The Drosophila dishevelled gene (dsh) encodes a secreted glycoprotein, which regulates cell proliferation, acting as a transducer molecule for developmental processes, including segmentation and neuroblast specification. We have isolated and characterized cDNA clones from two different human dsh-homologous genes, designated as DVL-1 and DVL-3. DVL-1 and DVL-3 putative protein products show 64% amino acid identity. The DVL-1 product is 50% identical to dsh and 92% to a murine dsh homologue (Dvl-1). Both human DVL genes are widely expressed in fetal and adult tissues, including brain, lung, kidney, skeletal muscle and heart. DVL-1 locus maps to chromosome 1p36 and DVL-3 to chromosome 3q27. DVL-1 locus on chromosome 1 corresponds to the murine syntenic region where Dvl-1 is located. DVL-1 and DVL-3 are members of a human dsh-like gene family, which is probably involved in human development. Although the precise role of these genes in embryogenesis is only conjectural at present, the structural and evolutionary characteristics suggest that mutations at their loci may be involved in neural and heart developmental defects.

INTRODUCTION

A single Drosophila dishevelled segment polarity gene (dsh) has been isolated (1). Dsh mediates cell–cell interactions during embryogenesis and adult fly development, which in turn determine the ultimate cell polarity and fate (1–3). Dsh is required for wingless (wg) function (4). wg is a segment polarity gene, homologous to the mammalian proto-oncogene Wnt-1, coding for a secreted protein (5–7). In Drosophila, dsh expression loss ends in segmentation defects identical to wg mutated embryos (8). Many Drosophila polarity genes are conserved during evolution. If dsh homologues are also conserved, they may have a key role in vertebrate morphogenesis as well. Only a mouse (Dvl-1) and a Xenopus dsh (Xdsh) homologous genes have been reported so far (9,10). They encode proteins with 50% identity to the Drosophila dsh. Dvl-1 and Xdsh protein sequence comparison shows 60% identity. Conservation is highest at the amino-terminus and at the central portion of the protein, where Dvl-1 and Xdsh are virtually identical. In particular, a sequence known as GLGF repeat or disc-large homology region (DHR), a motif which is common to proteins supposed to be part of cell junctions, is completely conserved (9,10). Dvl-1 also contains two RGD tripeptides. RGD motives are associated to recognition systems for cell signalling in proteins which have a role in cell adhesion (9). Only one of these RGD sequences is present in Xdsh (10).

The high dsh-like gene interspecies conservation suggests they act during common and precocious embryogenic events. In fact, Xdsh mRNA, injected into normal or ventralized Xenopus embryos, induces a complete body axis formation, and causes neuralization when overexpressed in the committed ectodermal cells (10). In situ hybridization studies indicate that the mouse Dvl-1 gene is uniformly expressed at high levels in the fetal central nervous system (CNS), also suggesting a general key role in CNS development (9). It is therefore likely that the dsh homologue(s), if expressed in humans, play important roles in embryonic patterning. Imbalance of such protein expression might be especially involved in neural developmental defects.

We report on the isolation of cDNA clones belonging to two different human dsh-like genes, and their respective chromosome locations. Our data indicate that other DVL sequences are present...
in the human genome and that they altogether may represent a more complex new gene family in humans.

RESULTS

Cloning and sequencing of the human DVL genes

In order to isolate human homologous cDNAs to the mouse Dvl-1 gene, we synthesized an oligonucleotide pair on the mouse cDNA 5′ translated sequence (DVL-1 5′ ATG GAC GAG GAG GAG ACG CCG TAC 3′ and DVL-3 5′ GAA ACC ACC CGG CCA TTG AAG CAG 3′). A 200 bp fragment (DVL-1,3), with high homology to the mouse Dvl-1 sequence (97%), was obtained by RT–PCR of human fetal brain total RNA, and used to screen a human adult caudate cDNA library.

