amino acids without WD repeats in between but which are predicted to code for a probable transmembrane domain (amino acids 451–467 as indicated by a box in Fig. 1) by PSORT II prediction due to the hydrophobic property of this stretch of residues, while most of the WD-repeat proteins characterized to date have their WD repeats clustered together as a single group (2, 10, 11, 32–35).

BLAST search shows that our protein is not homologous to any previously identified human WD-repeat protein including the recently cloned five WD-repeat proteins (*WDR1* through *WDR5*) of unknown function (35, 36), we refer to it as *WDR6* for WD-repeat protein 6. However, *WDR6* was found to be most closely related to the *A thaliana* hypothetical protein T7B11.12 (GenBank Accession No. AC007138), *S. pombe* putative elongation factor G (GenBank Accession No. AL133303) and a *S. cerevisiae* probable membrane protein YPL183c (PIR Accession No. S65195). Specifically, *Arabidopsis* T7B11.12 is 40% similar and 23% identical (expect value = 1×10^{-32}), *S. pombe* putative elon-

				Y						
				V						
				I		TS		I		
		S		L		SSN		VL		L IY
		A I	CI	W	[0-?]	DND	[0-?]	IVTAG		SVRLFN
CONSENSUS	POSITION	GHxxxV	xSV	xFx	x	PDG	x	xLASGS	xDx	TIKVWD
WD1	114-142	NMSDW**I**	WDA	RWL	EG			N**IA**L**A**L	GHN	**SV**V**LYD**
WD2	155-188	TDRCTL	S**S**A	C**LI**	G**D**AWKEL			**TIVAG**A	VSN	Q**LL**V**WY**
WD3	207-237	**GH**VGI**I**	**FS**M	SYL	E**S**KG			**LLATAS**	EDR	**SVRIW**K
WD4	256-284	**GH**SARV	WQV	K**LL**	E**N**			Y**LISAG**	EDC	VCL**VWS**
WD5	296-326	GHQGRG**I**	R**AI**	AAH	ERQA			W**VITGG**	DDS	G**IRLW**H
WD6	611-641	RGMNWL	AG**L**	RIV	**PDG**S			M**VILG**F	HAN	EFV**VW**N
WD7	772-815	N**HISSV**	**RAV**	AVW	GIGT**P**GGPQDPQPGLTA			H**VVSAG**	GRA	EMHC**FS**
WD8	857-892	DPETRY	**MSL**	AVC	ELDQPGLGP			L**VAA**A**C**	SDG	A**VRLF**L
WD9	908-945	H**H**KRC**V**	LK**V**	HSF	THEA**PN**QRRRL			LLC**SA**A	T**D**G	**SL**AF**WD**
WD10	978-1011	**AH**SCG**I**	N**SL**	HTL	**P**TREGHH			L**VASGS**	E**D**G	**SL**H**VF**V
WD11	1043-1072	**AH**AAH**V**	TG**L**	K**IL**	S**PS**			IM**VSAS**	I**D**Q	**RL**TF**WR**

FIG. 2. Comparison of the 11 WD repeats identified in human *WDR6* protein with the WD-repeat consensus sequence obtained from Smith, T., *et al.* (1999). The amino aids that are identical or similar to the consensus sequence are shown in boldface.

gation factor G is 37% similar and 21% identical (expect value = 4×10^{-32}), and yeast YPL183c is 37% similar and 19% identical (expect value = 2×10^{-22}) to human *WDR6,* respectively. Sequence similarity was found not only in the WD-repeat regions but also in the regions outside WD repeats, indicating *WDR6* is homologous to them. Although none of these three putative proteins was well characterized, three of them have been defined as WD-repeat proteins. Therefore, *WDR6* is a novel WD-repeat protein and probably belongs to a highly conserved sub-family of WD-repeat proteins in which *A. thaliana* protein T7B11.12 and yeast elongation factor G are its distantly related members.

Several different phosphorylation sites, myristoylation sites, and glycosylation sites were also identified in *WDR6* by motif search, but their significance remain to be determined.

