

EXPRESSION OF IQ-MOTIF GENES IN HUMAN CELLS AND ASPM DOMAIN STRUCTURE

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Genes encoding multiple IQ-motif proteins have been identified in the human genome and may be regulated by calmodulin (CaM). Three genes of unknown function, abnormal spindle-like primary microcephaly (ASPM), KIAA0036, and KIAA1023, were expressed strongly in nearly all transformed human cell lines and in a panel of 16 adult human tissues by reverse transcription polymerase chain reaction. However, ASPM gene expression was not detected in adult brain or skeletal muscle. To better understand function, the domain structure of ASPM was examined. Abnormal spindle-like primary (ASP) protein (abnormal spindle) of *Drosophila* spp, an orthologue of ASPM, is involved in mitosis, and mutations lead to abnormal spindles and inhibition of cytokinesis. Studies of ASP have indicated that a microtubule binding region exists on the N-terminal third of the protein. Reiterative searches of the protein database using PSI-BLAST identified a common putative microtubular binding domain of 240 residues designated as MTASP. This nearly "all alpha" domain occurs in >25 related proteins including ASP and ASPM. The major C-terminal region of MTASP is basic with conserved hydrophobic residues and terminates at a flanking actin binding (CH) domain. This region is somewhat similar to other microtubule binding proteins such as MAP1B, MAP2, and tau. Multiple IQ motifs and often a conserved C-terminal domain occur in the remaining sequence. The multidomain structure of ASPM suggests a role in the coordination of cell cycle events. The extensive expression of multiple IQ-motif genes and the absence of ASPM in nondividing adult brain and skeletal muscle also suggest a role in cell division. (*Ethn Dis.* 2005;15 [suppl 5]:S5-88-S5-91)

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INTRODUCTION

Several IQ-motif proteins are associated with spindle formation during cell division.¹ IQ-motif proteins are often regulated by calmodulin (CaM). CaM appears to be localized in the cytoplasm during interphase and associates with the spindle during mitosis.^{2,3} In *Drosophila* spp, abnormal spindle-like primary (ASP), or in mice, spindle hydroxyurea checkpoint abnormal (SHA1), associates with the mitotic spindle and contains multiple IQ motifs.^{4,5} These proteins may be important in the regulation of the mitotic spindle by calcium.

The human orthologue of SHA1 (or ASP, designated as ASPM for abnormal spindle-like primary microcephaly) was identified recently as a major determinant in human cerebral cortical size by positional cloning.⁶ The ASPM gene appears necessary for normal mitotic spindle function and neurogenesis during embryonic growth. Other multiple IQ-motif genes (KIAA0036 and KIAA1023) occur in the human genome, but have only a few IQ motifs compared to ASPM. The cellular function of these proteins is unknown. In this study, we examined the expression of these genes in human cells and tissues as measured by sensitive reverse transcription polymerase chain reaction (RT-PCR) and examined the domain structure of ASPM with focus on the microtubular binding domain.

METHODS

cDNA panels of normalized total RNA were isolated from eight human cell lines: 293, SK-OV-3, Saos-2, A-431, DU 145, H1299, HeLa, and MCF and from two sets of human tissues

