Research

Characterization of alternatively spliced products and tissue-specific isoforms of USP28 and USP25

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Abstract

Background: The ubiquitin-dependent protein degradation pathway is essential for the proteolysis of intracellular proteins and peptides. Deubiquitinating enzymes constitute a complex protein family involved in a multitude of cellular processes. The ubiquitin-specific proteases (UBP) are a group of enzymes whose predicted function is to reverse the ubiquitinating reaction by removing ubiquitin from a large variety of substrates. We have lately reported the characterization of human USP25, a specific-ubiquitin protease gene at 21q11.2, with a specific pattern of expression in murine fetal brains and adult testis.

Results: Database homology searches at the DNA and protein levels and cDNA library screenings led to the identification of a new UBP member in the human genome, named USP28, at 11q23. This novel gene showed preferential expression in heart and muscle. Moreover, cDNA, expressed sequence tag and RT-PCR analyses provided evidence for alternatively spliced products and tissue-specific isoforms. Concerning function, USP25 overexpression in Down syndrome fetal brains was shown by real-time PCR.

Conclusions: On the basis of the genomic and protein sequence as well as the functional data, USP28 and USP25 establish a new subfamily of deubiquitinating enzymes. Both genes have alternatively spliced exons that could generate protein isoforms with distinct tissue-specific activity. The overexpression of USP25 in Down syndrome fetal brains supports the gene-dosage effects suggested for other UBP members related to aneuploidy syndromes.

Background

Ubiquitin modification of protein substrates has a major role in a variety of cellular processes such as cell-cycle progression, DNA repair, antigen presentation, differentiation and development, transcriptional activation and selective degradation of damaged proteins (for review see [1-3]). Covalent attachment of ubiquitin molecules is the first step for degradation of the tagged protein via the 26S proteasome pathway. This is a finely regulated and highly specific process as an error in substrate recognition might compromise cell survival.

Studies on the specificity and regulation of ubiquitin-dependent protein degradation have mainly focused on the ubiquitinating enzymes. Nonetheless, there is increasing evidence that other enzymes that remove ubiquitin from the
ubiquitin-conjugates (deubiquitinating enzymes, DUBs) not only affect the fate and degradation of intracellular proteins but seem to be essential in the maintenance of cell-free ubiquitin pools [3-5]. Failures of the ubiquitin system have been implicated in many human diseases, among them some important neurodegenerative disorders and several carcinomas. The accumulation of ubiquitin adducts has been described in patients with Alzheimer’s, Huntington’s, and Parkinson’s diseases although the direct involvement of ubiquitinated protein aggregates in the pathological condition has not been proved. On the other hand, a missense mutation in the ubiquitin carboxy-terminal hydrolase L1 (UCHL1) has been found in a German family with Parkinson’s disease [6].

Two classes of deubiquitinating enzymes have been described: the UCHs (ubiquitin carboxy-terminal hydrolases) and the UBP s (ubiquitin processing proteases) [4,7,8]. The UCH family members share a small size, cleave ubiquitin from small peptides and amino acids and appear well conserved (40%) across species. In contrast, the UBP family gathers larger and distantly related enzymes that release ubiquitin from a wide range of ubiquitin-protein conjugates [5]. As the human UBP family is likely to be quite large, a systematic nomenclature has been proposed for these enzymes based on the abbreviation USP, for ubiquitin-specific protease [9].

In the UBP family, protein sequence comparisons have shown that homology is restricted to the regions encompassing the active site cysteine and histidine residues and other peptide segments putatively involved in catalysis or substrate binding [7,10]. More than 90 deubiquitinating enzymes have been characterized after data from genome sequencing projects [5]; as many as 16 are encoded in yeast, which is in agreement with the high specificity attributed to this family [2,3]. Although at present the overall homology among UBPs is low, subfamilies of closely related members (60% to 88% amino acid identities) begin to emerge [11-13].

Lately, we have identified a novel UBP member, named USP25, in the gene-poor region of human chromosome 21q11.2 and characterized the full cDNA sequence [14]. Isolation of the mouse homolog cDNA allowed us to perform northern and in situ hybridization analyses. Higher levels of USP25 expression in mouse were detected in the proliferative compartments of fetal brain and in maturing spermatocytes of adult testis, allowing the correlation of gene expression with high protein turnover.