Five independent cDNA clones were isolated. When the clones were initially sequenced at both ends, only one of them (4B) revealed a complete identity to the DVL1,3 probe, suggesting 4B represented the true human homologue of the mouse Dvl-1 (we designated it as DVL-1). 4B was 1600 bp long, and comprised a 150 bp 5′ UTR followed by a 1450 bp open reading frame (ORF), with 85% nucleotide homology to the mouse Dvl-1 cDNA. In order to get the complete human DVL-1 cDNA sequence, the same cDNA library was screened with a 200 bp probe representing the mouse DVL-1 3′ UTR. Sequencing of a 1800 bp clone (B8), partially overlapping with clone 4B, gave a full length ~3000 bp DVL-1 cDNA sequence, with 85% nucleotide homology to the mouse Dvl-1 in the longest ORF. Human DVL-1 and mouse Dvl-1 cDNA sequences quite differ at both the 5′ and the 3′ UTR, except at the very 3′ end, where a highly conserved 80 bp sequence is present, just preceding the poly(A) tail (Fig. 1).

The putative human DVL-1 gene product has a 92% overall identity to the mouse Dvl-1 protein, with a maximum 99% identity in the amino-terminal half, which includes both the DHR region and the two RGDs sequences. The carboxy-terminal half is slightly less conserved, with a 84% identity.

The remaining four cDNAs, isolated by DVL1,3 library screening, contained the same 5′ sequence, however, differing from DVL-1 (70% homology in the longest ORFs). In particular, a 2400 bp clone (1B) contained a complete coding sequence, in addition to a 150 bp 5′ UTR and a 150 bp 3′ UTR. As the 1B ORF sequence did not show very strong homology to either mouse Dvl-1 and to a second mouse dsh-like cDNA, designated as Dvl-2 (GenBank accession number MMU 24160), we numbered the 1B gene as the third human DVL gene (DVL-3), pending the isolation and characterization of the human homologue of mouse Dvl-2. 1B is not a full length clone, as it does not contain the complete DVL-3 3′ UTR. However, a second cDNA clone (9B), unfortunately rearranged in its central portion, was also isolated, with a DVL-3 5′ region, and a 3′ sequence identical to the very 3′ end of DVL-1 (Fig. 1). The 9B 3′ sequence may therefore represent the DVL-3 3′ UTR. As noted in Figure 1 both DVL-1 and DVL-3 cDNAs (and the mouse Dvl-1) would contain an identical 3′ terminal region, which might be a regulator sequence, typical of many members of the DVL gene family, for either the mRNA transcription or translation.

DVL-3 shows 74% nucleotide homology to the human DVL-1 and 71% to the mouse DVL-1. DVL-1 and DVL-3 share 64% amino acid identity (Fig. 2). The high level of similarity in the amino portion of all these proteins indicates that this region encodes essential domains for the activity of the whole DVL class of proteins. On the contrary, the sequence divergence of the carboxy terminal halves suggest these regions contain domains which are responsible for the specific single molecule activity.

Genomic Southern blot hybridizations of HindIII digested DNA with DVL-1 and DVL-3 cDNAs revealed two bands, respectively of 6.7 kb and 4.2 kb for DVL-1 and 5.8 kb and 1.8 kb for DVL-3.

Tissue expression of DVL-1 and DVL-3

Northern blot analysis of the DVL-1 expression using poly(A)+ RNA samples from various fetal and adult human tissues, revealed a single band of ~3 kb (Fig. 3A). Signal quantification was obtained by densitometric analysis, normalized to β-actin mRNA, and single tissue relative concentration values were determined as compared with the liver mRNA levels, where the weakest DVL-1 signals were detected. The highest expression levels were found in adult skeletal muscle and pancreas (15 and 10 times more than the liver, respectively) followed by heart muscle (3.5 times). Detectable levels were also found in adult brain, placenta, lung, liver and kidney (less than two times the liver) (Fig. 3A). The same expression pattern was observed in fetal tissues. Applicable quantitative differences between the adult and the fetal tissues present in the blot (brain, lung, liver and kidney) were not evident (Fig. 3B).

DVL-3 showed the same distribution of a 2.9 kb mRNA. Again, the highest expression levels were found in skeletal muscle (16 times the liver), in pancreas (11 times) and in the heart (nine times), the remaining tissues showing less than two times the liver expression levels (Fig. 3C). However, two additional 5.9 and 5.0 kb bands were also detected after hybridization to DVL-3 cDNA. The 5.0 kb form was prevalently expressed by skeletal muscle (18 times the liver), adult heart (seven times), and pancreas (2.7 times), and at lower levels in placenta (1.8 times). No such a signal was detected in the fetal tissues examined. The 5.9 kb form was found at highest levels in placenta (28 times the liver), in pancreas (13 times), in skeletal muscle (seven times), in kidney and lung (five times each). Fetal tissues showed relatively high levels of the 5.9 kb form, except the fetal brain where a low level of expression was detected (Fig. 3D).

