

Research article

# Identification and Characterisation of the Murine Homologue of the Gene Responsible for Cystinosis, *Ctns*

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## Abstract

**Background:** Cystinosis is an autosomal recessive disorder characterised by an intralysosomal accumulation of cystine, and affected individuals progress to end-stage renal failure before the age of ten. The causative gene, *CTNS*, was cloned in 1998 and the encoded protein, cystinosin, was predicted to be a lysosomal membrane protein.

**Results:** We have cloned the murine homologue of *CTNS*, *Ctns*, and the encoded amino acid sequence is 92.6% similar to cystinosin. We localised *Ctns* to mouse chromosome 11 in a region syntenic to human chromosome 17 containing *CTNS*. *Ctns* is widely expressed in all tissues tested with the exception of skeletal muscle, in contrast to *CTNS*.

**Conclusions:** We have isolated, characterised and localised *Ctns*, the murine homologue of *CTNS* underlying cystinosis. Furthermore, our work has brought to light the existence of a differential pattern of expression between the human and murine homologues, providing critical information for the generation of a mouse model for cystinosis.

## Background

Cystinosis is an autosomal recessive disorder characterised by an intralysosomal accumulation of cystine. The underlying metabolic defect is a defective transport of cystine across the lysosomal membrane [1, 2]. Cystine is poorly soluble and forms crystals in the lysosomes as its concentration increases. Affected individuals develop a de Toni-Debré-Fanconi syndrome around six-eight months and suffer a progressive decline of glomerular filtration rate with end-stage renal failure occurring before the age of ten [3]. Other clinical signs, such as severe growth retardation, ocular anomalies, diabetes, portal hypertension, hypothyroidism as well as muscular and neurological deterioration, appear due to the accumulation of cystine in different organs [2, 4]. Two other less

severe forms (juvenile and ocular nonnephropathic) have also been described and shown to be allelic by complementation studies [5]. To date, the most effective treatment of cystinosis is by the drug cysteamine which reduces intracellular levels of cystine [6, 7]. However, this treatment needs to be installed early on in the disease and at high doses, in order to be effective, and has a number of undesirable side effects.

The cystinosis gene was mapped to a 1 cM interval on the short arm of chromosome 17 between the markers D17S1798 and D17S1828 [8]. The identification of a 57 kb [9] deletion, encompassing the marker D17S829 in 30% of affected individuals, allowed us to delineate the gene interval to within the boundaries of this deletion [10]. Via a combination of large-scale sequencing and

exon-trapping techniques, a novel gene was identified in the minimum deletion interval. The detection of various mutations within the coding region of this gene, named *CTNS*, validated it as responsible for all three forms of cystinosis [11, 12, 13]. *CTNS* is composed of 12 exons and the predicted translation start site is situated in exon 3. The 2.7 kb *CTNS* transcript contains a 1101 bp open reading frame (ORF) predicted to encode a protein of 367 amino acids, named cystinosin. Computer modelling using several hydrophobicity algorithms predicts that cystinosin is an integral membrane-spanning protein with seven transmembrane domains preceded by seven potential N-glycosylation sites and an uncleavable signal peptide at the amino-terminal end, and followed by a lysosomal targeting signal (GY-DQ-L) at the carboxy-terminal end [10]. Taken together, these results suggest that cystinosin is a lysosomal membrane protein. However, as yet, the exact role of cystinosin in cystine transport is unknown and no animal model for cystinosis exists. Thus, as a first step towards the creation of such a model, we report here the isolation and characterisation of the murine homologue of *CTNS*.