Here we describe a human USP25 homolog, USP28, which maps to 11q23 and is preferentially expressed in heart and muscle. Tissue-specific alternatively spliced exons of USP25 and USP28 have been identified, in agreement with several isoforms described for vertebrate UBPs [13,15]. The functional deubiquitinating assay has been performed for the two members of the newly described UBP subfamily. Overexpression of USP25 as measured by real-time PCR in Down versus control fetal brains supports the gene-dosage effects reported for other UBP members.

**Results**

**Cloning of USP28**

A TBlastN comparison of USP25 sequence against nr database at the National Center for Biotechnology Information (NCBI) server revealed a homologous USP sequence on PAC clone pDJ356d6 (GenBank accession number AC002036) located at 11q23. Specific probes designed following this genomic sequence were used to screen 10^6 recombinant phages of a human fetal brain cDNA library (Clontech). Six positive clones were isolated, subcloned and sequenced. Four clone sequences were chimeric and were thus discarded. Two overlapping cDNA clones, 5A11 (2.3 kb) and 3A11 (1.5 kb), showed an exact match with chromosome 11 PAC clone pDJ356d6. Evidence that clones 5A11 and 3A11 did not encode the full-length cDNA was based on the protein alignment between the deduced amino acid sequence and USP25, the mRNA size obtained from northern analysis when probed with cDNA clone 5A11, and the absence of the strictly conserved UBP cysteine domain in either clone 5A11 or 3A11.

Three different strategies were followed to isolate the 5’ end of USP25 cDNA. First, a chromosome 11-specific forward primer (5.2 cr11F) was designed from the TBlastN analysis of USP25 (Figure 1). This sequence corresponded to the 5’-terminal sequence of the predicted exon encoding the cysteine domain in the chromosome 11 PAC DNA. A reverse oligonucleotide, 11race4, was also designed on the basis of the 3A11 cDNA clone sequence (Figure 1). An RT-PCR reaction using heart mRNA as template was subsequently performed with the forward and reverse primers. The expected 958 bp amplified fragment was subcloned in pUC18. Its sequence overlapped with 3A11 and 5A11 cDNA clones, thus allowing the characterization of an additional 908 bp. The overall cDNA sequence, although still incomplete, contained the cysteine domain, together with all other UBP reported signatures.

Second, 10^6 recombinant phages from kidney and placenta cDNA libraries (Clontech) were screened using three different 5’-USP28-specific probes obtained after PCR amplification with primers deduced from the TBlastN comparison to USP25 (Figure 1). Five different positive clones were isolated: four from kidney and one from placenta. All inserts were subcloned in pBLUESCRIPT SK+ (Stratagene). Sequence analysis revealed a further 318 bp, although the cDNA was still incomplete.

Third, a 5’ RACE experiment was performed on 10^9 phages from the kidney cDNA library with two specific reverse primers (11race6 and 11race7) (Figure 1) and two λgt10
Figure 1

USP28 nucleotide and amino acid sequences (GenBank accession number AF266283). Nucleotides and residues are numbered from the presumptive first ATG (methionine) codon. Specific primers used for cloning are also indicated. The alternative polyadenylation signal is underlined.

![Diagram](http://genomebiology.com/2001/2/10/research/0043.3)
vector-derived forward primers (GR1, GR2). The compar-
ison with the PAC clone PDJ105h16 sequence (htgs database)
allowed us to identify the first presumptive methionine.
However, as no further in-frame stop codons were detected,
the use of another initiation codon could not be ruled out.
The search for a putative TATA box in the upstream genomic
sequence rendered no positive results.

No cDNA clones with a poly(A)+ tail could be isolated, but
BlastN comparisons against the dbEST database allowed the
identification of an overlapping EST (AI337094) containing
the polyadenylation signal.

**USP28 cDNA (accession number AF266283)** is at least 3,624
bp long and encodes 1,077 amino acids with a relative molec-
ular mass of 122.4 kDa. The gene is organized in 25 exons and
exon-intron boundaries were determined after comparison
with the human htgs database (Table 1). Average exon length
was 130 bp, the shortest being 71 bp and the longest 229 bp.
Nucleotide and amino acid identity percentages between **USP28** and
**USP25** (Figure 2) are 55.77% and 51.36%, respectively.
**USP28** is more similar to **USP25** than to any other known
UBP. Besides, exon-intron boundaries between **USP28** and
**USP25** are highly conserved (Figure 2). The homology
between **USP28** and **USP25** with other UBP family members
appears to be confined to the reported conserved domains.