RT–PCR studies were also performed on some adult and fetal tissues using simultaneous amplification of β-actin and either DVL cDNA, followed by co-amplification of both DVL-1 and
**Figure 2.** Aligned sequences of the putative DVL-1 and DVL-3 protein products. Sequence identity are represented by vertical lines. The underlined region corresponds to the conserved dgl sequence. RGD recognition sequences are indicated by asterisks. (DVL-1 cDNA GenBank accession number U46461; DVL-3 cDNA GenBank accession number U49262).

DVL-3 (Fig. 4). The intensity of the amplified fragments, corrected for their respective length, was compared by scanning with a Bio-Image (Millipore) computerized Densitometer, within the exponential phase of the PCR reaction. DVL-1/DVL-3 ratio was about 1 for most tissues examined (skeletal muscle, fetal brain, adult heart, fibroblasts and chorionic villi), with the exception of the fetal heart, where it dropped to 0.7.

DVL-1 expression in early human CNS (fifth post conceptional week) was investigated by in situ hybridization (Fig. 5). Signals were predominantly found in the neural tube (in particular the dorsal portions) indicating a possible role, at that stage, for the maturation of the neural elements. No appreciable levels of hybridization signal was detected in other tissues.

**Chromosome localisation of DVL-1 and DVL-3**

DVL-1 specific primers were designed on the 3'UTR and used to amplify by PCR a rodent–human somatic cell hybrids panel. Control rodent genomic DNA showed a PCR product which differed in size from the expected human product (data not shown). The latter was detected in human chromosome 1 containing hybrids. Similarly, using DVL-3 specific primers, DVL-3 was assigned to chromosome 3 (data not shown).
**DISCUSSION**

We report the cloning of human cDNAs encoding for two homologues of the *Drosophila dsh* gene, and their mapping at different loci in the human genome. The only other known vertebrate *dsh* homologues so far described are the *Xenopus Xdsh* and the mouse *Dvl-1* gene (9,10). A second mouse gene (*Dvl-2*) has been deposited on GenBank, but no report has been issued yet. On the basis of the extensive nucleotide sequence homologies, the putative peptide sequence resemblance and the map position (human *DVL-1* and mouse *Dvl-1* genes map on known regions of synteny), human *DVL-1* should correspond to the mouse *Dvl-1* gene. No human *Dvl-2* homologous cDNA has been isolated yet.

The second *dsh*-like gene we characterized and mapped has no mouse counterpart yet. We therefore designated it as *DVL-3*. It is likely that the human *dsh*-like gene family is much wider than the single *Drosophila dsh* gene. We have mapped a *DVL* 3′ UTR-like sequence (90% identical to the *DVL-1/9B* sequence in Fig. 1) on the DiGeorge syndrome critical region (DGS/SRO), on chromosome 22q11 (11). We also isolated a chromosome 22 cosmid clone, which cross-hybridizes to the *DVL-1* locus on chromosome 1 in FISH experiments. This cosmid, which maps well outside the DGS/SRO, specifically hybridizes to sequences representing the 5′ coding region of *DVL-1* cDNA (unpublished data). Moreover, the detection of multiple hybridization bands in *DVL-3* Northern blotting experiments, which are very different in size, could also indicate, rather than the presence of different alternatively spliced forms of the same transcript, the presence of other very closely related genes. However, as we could not isolate any cDNA clone representing the 5.0 and the 5.9 kb ‘*DVL-3* forms’ so far, this issue remains still open to discussion.

**Figure 5.** (A) *In situ* hybridization with *DVL-1* oligonucleotide on a coronal section of the neural tube, obtained from a human fetus at 5.5 p.c.w. NT = neural tube. (B) Blue toluidine staining of the adjacent section. The slightly different pattern of the adjacent sections is due to the sectioning procedures (cryostat, non-embedded sections).