## Results and Discussion

In order to identify the murine homologue of *CTNS*, *Ctns*, a E11.5 mouse embryonic cDNA library (Clontech) was screened with a probe spanning the entire ORF of the human *CTNS* gene. Three overlapping cDNA clones were isolated and completely sequenced. The reconstructed sequence was homologous to a region of the human *CTNS* cDNA from base pair 565 to 2388. RACE (rapid amplification of cDNA ends) PCR of an E13.5 adaptor ligated-cDNA library (kindly provided by D. Weil, Institut Pasteur, Paris, France) was employed to isolate the remainder of the 5' and 3' murine cDNA sequences using the *Ctns* cDNA primers 1.1 (5'-ACC AAG AAC CGG ATC CTG GGG CA-3') and 2.1 (5'-GCC CTC TTT CCT ACC TCC ACT TTC TGA-3'), coupled with the adaptor primer AP1, and the nested primers 1.2 (5'-GCA TTT TTC TCT CCG CGA GGC AC-3') and 2.2 (5'-GTC AAT CAG TAA GCT GCC CTG GAT G-3'), coupled with AP2, for the amplification of the 5' and 3' ends, respectively (Marathon-Ready cDNA kit, Clontech).

The resulting consensus *Ctns* cDNA sequence is 2464 bp long (GenBank accession number-AJ250670) with a 19 bp 5' non-coding region, a 1344 bp 3' non-coding region and a 1101 bp ORF which, like *CTNS*, is predicted to encode a 367 aa protein. The *Ctns* translation start site, which likewise lies within a Kozak consensus sequence (GAGACCAatgA), and the polyadenylation site are predicted to be equivalent to those deduced for *CTNS*. All of the 5' cDNA sequences amplified by RACE-PCR began at the same position, i.e. with a sequence homologous to the beginning of the *CTNS* exon 3. No sequences homologous to the *CTNS* untranslated exons 1 and 2 were de-

tected. Moreover, two EST clones (GenBank accession numbers gi9165096 and gi6083297) containing 69 and 126 bp of upstream sequence, respectively, preceding the beginning of the 5' non coding region that we identified, also do not show homology with the *CTNS* exons 1 and 2. At the nucleotide level, *Ctns* shows 83% homology with *CTNS* for the ORF and 58.8% homology over 1257 bp for the 3' untranslated region. The predicted encoded amino acid sequence shows 83.9% identity and 92.6% similarity with cystinosin (Figure 1A). The uncleavable signal peptide, seven amino-terminal N-glycosylation sites, seven transmembrane domains and lysosomal targeting signal of cystinosin are conserved in the murine sequence.

In order to determine the exon/intron structure of *Ctns*, the 129/Sv Ev Tac flor DNA genomic library (Stratagene) was screened with a cDNA probe homologous to the *CTNS* exons 7 to 12 and four clones were isolated. Sequence analysis of their inserts demonstrated that the four overlapping clones spanned all the *Ctns* exons. The *Ctns* gene spans 14 kb is composed of ten exons and its exon/intron boundaries are identical to that of *CTNS* (Figure 1B). A combination of Long Range PCR (Expand Long Template PCR System, Boehringer Mannheim) and sequencing permitted an estimation of intron sizes. A comparison of the sizes of *CTNS* and *Ctns* introns showed that, with the exception of the human introns 3 and 5, intron sizes were similar between both species (Figure 1B).

The *Ctns* expression pattern was determined by hybridisation of a probe spanning exons 4 and 5 to a Clontech multiple tissue northern blot (MTN 7762-1). A transcript with an expected size of 2.5 kb, highly expressed in kidney and liver, less expressed in heart, and weakly expressed in brain, spleen, lung and testis was detected (Figure 2); a proportionally faint approximately 4.4 kb transcript could also be seen, reminiscent of the additional 4.4 kb *CTNS* transcript detected in human pancreas [10]. In contrast to the *CTNS* transcript, no expression was detected in skeletal muscle (Fig. 2A) whereas hybridisation with a  $\beta$ -actin cDNA control probe confirms the loading of RNA for all tissues (Fig. 2B). A possible explanation for this differential pattern of expression is that the promoter situated upstream of the *CTNS* exon 1 has been lost during the course of evolution and the working murine promoter does not direct the same tissue expression, as has been proposed for *IGF II* [14]. Similarly, it is possible that sequences corresponding to the *CTNS* exons 1 and 2 have also been lost during evolution or, alternatively, they exist in the form of pseudo-exons in the upstream genomic sequence. However, low stringency hybridisation of the genomic clones containing the *Ctns* gene with a probe corresponding to the *CTNS* exons 1 and 2, did not detect any homologous se-