**Expression analysis of USP28 in human tissues**
Northern analysis for **USP28** was performed using the cDNA
clone 5A11 as probe (Figure 3). A single transcript of approx-
imately 4.5 kb was identified in heart and skeletal muscle
after short film exposure. At longer exposures a transcript of
approximately 4.5 kb was identified in heart and skeletal muscle
and the amplified products were subcloned and sequenced.

**Alternative splicing of USP25 and USP28**
Isoforms generated by alternative splicing have been
described for some vertebrate UBP family members. The expres-
sion analysis of the isolated **USP25** cDNA clones revealed four alterna-
tively spliced exons. Three of those generated an in-frame
stop codon in some human fetal brain cDNA clones and were
located between exons 3 and 4, 10 and 11, and 17 and 18
(exons numbered according to [14]). The fourth alternatively
spliced product corresponded to exon 19 and added 32
amino acids to the protein product (exon 19 has been
renamed 19b in this work). It had been previously identified
in a single I.M.A.G.E consortium neuroepithelium cDNA
clone (id: AA209364 [16]). In order to verify all these puta-
tive exons, specific sets of primers (Table 2) were designed
for RT-PCR amplification on 24 different tissues (Multiple
Tissue cDNA Panels, Clontech).

The additional sequences between exons 3-4 and 17-18 were
not further observed in any tissue tested. However, the
alternative sequence between exons 10 and 11 (named exon
10b) was clearly detected and verified by sequencing in testis
(Figure 4a), where both transcripts (exons 10-11 and 10-10b-
11) had been amplified at similar levels. Exon 10b was also
detected, albeit faintly, in small intestine, spleen and periph-
eral leukocytes. This exon encompasses 124 bp and gener-
ates a stop codon in the open reading frame (ORF) at
nucleotide 1,453. Therefore, this transcript would produce a
361 amino acid truncated protein where the cysteine, but not
the histidine UBP domain, would be preserved.

**RT-PCR experiments performed with specific primers, 321F-
321R (located in exons 17 and 19b, respectively) and N1-121R
(in exons 18 and 20, respectively) (Table 2), allowed us to
visualize three mRNA isoforms. The shortest, already identi-
fied in many expressed sequence tags (ESTs), showed exon
18 directly fused to exon 20 and was detected in all the 24
different tissues tested (Multiple Tissue cDNA Panel, Clon-
tech). The second isoform contained exons 18-19b-20, of
intermediate size and corresponded to the reported USP25
[14]. This isoform was detected in all the assayed tissues
except fetal and adult muscle and heart (Figure 4a). Finally,
the longest isoform was only detected in fetal and adult
muscle and heart mRNAs and included a new exon (named
19a) which was always fused to 19b (18-19a-19b-20) in these
tissues (Figure 4a). Exon 19a contained 114 bp that added 38
amino acids to the reported USP25 protein.

**Primer selection**
Primers 5A11.2 and 5A11.1 from **USP28** (Table 2), located
respectively in exons 16 and 24, were used to verify the homolog of the USP25 muscle and heart isoform (exons 18-
19a-19b-20). RT-PCRs were performed in different tissues
and the amplified products were subcloned and sequenced.

**Alternative splicing of USP25 and USP28**
Two **USP28** sequences were detected in adult and fetal
muscle, heart and brain. The shortest fragment corre-
sponded to the reported **USP28** cDNA containing exon 19b
(Figure 4b). The longest contained a new exon (named 19a)
(Figure 4b), which introduced 62 amino acids to the
protein product (19a-19b-20). RT-PCRs were performed in different tissues
and the amplified products were subcloned and sequenced.

To test the ubiquitin-specific protease activity of **USP28**, an
assay was performed using anti-Ub-M-gal as probe (Figure 5, lanes 5, 6 and 7). The expected, cells with the control pGEX-4T-1 vector failed to
cleave Ub-M-β-gal (lane 4). The endogenous *E. coli* XL1-blue β-galactosidase (lacking the α peptide, and thus producing a shorter protein) was also detected (lanes 2 and 8).

