A G339R mutation in the *CTNS* gene is a common cause of nephropathic cystinosis in the south western Ontario Amish Mennonite population

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EDITOR—Nephropathic cystinosis (MIM 219800) is a rare autosomal recessively inherited lysosomal storage disorder with a newborn incidence of about 1 in 100 000-200 000 in the general population (OMIM). Cystine accumulates in lysosomes because of dysfunctional cystinosin mediated transport of cystine out of lysosomes. The accumulation of cystine results in damage to several organs with renal damage being the most pronounced in the first decade of life. Patients with cystinosis experience both tubular dysfunction (renal Fanconi syndrome) and glomerular deterioration. Renal Fanconi syndrome usually occurs within the first year of life with glomerular deterioration progressing throughout the first decade of life resulting in end stage renal failure.[1]

The *CTNS* gene was mapped to chromosome 17p13 and subsequently isolated and characterised to have 12 exons spanning 23 kb of genomic DNA.[2 3] The most common mutation that causes cystinosis is a large deletion that encompasses exons 1-10.[4] Originally, this deletion was described as 65 kb long but the size has been recently refined to 57 257 bases.[5] Forty four percent of 108 American based patients with nephropathic cystinosis were homozygous for this deletion.[6]

At least seven children in the Old Order Amish population in south western Ontario, Canada have been diagnosed with nephropathic cystinosis. This population is a culturally isolated population founded in 1824 by emigrants from Bavaria and Alsace-Lorraine. Further immigration occurred from the same regions and more recently from the United States.

Within this Amish community, we have recently diagnosed four children with cystinosis, two sibs in two families not known to be related. In each family the parents are consanguineous. The proband presented at 14 months of age with protracted vomiting and dehydration, polyuria, polydipsia, and failure to thrive. On initial investigations he had a metabolic acidosis, hypophosphataemia, hypokalaemia, and glucosuria. Slit lamp examination of his eyes showed corneal crystals. Urine amino acid analysis indicated a generalised amino aciduria. At presentation he had no evidence of renal insufficiency. A leucocyte cystine level done before the initiation of treatment was 1.99 nmol ½ cystine/mg protein. Patients with untreated cystinosis usually have greater than 2.0 nmol ½ cystine/mg protein (Dr J A Schneider, San Diego). His younger sister was diagnosed at 8 months when she presented with a similar clinical history and a leucocyte cystine of 1.19 nmol ½ cystine/mg protein before the initiation of treatment. Leucocyte cystine concentrations were measured at the Cystine Determination Laboratory, UCSD, La Jolla, CA using the cystine binding protein assay.

DNA was isolated from blood specimens that were obtained after receiving consent from the parents. Mutations were identified in the *CTNS* gene by PCR amplification and direct sequencing (ABI PRISM Model 377 sequencer) of exons 3-10 and PCR amplification for detection of the 57 257 base deletion using flanking primers as listed in table 1.

A mutation, 1354 G→A, was identified in exon 12. This mutation results in the loss of an *Ava*I restriction site. The proband was homozygous for the 1354 G→A mutation as shown in fig 1 and no other mutations were identified. This mutation results in a glycine 339 to arginine amino acid change in a transmembrane region of cystinosin. All four cystinosis patients from the two families were homozygous for this mutation and an unaffected sister was heterozygous.

The G339R mutation has been previously identified in one allele in a compound heterozygous patient of Italian ancestry.[6] Further evidence that this mutation is pathogenetic is that glycine 339 is an amino acid which is conserved between *C elegans* and humans in cystinosin.[6] In our patients, homozygosity for the G339R mutation seems to be associated

Table 1 Primers for amplifying CTNS exons and testing for the 65 kb deletion

Exon	*Direction*	*Sequence*
3	Forward	5'- CAG ATT GTC TAC AGG GAG CT -3'
	Reverse	5'- CTT GGC AAC AAA CAG ATC AG -3'
4	Forward	5'- CTG ACC CAG TGC CTC ATG TC -3'
	Reverse	5'- GAG CTG AGC ACA GCG CCA -3'
5	Forward	5'- TCC AGC TTC TCA GCA GTA AT -3'
	Reverse	5'- ACC TAG CAT TTC CCT ACC C -3'
6	Forward	5'- GCG GGG TCC TCG GTA ACT G -3'
	Reverse	5'- CAG CAC GGC CCC CTT CT -3'
7	Forward	5'- AGT CTC CTT CAG AAG CCC AG -3'
	Reverse	5'- GGC AGA CAG AAG GGT AGA GG -3'
8	Forward	5'- CCC TGC CCT GTC TTG TCC -3'
	Reverse	5'- CAG AGA TGT AGG GCA GGC AA -3'
9	Forward	5'- CAT CTC TGC CCA CAT GGC GT -3'
	Reverse	5'- GCT CTG CCG TGT CTT CTG TC -3'
10	Forward	5'- GGC CTC TGT GTG GGT CC -3'
	Reverse	5'- GGC CAT GTA GCT CTC ACC TC -3'
11	Forward	5'- GCC CTC CGT CTG TCT GTC CG -3'
	Reverse	5'- GCC CGA TGC CCC AGC -3'
12	Forward	5'- TCG GAG ACC CAA CCA AGT TT -3'
	Reverse	5'- TGG CCC CAG GAG CAG AGT GG -3'
LDM-1*	Forward	5'- CCG GAG TCT ACA GGG CAC AG -3'
	Reverse	5'- GGC CAT GTA GCT CTC ACC TC -3'
D17S829*	Forward	5'- CTA GGG GAG CTG GTT AGC AT -3'
	Reverse	5'- TGT AAG ACT GAG GCT GGA GC -3'