(multiple tissue cDNA [MTC] panel 1: heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas and MTC panel 2: spleen, thymus, prostate, testis, ovary, small intestine without mucosal lining, colon with mucosal lining, and peripheral blood leukocytes) were obtained from BD Biosciences (Palo Alto, Calif). An additional cell line, human leukemia THP-1 cells were cultured and harvested in exponential growth phase. Total RNA of the THP-1 cells was isolated by the RNeasy Midi Kit (Qiagen Inc.). For the RT-PCR, the Omniscript reverse transcriptase kit (Qiagen Inc.) was used for first strand cDNA synthesis using oligo dT (27 mer) primer. RT-PCR was performed on the isolated cDNA with specific primer sets: PG1 (5'-AGGGCTGCATCAGTAATACAG3' and 5'-GGAATGCCAAGCGTATC CATC-3'); KIA136 (5'-GGCATG CTGGTAGACTAGGG-3' and 5'-GAAAACAGGGAAATGTCCTTCA-3') and KIA1023 (5'-CACCTCTAC CTGGGGATGAC-3' and 5'-TCTTT CCAGTTCGTGGTTTCT-3'), for the human ASPM (AF509326), KIAA0036 (NM_014642), and KIAA1023 (NM_152558) genes, respectively. The PCR product for ASPM was verified by dye termination cycle sequencing of isolated DNA (Veritas, Inc, Gaithersburg, Md). The T_m of these primers ranged from 58°C to 60°C, and the amplified regions ranged from ≈157 to 983 bp. Touch-down PCR was performed in 1× PCR buffer (PCR Master Mix, Qiagen Inc) in a Mastercycler, Personal (Eppendorf). Human placental and other supplied cDNA were used as tissue controls. The level of glyceraldehyde 3-phosphate dehydrogenase was used as a positive internal tissue control by using specific primers (5'-TGAAGGTCCG GAGTCAACGGATTTGGT-3' and

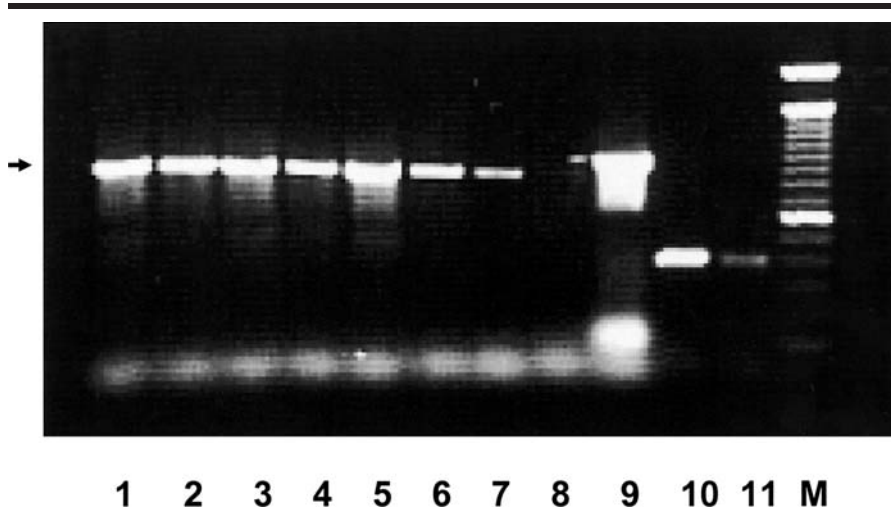


Fig 1. Expression of ASPM gene in human cell lines. Primer set, PG1, and cDNA isolated from human cell lines: 293, SK-OV-3; Saos-2, A-431, DU 145, H1299, HeLa and MCF7, respectively were used to detect ASPM (891 bp (arrow), lanes 1-8) by RT-PCR. Controls include glyceraldehyde 3-P dehydrogenase of human placental tissue (983 bp, lane 9) and rat pseudogene of calmodulin (387 bp, lanes 10-11). The 100 bp markers are in lane M with lower bright marker at 600 bp

5'-CATGTGGGCCATGAGGTCCA
CCAC-3'). The RT-PCR products
were electrophoresed in 1.0% agarose
gels in .001% ethidium bromide in

Tris-acetate-EDTA buffer. Multiple
protein sequence alignments were per-
formed by Higgins and Sharp⁷ and
Schuler et al.⁸