Overexpression of *USP25* in Down syndrome patients
As *USP25* is located on chromosome 21, its overexpression could be presumed in Down syndrome (DS) patients. In order to assess the expression level of *USP25* in DS versus control samples, we analyzed eight independent fetal brain samples (four trisomic and four disomic) by real-time quantitative PCR. An average of 1.7-fold overexpression of *USP25* was shown in trisomic versus disomic samples.

Discussion
Database homology searches with the reported *USP25* [14] have led to the characterization of a new UBP member in the human genome, named *USP28*, which maps at 11q23. Structural comparisons at the genomic and protein levels of *USP25* and *USP28*, and the deubiquitinating enzymatic assays, allowed the definition of a new UBP subfamily.

Sequence alignments of UBPs have been hindered by the few shared conserved segments, which need specific computer programs to be identified. Nonetheless, several UBP subfamilies have been reported so far. DUB1 and DUB2, with 88.4% amino acid identities, is one of those. Similarly, alignments of UBP41, UBP46, UBP52 and UBP66 from chick skeletal muscle suggest a new subfamily. In our case, the alignment of the newly reported *USP28* with *USP25* showed homologies beyond the conserved UBP domains and amino acid identities amounting to 51%. In addition, the extensive alignment at the amino-terminal segment supports the proposed *USP28* translation initiation site.

USP25 and USP28 share the exon-intron distribution and those intron positions with an inaccurate match, located between exons 1-2, 13-14 and 17-18 (Figure 2), could be explained by slippage of the donor or acceptor splice sites. All these data strongly

### Table 1

Genomic organization of *USP28*

| Exon | Acceptor splice site | Donor splice site | Intron length (bp) |
|------|----------------------|-------------------|-------------------|
| 1    |                      | GCTCG/gtgggtccgg  | >14,000           |
| 2    | 58-135               | aaatatagcAGCTA    | TGAAG/gtgggtccc   | 1,635          |
| 3    | 136-268              | tccctacagGCAG     | AGCAA/gttagcaca   | 10,737         |
| 4    | 269-374              | tatctacagAGATT    | AAGCA/gtattgcat   | 905            |
| 5    | 375-534              | caacataagGATGC    | TTCAG/gtargatct   | 6,262          |
| 6    | 535-621              | tgttttacgTCTCT    | ATACA/gtargtgct   | 691            |
| 7    | 622-759              | tttctacagGAAAA    | AGCAG/gtacaaaaa   | 1,426          |
| 8    | 760-833              | tctctatagCAAGA    | GTTAA/gttagtgcc   | 976            |
| 9    | 834-910              | caaaagcagCAGTC    | TGAAG/gttaaagttcc| 1,515          |
| 10   | 911-1,059            | cttgctcagGAAAA    | AAGAG/gtactgagga  | 1,836          |
| 11   | 1,060-1,187          | tgtacactagCGTGG   | GACAG/gttagtgctc  | 3,532          |
| 12   | 1,188-1,283          | attaccttagGTACA   | GAAAG/gttagtgctg  | 5,763          |
| 13   | 1,284-1,463          | tgttgtgataGTATG   | GAAAAG/gtatgaaaaa | 2,282          |
| 14   | 1,464-1,672          | tcaagtaacAGAAA    | ACAAG/gtattgctt   | 1,213          |
| 15   | 1,673-1,743          | ttcacacagATTATA   | GTCAAG/gttagaatgaa| 1,380          |
| 16   | 1,744-1,972          | tgcctacagGTGCC    | TGCAG/gttaaaagat  | 3,021          |
| 17   | 1,973-2,164          | ttccttactagAGGCA  | ACAAG/gtactgagga  | 625            |
| 18   | 2,165-2,304          | ttctttacagAGCCT   | GTGAG/gttagaagga  | 1,713          |
| 19   | 2,305-2,400          | ctttgtgtagGTGAT   | TTAAG/gtacttctgt  | 1,443          |
| 20   | 2,401-2,579          | cctttaatagGCATT   | GAAAG/gttagaagag  | 116            |
| 21   | 2,580-2,658          | tccccacacagATCAA  | ACAAG/gttaaggttt  | 795            |
| 22   | 2,659-2,738          | tctttttacagAAGTG  | GGAAA/gtatttgcc   | 516            |
| 23   | 2,739-2,862          | tttcttacagGTACC   | TTCTG/gttagtctg   | 1,479          |
| 24   | 2,863-3,058          | ttacctgtagGAGCT   | TGCAG/gttagctc    | 2,068          |
| 25   | 3,059                | ccctccacagAAAAT    |                  |                |

The donor and acceptor splice site signals are indicated in bold.
Figure 2
Protein alignment of USP25 and USP28. Amino acid identities are boxed in black and conserved amino acid changes are boxed in gray. The exon-intron boundaries in each gene are marked by arrowheads.
support a common ancestry for USP25 and USP28, and suggest that the minor variations observed may contribute to functional differences.