*Sequences from Anikster *et al*.[4]

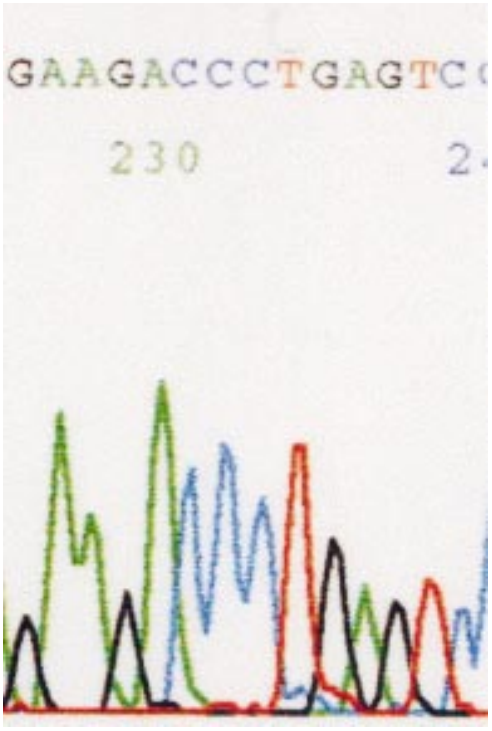

Figure 1 Part of the DNA sequence of exon 12 showing that the patient is homozygous for the 1354 G→A mutation. The sequence is in the reverse direction with the normal sequence across this region being GAAGACCCCGAGTC.

with a relatively low concentration of leucocyte cystine.

Germany is likely to be the country of origin for the common 57 257 base deletion in the *CTNS* gene.[5] The Amish Mennonite population originated in Germany but appears to have the G339R mutation exclusively rather than the 57 257 base deletion. This may reflect a founder effect but there are no data to indicate from whom or when the founder allele originated. Cystinosis does not appear to be present in the Amish population of Pennsylvania, suggesting that the mutation may have originated in a founder who emigrated to south western Ontario directly from Europe. A study of other populations that are related to the population from which this Amish community is derived would be helpful in this regard.

There are no data on the incidence of cystinosis or the prevalence of the G339R allele in the south western Ontario Amish Mennonite community. Our awareness of seven cases in the past 10 years suggests an incidence far greater than that of the general population.

There is evidence that the earlier that cysteamine therapy is started the less cystine accumulates in tissues. Markello *et al*[7] showed that the treatment of children with cystinosis with cysteamine before the onset of end stage renal disease resulted in a delay in the need for renal replacement therapy when compared to children not treated or not compliant with therapy. Early therapy has also been shown to prevent hypothyroidism[8] and the accumulation of cystine in muscle.[9]

If this Amish Mennonite community wishes, the determination of the frequency of the G339R allele within the population using the *Ava*I restriction site would enable the prediction of the population incidence of cystinosis. This incidence may be high enough to justify targeted newborn screening and early institution of management.

Electronic database information: Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for cystinosis (MIM 219800)).

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1 Gahl WA, Schneider JA, Aula P. Lysosomal transport disorders: cystinosis and sialic acid storage disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited diseases*. 7th ed. New York: McGraw Hill, 1995:3763-97.
2 Cystinosis Collaborative Research Group. Linkage of the gene for cystinosis to markers on the short arm of chromosome 17. *Nat Genet* 1995;**10**:246-8.
3 Town M, Jean G, Cherqui S, Attard M, Forestier L, Whitmore SA, Callen DF, Gribouval O, Broyer M, Bates GP, van't Hoff W, Antignac C. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat Genet* 1998;**18**:319-24.
4 Anikster Y, Lucero C, Touchman J, Huizing M, McDowell G, Shotelersuk V, Green ED, Gahl WA. Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (*CTNS*). *Mol Genet Metab* 1998;**66**:111-16.
5 Touchman JW, McDowell G, Shotelersuk V, Bouffard GG, Beckstrom-Sternberg SM, Anikster Y, Dietrich NL, Maduro Gahl WA, Green ED. The genomic region encompassing the nephropathic cystinosis gene (CTNS): Complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. *Genome Res* 2000;**10**:165-73.
6 Shotelersuk V, Larson D, Anikster Y, McDowell G, Lemons R, Bernardini I, Guo J, Thoene J, Gahl WA. CTNS mutations in an American-based population of cystinosis patients. *Am J Hum Genet* 1998;**63**:1352-62.
7 Markello TC, Bernardini IM, Gahl WA. Improved renal function in children with cystinosis treated with cysteamine. *N Engl J Med* 1993;**328**:1157-62.
8 Kimonis VE, Troendle J, Rose SR, Yang ML, Markello TC, Gahl WA. Effects of early cysteamine therapy on thyroid function and growth in nephropathic cystinosis. *J Clin Endocrinol Metab* 1995;**80**:3257-61.
9 Gahl WA, Charnas L, Markello TC, Bernardini I, Ishak KG, Dalakas MC. Parenchymal organ depletion with long-term cysteamine therapy. *Biochem Med Metab Biol* 1992;**48**:275-85.