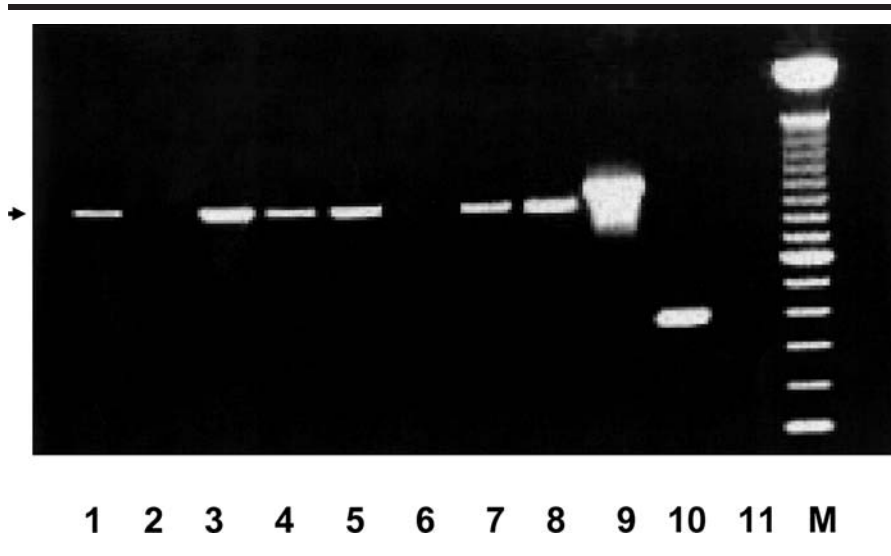


Fig 2. Expression of ASPM gene in human tissues. Human tissues: heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, respectively and primer set PG1 were used to detect ASPM by RT-PCR (891 bp(arrow), lanes 1-8). Controls include glyceraldehyde 3-P dehydrogenase of control cDNA (lane 9), a rat calmodulin pseudogene (387 bp, lane 10) and negative control (lane 11). The 100 bp markers are in lane M with lower bright marker at 600 bp

RESULTS

The three IQ-motif-containing genes ASPM, KIAA0036, and KIAA1023 were widely expressed in adult human cells and tissues. Abnormal spindle-like primary microcephaly (ASPM) was expressed strongly in all cell lines as measured by RT-PCR, but only weakly or negligibly in MCF-7 cells (Figure 1). KIAA0036 and KIAA1023 were expressed robustly across all human cell lines, with weaker expression of KIAA0036 in HeLa and MCF-7 cells; human THP-1 cells had a high expression of all three genes as well (data not shown). In the examined human tissues, both KIAA0036 and KIAA1023 were expressed ubiquitously at moderate-to-high levels. Abnormal spindle-like primary microcephaly (ASPM) was also expressed strongly in all tissues with the exception of skeletal muscle and brain (Figure 2).

To better define the possible function of the IQ-motif genes, bioinformatic analysis of the ASP/ASPM proteins was performed. The other two IQ-motif genes lack defined domains other than the IQ motif and were not examined at this time. Previous binding studies of different expressed regions of the ASP protein revealed the presence of a strong microtubule-binding domain in the N-terminal third of the protein even at high salt concentrations.⁵ We performed sequence analysis studies of this protein family and identified a common putative microtubule binding domain which has been designated as MTASP. This domain is present in the N-terminal region of ASP, ASPM, and related proteins. The only other homologous blocks in the N-terminus was a small region of 80-90 residues near the N-terminus and the calponin/actin binding (CH) domain. TBLASTX searches⁹ of the nonredundant and EST databases revealed orthologues of ASP and ASPM throughout vertebrates that extend to *Ciona intestinalis*, *C. elegans*, and plants. The MTASP do-

main was identified by reiterative searches of the protein nonredundant database by using PSI-BLAST.¹⁰ Because of the presence of several other high homology motifs and domains in ASP, the N-terminal fragment of ASP associated with microtubular binding was used as the initial query for searching the database. The segment (1 to 788) of the N-terminus of the *Drosophila* ASP protein (1861 total residues, accession number, T13845) was used as a query that terminated near the beginning of the CH domain (residues 745–875). The search identified ≈25 related sequences after four rounds of PSI-BLAST searching. The sequences were highly related to the *Drosophila* query sequence with E values 2×10^{-5} or less. When compared to *Drosophila* ASP, the domains from *Aspergillus nidulans* and *C. elegans* were the most divergent, with <19% overall alignment identity, whereas comparisons with vertebrate ASPMs ranged