Northern analysis showed abundant USP28 expression in adult skeletal muscle and heart (Figure 3). The transcript size (4.5 kb approximately) was longer than expected from cDNA analysis, although this variability could be due to distinct polyadenylation and transcription initiation sites, as reported for USP25 and other UBPss [17].

The high specificity attributed to individual members of the ubiquitin proteolytic system and the structural complexity of USP25 and USP28 prompted us to analyze the tissue-specific mRNA isoforms and the cellular localization of the proteins. Alternative splicing had been also reported for other UBP members, such as USP3 [17], USP4 (previously named UnpEL-UnpES [13]), USP5 (previously named ISOT-1/2 [18-20]), USP9X (previously named DFFRX [15]) and USP15 [9]. Some cDNA clones of USP25 and USP28 contained additional exons that introduced in-frame stop codons, similarly to what has been reported for USP15 and USP3. These transcripts most probably originated from splicing errors and were fortuitously cloned, as they did not appear after northern or RT-PCR analyses. Besides, the absence of the conserved domains in the truncated proteins would compromise functionality. Nonetheless, the USP25 RT-PCR assays in testis produced comparable amplification levels of sequences containing either exons 10-10b-11 (10b introduces an in-frame stop codon) or exons 10-11. This argues in favor of a tissue-specific function for the truncated protein, possibly related to substrate availability and/ or enzyme activity.

Exon 19b is present in all USP28 transcripts in all the assayed tissues. In contrast, exon 19b from USP25 is present in all tissues but not in all transcripts, thus constituting an alternatively spliced exon. The high degree of sequence homology of exon 19b from both genes supports its functional relevance. In addition, the tissue specificity shown for exon 19a of USP25 (muscle and heart) and USP28 (muscle, heart and brain) could confer the enzyme a tissue specificity to deubiquitinate a ubiquitous substrate. This would apply to a widely expressed gene whose function was only relevant in some tissues, as suggested for Fam [20]. Alternatively, the tissue-specific exon would bestow on the enzyme the ability to recognize a tissue-specific substrate, as suggested for Faf [20].

The specific subcellular localization reported for some deubiquitinating enzymes may imply spatial restriction of either the locus of action or the accessibility to the substrate but it might also indicate regulation of cellular processes where ubiquitylation plays a role unrelated to protein degradation (for a recent review see [21]). In our case, preliminary subcellular localization experiments with protein fusions to GFP showed that USP25 was cytosolic (data not shown) and did not support an involvement outside the ubiquitin-proteasome pathway.

Although several deubiquitinating enzymes have been shown to contribute to development and differentiation (that is, Faf (Drosophila Fat facets) [15] and UBP43 [22]), the specific function of most family members remains unknown. Homology searches of the domains conserved...
between different UBPs would help to elucidate the function of new members and define the substrate-specific domains. The contribution of USP25 to Down syndrome pathogenesis is still unclear. However, its overexpression (1.7-fold ± 0.13, P < 0.05 according to the Mann-Whitney test) in Down syndrome with respect to control fetal brain samples would support its involvement in the pathology. In fact, several UBPs have shown gene-dosage effects, such as USP9Y (whose gene is located in the Y-chromosome pseudoautosomal region and is involved in male azoospermia [23]) and other USPs related to aneuploidy syndromes: DFFRX in Turner syndrome [15] and USP18 in DiGeorge syndrome [24]. On the other hand, in vitro overexpression or inhibition of some ubiquitin-specific proteases has led to programmed cell death, supporting the idea that their activity is dose dependent [25].

Materials and methods

USP28 cDNA cloning

To screen cDNA libraries from human fetal brain, placenta and kidney (Clontech), specific primers of the USP-like region
on chromosome 11 were designed (Figure 1) after comparison of USP25 cDNA against AC002036, a PAC that contained genomic DNA from chromosome 11. Approximately, 10^6 phages of each library were plated and transferred onto Nyoron membranes (HybondN, Amersham-Pharmacia Biotech).