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Human cystinosin MIRNWLTIFILFPLKLVEKCESSVSLTVPPVVKLENGSSTNVSLTLRPPINATLVITFEI
Mouse cystinosin MRRNWLLILTLFLLMFIEKYESTVSLTAPPTVKLENGSSTNVDTLGHPISTLVITFEV
* **** *: ** * ::** **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*
Human cystinosin TFRSKNITILELPDEVVPPGVTTSSSQVTSQNVGQLTVYLHCNHSNQTGPRIRFLVIRS
Mouse cystinosin TFRSKNLTIVELPDEVIVPRGKVASFQVTSQNIQVTVFLHCNHSNQTCPRIIRFLVIHS
*****:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*
                tm1                                tm2

Human cystinosin SAISIINQVIGWIYFVAWSISFYPPQVIMNWRKRSVIGLSFDFVALNLTGFVAVSVFNIGL
Mouse cystinosin RIVSIINQVIGWIYFMAWSVSFYPPQVIQNWRKRSVIGLSFDFLALNLTGFVAVSVFNIGL
:*****:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*
                tm3

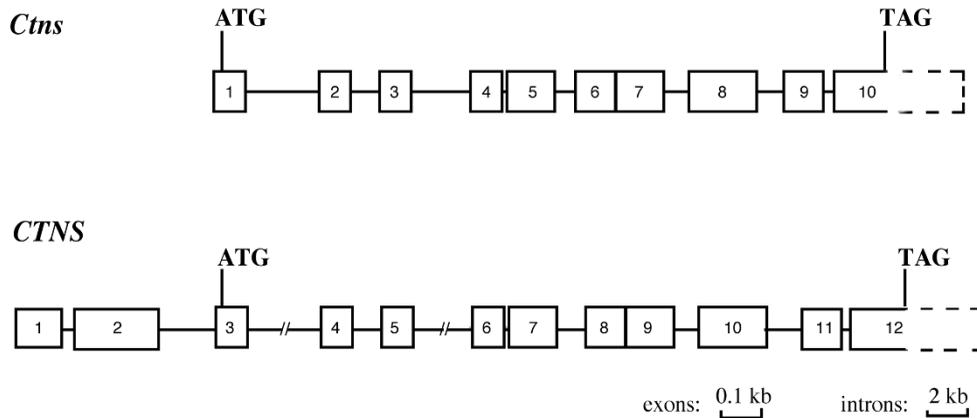
Human cystinosin LWVPYIKEQFLLKYPNGVNPVNSNDVFFSLHAVVLTLLIIVQCCLYERGGQRVSWPAIGF
Mouse cystinosin LWVPYIQEEFLLKYPNGVNPVDSNDAFFSLHAVALTLIVILQCCLYERGNQRVSWPSIGF
*****:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*
                tm4                                tm5

Human cystinosin LVLAWLFAFVTMIVAAGVITWLQFLFCFSYIKLAVTLVKYFPQAYMNFYKSTEGWSIG
Mouse cystinosin LVLAWLFLVVTMIVAAGVITWLQFLFCFSYIKLIIITLIKYFPQAYMNFYKSTKGWSIG
*****:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*
                tm6                                tm7

Human cystinosin NVLLDFTGGSFSLQMFQSYNNDQWTLIFGDPKFKGLGVFSIVFDVVFVFIQHFCLYRKR
Mouse cystinosin GVLLDFTGGSFSLQMFQSYNNDQWTLIFGDPKFKGLGVFTIFFDVVFVFIQHFYLYRKK
.*****:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:* **:*