*DV L-1* and *DV L-3* cDNAs were more finely mapped, to bands 1p36 and 3q27 respectively, using the cloned cDNAs for FISH analysis (Fig. 6A and B).

*DV L-1* and *DV L-3* expression is widespread, both in adult and in fetal tissues, with particularly high levels in heart and striated muscle, as demonstrated by Northern analysis. *In situ* experiments during early human embryogenesis indicate that *DV L-1* is more selectively expressed in the neural tube, in particular in its dorsal portion, at those very precocious developmental stages. These data somehow reminds the abundant *Dvl-1* expression observed in mouse embryo where localization is strong throughout the neural folds and in mouse fetus where it is very abundant in the spinal cord, in particular on the ventral horns (9).

Interestingly, two axonal forms of the Charcot–Marie–Tooth disease (*CMT2A* and *CMT2B*) have been mapped to chromosomes 1p35–p36 and chromosome 3q, respectively (12,13). The main pathological feature of these peripheral nerve hereditary disorders is a motor neuron axonal degeneration. 1p35 region is also frequently involved in somatic loss of heterozygosity associated with neuroblastomas, suggesting a tumor suppressor gene is present in the region (14). Although at least four candidate genes (*DAN, ID3, CDC2L1* and *TNFR2*) have been proposed for this locus, a consensus region of deletion (CDR) has been described, excluding all these genes (14). One domain of *DV L-1* is similar to a portion of the *discs large-1* (*dgl*) protein, a *Drosophila* tumor suppressor gene (15). This feature, as well as its possible role as a neural differentiation factor (10), would make *DV L-1*, if positioned within the CDR, a good candidate gene for neuroblastomatous transformation.

One of the main roles of *DV L* proteins may be the involvement in vertebrate embryos dorsoventral patterning (10). Three human congenital malformation syndromes, Schwartz–Jampel, De Lange 1, and frontonasal dysplasia, have been mapped in the *DV L-1* and *DV L-3* gene locations (16–18). The phenotype of
Figure 6. FISH mapping of the DVL-1 gene to chromosome 1p36 (A) and DVL-3 gene to chromosome 3q27 (B). cDNA B4 and 1B were used as probes for DVL-1 and DVL-3, respectively.

these diseases is consistent with defects that might be expected from aberrant expression of DVL genes during development. DVL-1 and DVL-3 are likely to be important regulators of embryonic development. A mutation screening and future mapping of additional diseases to the 1p36 and 3q27 regions will clarify the role of DVL genes in these diseases. Further studies of DVL-1 and DVL-3 expression during human embryogenesis, mixexpression and loss-of-function experiments in mice will provide insight into the normal activity of these genes and their potential role in human congenital diseases.

MATERIALS AND METHODS

General procedures

Purification of nucleic acids, restriction analysis, gel electrophoresis, DNA ligation, cloning, subcloning and Sanger sequencing (automated), probe radiolabelling, northern and Southern analysis, library screening were performed according to established protocols (19). The human adult caudate cDNA library was purchased from Stratagene. Northern blot analysis was performed on commercially available filters containing about 2 µg per lane of poly(A)+ enriched RNAs from adult and fetal tissues (Clontech, Bios Laboratories). Hybridizations were conducted at 65°C in 125 mM sodium phosphate (pH 7.2), 250 mM NaCl, 7% SDS, 10% PEG for Southern blots and at 42°C in 50% (v/v) formamide, 125 mM sodium phosphate (pH 7.2), 0.1% SDS, 5× Denhardt’s solution, 100 g/l denatured ssDNA for Northern blots. Southern filters were washed at the same temperature of hybridization with a final stringency ranging from 0.5× SSC to 0.1× SSC and at 50°C in 0.25× SSC/0.2% SDS for Northern filters. Automatic DNA sequencing was performed on an Applied Biosystems 370A apparatus.