Probes were labeled by random hexamer priming with [α-32P]dCTP. A solution containing 50% (v/v) formamide, 5x SSC, 5x Denhart’s, 0.1% (w/v) SDS and 100 μg/ml denatured salmon sperm was used to pre-hybridize and hybridize the filters for 2 h and 18 h, respectively. Washes were performed as follows: two washes in 2x SSC/0.1%SDS at 65°C for 10 min and two times in 1x SSC/0.1%SDS at 65°C for 10 min. Filters were exposed during two days with double screen in order to amplify the positive signals. Positive clones were subcloned into pBLUESCRIPT SK+ (Stratagene), sequenced in an ABI377 automatic sequencer (Applied Biosystems). Sequences were analyzed using the BLAST software at the NCBI [26,27].

**Northern blot analysis of USP28**

The cDNA clone 5A11 (from nucleotides 1,473 to 3,618, Figure 1) was used to probe a human multiple tissue northern blot (Clontech). Hybridization and washes were carried out according to the manufacturer’s protocol. A control actin probe was used to assess the amount of RNA poly(A)^+ loaded in each lane.

**Specificity of alternative splicing events**

A set of primers corresponding to exon sequences of USP25 and USP28 (Table 2) (USP25 primers numbered according to [14]) were designed for RT-PCR experiments on 24 samples from different tissues (Multiple Tissue cDNA Panels, Clontech). Amplification was performed in a total volume of 25 μl containing: 2.5 μl of template cDNA, 200 μM dNTPs, 5 pmol of each primer, 1x Taq Platinum buffer, 1.5 mM MgCl2 and 1U Taq Platinum (GIBCO-BRL). After denaturing at 94°C for 3 min, two-step PCR was carried out for 35 cycles at 94°C for 30 sec and 58°C for 30 sec. Final extension was for 5 min at 72°C. Bands differing from the expected size were subcloned in pBLUESCRIPT SK+ (Stratagene) and subsequently sequenced in an ABI377 automatic sequencer (Applied Biosystems). Sequences were analyzed using the BLAST software at the NCBI [26,27].

**Assay for ubiquitin-specific protease activity**

The ubiquitin-specific protease activity of USP25, USP25-specific heart and muscle isoform, which contains the additional exon 19a (USP25 isoform), and USP28 was determined as described [10,11]. The three corresponding cDNAs were cloned in-frame in pGEX-4T-1 (Amersham Pharmacia Biotech) downstream from the glutathione-S-transferase (GST) coding region. Plasmid pACYC184 Cm' expressing UbMet-β-gal protein fusion (pAC-M-β-gal) was kindly provided by M. Hoschtrasser. E. coli XL1blue bacteria were co-transformed with pAC-M-β-gal and either pGEX-4T-1-USP25 or pGEX-4T-1-USP28 or pGEX-4T-1-USP28. Amp' and Cm' colonies were grown and induced for 3 h with isopropyl-β-thiogalactopyranoside (final concentration 1 mM). Total protein extracts were analyzed by western blotting with anti-β-galactosidase rabbit polyclonal antibody (Cappel).

**Overexpression of USP25 in Down syndrome patients**

Total RNA from 5 mg of fetal brains of Down and control samples (three samples each) were obtained using ABI PRISM 6700 Automated Nucleic Acid workstation. RT-PCRs were produced according to Taqman Reverse transcription reagents (Roche Molecular Systems). Quantitative PCRs were performed using the Universal Master Mix (Applied Biosystems) following the manufacturer’s specifications. The real-time amplification was analyzed by the ABI Prism 7700 Sequence detection system. USP25 primers and the Taqman probe (using FAM as reporter and TAMRA as quencher) were designed according to the Primer Express software. Forward primer: 5'-GATGAAAGGTGTCAACAATGAAA-3'; reverse primer: 5’-CCACTCTCATATTCCTCAAGTTT-3'; TaqMan probe: 5’-TCAAGCCAAACTGGAAATGATAAAACCT-GAAGA-3'. To normalize the USP25 quantitative determinations, GAPDH (Applied Biosystems) was used as endogenous control. The standard deviation of the disomic and trisomic samples was 0.08 and 0.13, respectively. The overexpression (1.7 fold) of USP25 in trisomic fetal brains shows statistical significance according to the Mann-Whitney test (P < 0.05).

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