Tissues distribution of human WDR6. To determine the size of the full-length transcript and tissue distribution of human *WDR6,* Clontech MTN blots and a MTE array were probed with *D31.* It was demonstrated that a transcript of approximately 4.4 kb is widely expressed in human tissues including pancreas, kidney, brain, liver, heart, and skeletal muscle. A much more abundant expression was observed in pancreas when compared with the expression of the other tissues (Fig. 3A). A transcript of about 9.5 kb was evident in tissue from pancreas. To test if *WDR6* is also expressed in smooth muscle, a muscle MTN blot was probed with *D31* probe. A similar level of the expres-

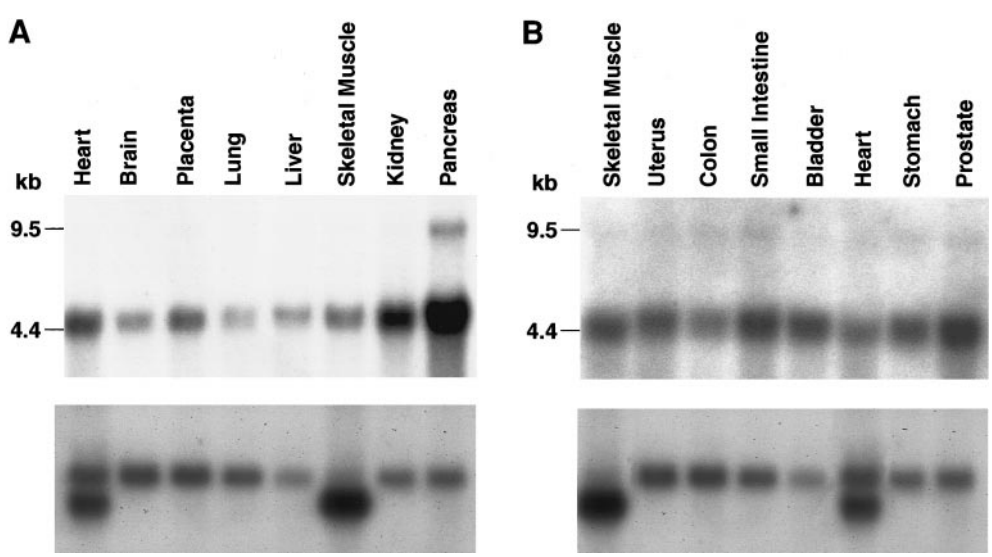
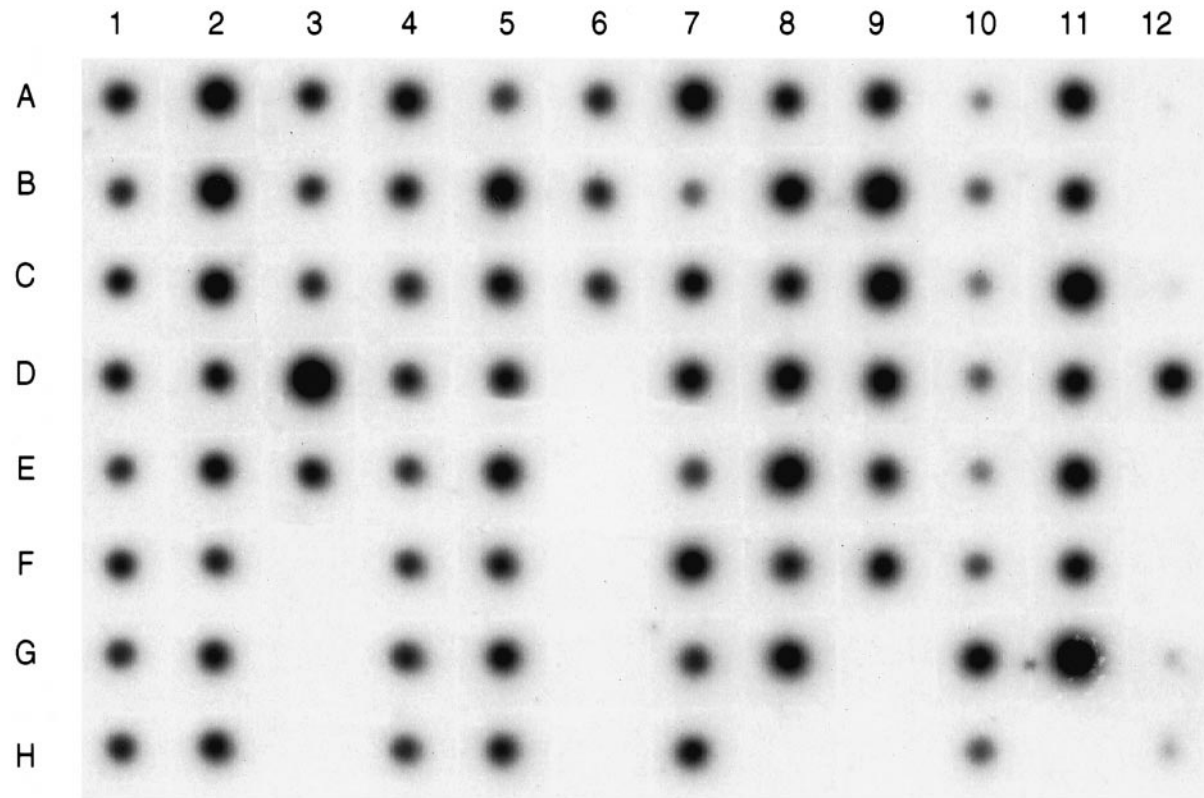