Human cystinosin PGYDQLN
Mouse cystinosin PGYDQLN
*****
    
```

**A**

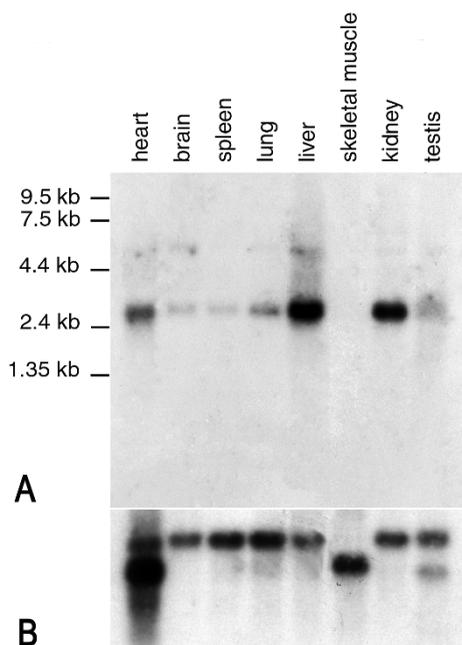


**B**

**Figure 1**

Comparison of the human and murine *CTNS* genes and encoded products. **(A)** Alignment of the human and murine cystinosin sequences. Asterisks indicate identical amino acid residues. Colons indicate residues of high similarity and fullstops residues of low similarity. The uncleavable signal peptide is boxed, the N-glycosylation sites circled, the transmembrane domains overlined and the lysosomal targeting signal is in bold. **(B)** Schematic representation of the gene structure of *CTNS* and *Ctns*. The exons are indicated by solid boxes, the 3' non coding regions by a dashed box and the introns by a solid line. *Ctns* exon sizes are identical to those reported for *CTNS* [9]. Intron sizes are as follows: i) *Ctns* intron 1-3.5 kb; 2-1.5; 3-3; 4-0.127; 5-0.8; 6-0.095; 7-1.1; 8-1.15 and 9-0.35. ii) *CTNS* intron 1-0.257 kb; 2-2.8; 3-8; 4-1.54; 5-6; 6-0.115; 7-1.2; 8-0.085; 9-1.2; 10-1.683 and 11-0.26.

quences thus providing support for the former hypothesis. Moreover, comparison of 1.1 kb of genomic sequence immediately upstream of the murine exon 1, which is hypothesised to contain the *Ctns* promoter, with the *CTNS* intron 2 sequence detected a region of significant homology (66.7% over 306 bp). These results suggest the possible presence of an alternative *CTNS* promoter. Such regions of homology between the regulatory sequences of other human and murine homologue, such as *WT1* or *PAX-2*, have also been described [15, 16].



**Figure 2**  
Northern blot analysis of *Ctns* expression. (A) A 2.5 kb transcript, accompanied by a faint 4.4 kb transcript, can be seen in all tissues except skeletal muscle upon hybridisation with a *Ctns* specific probe. (B) Following hybridisation with a  $\beta$ -actin cDNA control probe, a 2.0 kb transcript can be seen for brain, spleen, lung, liver and kidney, a 2.0 kb and a 1.8 kb transcript can be seen for heart and testis, and a 1.8 kb transcript can be seen for skeletal muscle.

The chromosomal localisation of the *Ctns* gene was determined by PCR amplification of the mouse T31 irradiation hybrid panel [17] with the primers LOC/1 (5'-TGT GGC CCA TGG ACT TGA AG-3') and LOC/2 (5'-CAA TCT GGC AGG CAC CTC A-3') situated in the 3' non-coding region. Two point lod score analysis resulted in a significant score of 5.37 and 5.36 with the chromosome 11 markers D11Mit279 and D11Mit7, respectively. A highly

significant score of 12.25 and 11.09 was obtained with the ESTs AA589579 and R74628, respectively. R74628 maps distal to D11Mit7 and both of these markers are located within two contigs which lie in the region of synteny with human chromosome 17. Hence, this leads us to assume that *Ctns* also lies in the region of chromosome 11 syntenic with chromosome 17 which contains the human *CTNS* gene.

## Conclusions

Although, the encoded products of *CTNS*, the gene underlying nephropathic cystinosis, and its murine homologue, *Ctns*, are highly conserved between man and mouse, these genes have a differential expression pattern. This information is crucial for the analysis of a mouse model for cystinosis which, in the absence of a spontaneous mouse mutant, we are currently generating in order to study the pathogenesis of cystinosis *in vivo*.

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