In situ hybridization (ISH) of human embryonic tissue sections

Tissues from chromosomally normal first-trimester spontaneous abortions specimens (5.3 weeks of gestational age) were obtained according to the current Italian regulation. Tissues were washed in PBS and frozen in liquid nitrogen. Fourteen µm thick sections were cut in a cryostat (Leitz Kryostat 1720) at −20°C and twad-mounted on to preclemed microscope glass slides (ProbeOn, Fisher Scientific, Pittsburgh, USA). Oligonucleotide probe with sequence complementary to mRNA encoding DVL-1 (5′ CGC TCC TTG AAG CCC TCC ACG TGT GTG TAC AGC CAG TCC ACC AC 3′) was synthesized and purified through Pharmacia NAP-10 column. The oligonucleotide probe was labeled at the 3′-end with 35S-dATP (New England Nuclear, Boston, MA) using terminal deoxynucleotidiltransferase (IBI, New Haven, CT, or Amersham, England) in a buffer containing 10 mM CoCl2, 1 mM dithiothreitol (DTT), 300 mM Tris base, and 1.4 M potassium cacodylate (pH 7.2). Afterwards, the labeled probe was purified through Nensorb-20 columns (New England Nuclear) and DTT was added to a final concentration of 10 mM. The specific activity obtained ranged from 1 to 4×106 d.p.m./ng oligonucleotide. Sections were brought to room temperature, air dried, covered with a hybridization buffer containing 50% formamide, 4× SSC, 1× Denhardt’s solution, 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulfate, 500 g/ml heat-denatured salmon sperm DNA, 200 mM DTT and 40 ng/l of the labeled probe. The slides were placed in a humid chamber and incubated for 15–20 h at 50°C. Afterwards, the sections were rinsed in 1× SSC at 55°C for 1 h with six changes and washed in the same buffer for 1 h at room temperature. Finally, the slides were rinsed in distilled water and 60 and 95% ethanol (2 min each) and air dried. The sections were dipped in NTB2 nuclear track emulsion (Kodak). Sections were exposed for a period of time of 3 weeks before being developed with Kodak D19 for 3 min and fixed with G333 (AGFA Gevaert, Leverkusen, Germany) for 10 min. The tissue was counterstained with toluidine blue. Emulsion-dipped tissue sections were examined in a Nikon Microphot-FX microscope equipped with dark-
bright-field condensers and photographed with Tri-X 100 (Kodak) black and white film.

**Somatic cell hybrids and FISH mapping**

DVL-1 and DVL-3 were mapped respectively to chromosomes 1 and 3 using somatic cell hybrids from BIOS Laboratories and the monochromosomal somatic cell hybrid panel (UK Human Genome Mapping Project Resource Centre). The following primers were used to amplify human-specific DVL-1 and DVL-3 DNA fragments: DVL-1, sense (5′ AGC CGT GAC GGG ATG GAC AAC G 3′), and antisense (5′ TCA AGC CGT CTG AGG GC 3′); DVL-3 sense (5′ CTT CCG CAT GGC CAT GGG AAA C 3′) and antisense (5′ GAC GGA ATG AGA GGA GGA AC 3′).

Metaphase chromosomes were prepared from human peripheral blood lymphocytes obtained from normal individuals as described (20). Purified cDNA probes were labeled with biotinylated dATP and digoxigenated dUTP by nick translation (Life Technologies Inc., USA) and hybridized to chromosome spreads according to routine procedures. Chromosomes were counterstained with DAPI and visualized as described (20). At least 25 metaphases for each probe were scored. Images were CCD captured and merged through a Vidas Image Analyzer (Zeiss, Germany).

**Sequence analysis**

Data bank search (GenBank, GenEmbl, SwissProt and PIR) were run through the BlastX and BlastN network service. Sequence analysis was performed using the GCG package. For pairwise alignments, the gap program was used. Known protein motifs were searched with PROSITE.

**ACKNOWLEDGMENTS**

We are grateful to D. Sussman and T. Wynshaw-Boris for communicating results prior to publication and for useful comments on this work. We thanks the UK Human Genome Mapping Project for reagents and computer resources, M. Gennarelli and M. Lucarelli for experimental help. This work was supported by grants from EEC, Biomed-1 Project, MURST, Gennarelli and M. Lucarelli for experimental help. We thanks the UK Human Genome Project for reagents and computer resources, M. Mingarelli, R., Scarlato, G., Scambler, P., Dallapiccola, B. (1996) Human homologous sequences to the Drosophila dishevelled polarity gene are deleted in DiGeorge Syndrome. *Am. J. Hum. Genet.* 58, 722–729.

**REFERENCES**