FIG. 3. Northern blot analysis of human *WDR6* gene expression in various human tissues. Human multiple tissue Northern blots (Clontech) were probed with ^{32}P-labelled *WDR6* (nucleotide 1260-2651). A major transcript about 4.4 kb was shown across all the tissues tested. Two μg of poly-A RNA from each tissue were loaded on each lane of the blot. The constant amount of mRNA was demonstrated by hybridization with a control β-actin cDNA probe as shown in the lower panel. The film was exposed at -70°C for 16 h.



	1	2	3	4	5	6	7	8	9	10	11	12
A	whole brain	cerebellum, left	substantia nigra	heart	esophagus	colon, transvers	kidney	lung	liver	leukemia, HL-60	fetal brain	yeast total RNA
B	cerebreal cortex	cerebellum, right	accumbens nucleus	aorta	stomach	colon, descending	skeletal muscle	placenta	pancreas	HeLa S3	fetal heart	yeast tRNA
C	frontal lobe	corpus callosum	thalamus	atrium, left	duodenum	rectum	spleen	bladder	adrenal gland	leukemia K-562	fetal kidney	E. coli rRNA
D	parietal lobe	amygdala	pituitary gland	atrium, right	jejunum		thymus	uterus	thyroid gland	leukemia, MOLT-4	fatal liver	E. coli DNA
E	occipital lobe	caudate nucleus	spinal cord	ventricle, left	ileum		peripheral blood leukocyte	prostate	salivary gland	Burkitt's lymphoma, Raji	fetal spleen	Poly r(A)
F	temporal lobe	hippo-campus		ventricle, right	ilocecum		lymph node	testis	mammary gland	Burkitt's lymphoma, Daudi	fetal thymus	human Cot-1 DNA
G	p.g.*of cerebral cortex	medulla oblongata		inter-ventricular septum	appendix		bone morrow	ovary		colonrectal adeno-carcinoma, SW480	fetal lung	human DNA 100 ng
H	pons	putamen		apex of the heart	colon, ascending		trachea			lung carcinoma, A549		human DNA 500 ng

FIG. 4. mRNA expression of human *WDR6* as detected with human multiple tissue expression array (Clontech). Human *WDR6* is ubiquitously expressed as shown in upper panel. mRNA from 76 different human tissues as indicated in lower panel was loaded on the nylon membrane. mRNA levels were normalized versus eight different housekeeping gene makers by the manufacture, thus the blot is quantitative. The blot was hybridized with cDNA probe of *WDR6* under high stringency conditions and exposed to film for 16 h.

