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

ABSTRACT

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.

INTRODUCTION

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. 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 occuring before the age of ten. 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. Two other less severe forms (juvenile and ocular nonnephropathic) have also been described and shown to be allelic by complementation studies. To date, the most effective treatment of cystinosis is by the drug cysteamine which reduces intracellular levels of cystine. 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. The identification of a 57 kb deletion, encompassing the marker D17S829 in 30% of affected individuals, allowed us to delineate the gene interval to within the boundaries of this deletion. Via a combinaton 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. 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-teminal end, and followed by a lysosomal targetting signal (GY-DQ-L) at the carboxy-terminal end. 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.

CONCLUSION

Conclusions Although, the encoded products of CTNS, the gene underlying nephropathic cystinosis, and its murine homologue, Ctns, are highly conserved between man and mouse, they 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.