between 24% and 27%. No orthologues of ASPM or ASP containing the MTASP domain were found in yeast. The closest related protein family in yeast was the IQGAP protein that lacks the MTASP domain, but does contain the CH domain and multiple IQ motifs and additionally a RasGap domain near the C-terminus.

The MTASP domain of the identified proteins is ≈240 residues in length. The ASP sequence was predicted with PHD¹¹ to be nearly all alpha-helix (62%), and most of the remainder consisted of loops with <3% extended conformation. No coiled coil structure or transmembrane domains were predicted. The nine predicted regions of alpha-helix extend throughout the entire MTASP domain and vary in length from 12 to 29 residues. An adjacent CH domain (pfam00307) was identified in all sequences with the exception of ASPM of *Arabidopsis* spp, but the CH domain was present in an *Oryza sativa*

orthologue. The putative MTASP domain of human ASPM extends from residues 659–904 based on comparisons among this family. A summary diagram of the domain architecture of human ASPM compared to orthologues in other species is shown in Figure 3. In human ASPM and its vertebrate orthologues, the C-terminal region of ≈220 residues is highly conserved and is designated the Sha1 C-terminal homology domain or SCH domain of unknown function.

DISCUSSION

In summary, the three multi-IQ-motif proteins examined in this study were expressed widely in adult human tissues and transformed cell lines. The ASPM gene was expressed in nine transformed cell lines and 16 adult tissues but was absent in skeletal muscle and brain. Bond et al reported that in mice, ASPM was widely expressed at embryonic days 11–17 within the cerebral cortical ventricular zone, that expression was greatly reduced at birth, and that by postnatal day 9 expression was limited only to discrete cells scattered within the neocortex.⁶ This finding would support our inability to detect ASPM in adult brain tissue. cDNA was prepared from different regions of adult mouse brain, but ASPM was still not detected by using RT-PCR with mouse-specific primers, whereas control adult spleen and mouse embryonic tissue yielded strong expression. Lüers et al found the orthologue of ASPM to be expressed in adult mouse tissues (testes, ovary, and spleen) by Northern blot analysis.¹² The greater sensitivity of RT-PCR compared to Northern blot analysis may reflect the more widespread detection in this study.

Although the three genes have multiple IQ motifs, they vary widely in their structure and other properties. These proteins may function as scaffolding proteins in the formation of multiple