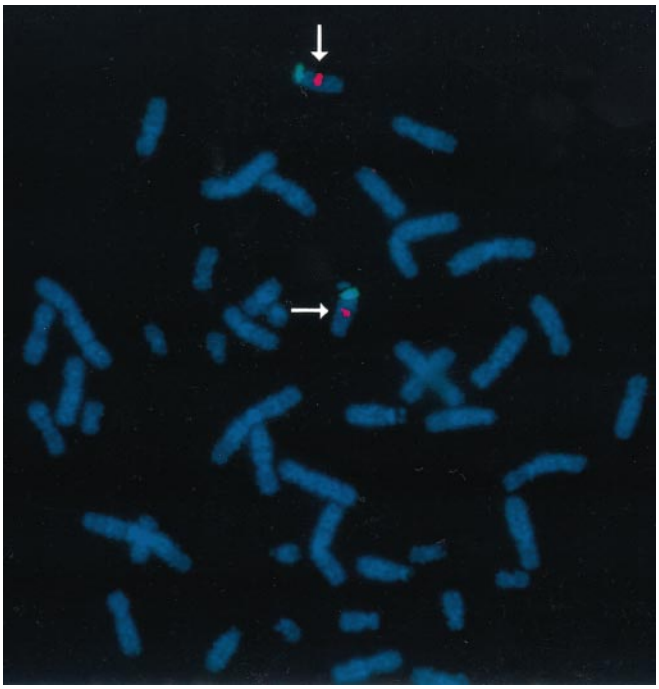


FIG. 5. Chromosomal localization of *WDR6* gene on chromosome 15q21 by FISH. Metaphase spreads of karyotypically normal blood lymphocytes were hybridized with BAC clone 515O6 harboring the *WDR6* gene as described in the text. The arrow indicates the specific hybridization signal for clone 515O6 on the long arm of chromosome 15 in a metaphase cell (red signal). The chromosome 15 specific classic satellite probe D15Z1 (fluorescent green), which maps to the short arm of chromosome 15, confirms the assignment of *WDR6* to 15q.

sion was evident among eight different muscle tissues including uterus, colon, bladder, and prostate (Fig. 3B). MTE analysis showed that *WDR6* was expressed not only in both atria but also in both ventricles as well as all other adult and fetal tissues examined (Fig. 4). It appeared that *WDR6* was particularly abundantly expressed in pituitary gland, and fetal lung (20 to 25 weeks gestation). ESTs homologous to the *WDR6* were found in cDNA libraries from whole embryo and diverse adult tissues in mouse and human (Unigene Mm. 29493 for mouse; Hs. 8737 for human), which further indicates that *WDR6* is a ubiquitously expressed gene.

Chromosome localization of WDR6 gene. Human *WDR6* hybridized to the long arm of chromosome 15 (15q21), confirmed by two-color FISH using a classical satellite probe (D15Z1) which maps to the short arm of chromosome 15 (Fig. 5). The probe hybridized to the centromeric half of chromosome 15q, placing it roughly to band 15q21.

DISCUSSION

We identified and cloned a novel human WD-repeat gene, *WDR6*. This novel gene is mapped to chromosome 15q21 and is ubiquitously expressed in fetal and adult human tissues. Sequence analysis established that *WDR6* belongs to WD-repeat protein family and is highly conserved from yeast, *A. thaliana*, and mouse. The high level of conservation and ubiquitous expression suggests that *WDR6* is likely to be an important gene.

The WD-repeat proteins are a rapidly expanding protein family (http://bmerc-www.bu.edu/wdrepeat/members.html). There are at least 123 proteins identified in the protein database (SWISS-PROT/TrEMBL) to have more than four WD repeats in their amino acid sequences. A total of 205 putative WD-repeat proteins have been predicted from the genomes of *A. thaliana*, *C. elegans*, and *S. cerevisiae*. Interestingly, seven WD-repeat proteins were also identified in prokaryotes like *Synechocystis* in contrast to a previous claim that WD-repeat proteins are only found in eukaryotes (1, 2). Based on the distribution of the WD repeats, WD-repeat proteins were grouped into two classes: one in which the proteins are composed almost entirely of the WD-repeat domains; a second group in which WD repeats are restricted to either the N-terminus or the C-terminus (2, 14). Proteins of the first group is often seen in the G-protein β subunits (10) and some other signal transduction related proteins such as the receptor for activated protein kinase C (4). The proteins in the second group are involved in functions as diverse as RNA processing, transcription regulation, vesicular trafficking, cytoskeletal assembly, and cell division control (3, 5–9, 37).

The primary structure of *WDR6* is unique in that its 11 WD repeats are clustered into two distinct groups separated by a probable transmembrane domain. Since a group of four WD repeats is able to form a structural unit, typically a β-propeller fold (1, 38, 39), it seems possible that *WDR6* may form two separate structural units across the plasma membrane. WD-repeat proteins represent a large protein family with similar sequence repeats and predicted three dimensional structure. One possible common functional theme is that the WD-repeat propeller structures create a stable platform that can form reversible complexes with several proteins and in so doing, coordinate sequential and/or simultaneous protein interactions (1, 2). However, these proteins exhibit a high degree of functional diversity. Therefore, currently it is very difficult to assign a specific function to a particular WD-repeat protein based on protein sequence analysis alone. *WDR6* may be important for developmental and physiological processes, although its precise function remains to be defined by further experimental studies.

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