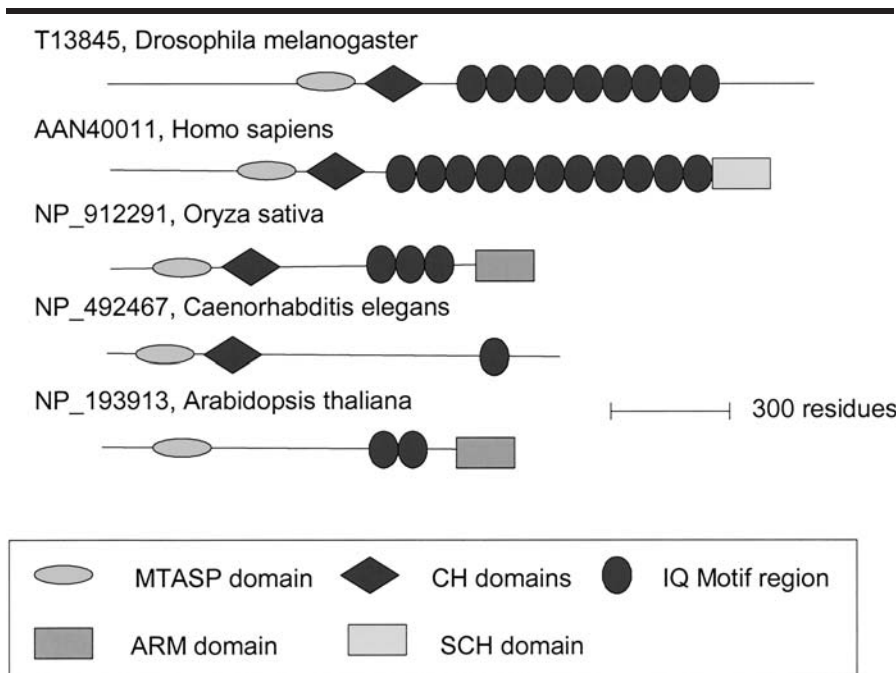


Fig 3. The multidomain architecture of MTASP domain proteins. The protein representations are drawn approximately to scale with the exception of the human ASPM (AAN40011) which is scaled to 50% relative to the others due to its size. In some instances due to their high frequency, the depicted IQ motifs represent multiple motifs and not the exact number. These motifs or domains are listed as a key in the box at the bottom of the figures

protein assemblies that may regulate specific phases of cell division. At least the ASPM gene appears to function in this manner based on previous studies.^{4,6} In addition, the ASPM gene appears especially important in embryonic neurogenesis within the cortex.

This family of ASP and ASPM proteins has a similar domain architecture. Only three highly conserved regions (a small conserved 80- to 90-residue homology, MTASP, and CH domain) exist in the N-terminal portion of ASPM across species. In ASP, the more conserved C-terminal of the MTASP domain is basic, like other microtubular binding regions found in MAP1B, MAP2, and tau. The MTASP domain does not contain any of the characteristic repeated elements or short reiterated motifs found in other microtubule binding proteins.^{13,14} The MTASP appears to be an additional class of binding domain with a distinct microtubular interaction. Charged residues are found throughout the MTASP domain with highly conserved glycine residues in three of the nine helices. A strongly hydrophobic cluster of residues (LLLLVCFL, human ASPM) is found in the last predicted helix near the end of the domain.

The multidomain structure of ASP and ASPM including the putative microtubular binding domain, MTASP of ASP and ASPM and related proteins (Figure 3), appears similar. The MTASP domain terminates near the start of a CH domain. Sequence similarities in a portion of the MTASP domain that includes the CH domain have been identified as a eukaryotic orthologues group (KOG) for four of the seven eukaryotic genomes in the group (see reference KOG0165 in the Conserved Domain Database).¹⁵ The multiple IQ motifs that are often associated with CaM binding and are defined here as [FILVM]QXXXR range in number from ≈ 29 for *Drosophila* spp and 68 for humans with 1–8 in *C. elegans* and plant

sequences. The motifs are distributed with striking periodicity throughout most of the remaining sequence (65% occur every 22–28 residues in human ASPM). The multiple IQ motifs have been identified as KOG0160. The SCH domain is located at the C-terminus in human ASPM. The SCH domain is conserved among vertebrates with 72% protein identity between the human and mouse ASPM compared to an overall identity of 67%. In plant proteins, an ARM domain is located in this region. The ARM and SCH domains may have a similar function of promoting protein-protein interactions. Some variation exists in the composition of the domain architecture of these proteins. All ASP/ASPM-like proteins from different species possess both a MTASP domain and IQ motif(s). The CH domain is present in all identified proteins except the orthologue of *Arabidopsis* spp. The SCH domain was found only in the vertebrate proteins. The ARM domain occurs in plant proteins, but is absent in *D. melanogaster* and *C. elegans*. Thus, the domain structure of ASPM appears similar over considerable evolutionary distances among species especially vertebrates. Understanding the molecular mechanism of microcephaly and mental retardation that arise in fetal alcohol syndrome and developmental disorders will be valuable in minimizing infant mortality and health disparities and optimizing treatment.

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