Glycogen storage disease: report of 17 cases from southern India

Abraham Koshy,* Kannan Ramaswamy,* Marjorie Correa,** Swarna Rekha***

Departments of *Gastroenterology, **Pathology, ***Pediatrics and #Molecular Biology Unit, Institute of Population Health and Clinical Research, St. John’s Medical College Hospital, Bangalore 560 034

Background: There are only four reports of glycogen storage disease (GSD), totalling six cases, from India. **Objective:** To determine the clinical phenotypes of children diagnosed with GSD in southern India. **Methods:** Liver biopsy reports from 1994 to 2005 were reviewed and GSD was confirmed in 17 patients. All 17 patients were tested for the three commonest GSD 1a mutations by restriction fragment length polymorphism: R83C, Q347X and G727T. **Results:** They presented at mean age of 15 months (range, birth to 46 months) with hypoglycemia, hepatomegaly and delayed milestones. None of the patients showed R83C, Q347X or G727T mutation. **Conclusion:** Glycogen storage disease may not be rare in India. The commonest 1a mutations are probably rare here. [Indian J Gastroenterol 2006;25:182-184]

Glycogen storage disease (GSD) is caused by inherited defects in one of the enzymes involved in the synthesis or degradation of glycogen. Liver and muscle, with abundant glycogen, are the most commonly and seriously affected. Eight types of GSD are known. Symptomatic liver involvement is most common in types I and III, both of which are characterized by hypoglycemia and hepatomegaly.

The overall frequency of all forms of GSD in Europe, Japan and USA is 1 in 20,000-25,000 live births. However, a literature search revealed only four reports from India, totalling six patients.

**Methods**

To determine the clinical phenotypes of children with GSD, we reviewed the reports of 632 liver biopsies done in patients aged below 19 years for various indications during the years 1994 to 2005. Case records of 30 patients with a probable diagnosis of GSD on liver biopsy were identified. Those patients who had not attended the out-patient clinic since December 2001 were invited for a follow-up visit, during which recording of a detailed history, clinical examination, and tests for blood sugar, liver function tests, lipid profile and urinary ketones were done.

Thirteen cases were excluded – 4 had only fatty liver, one each had gangliosidosis and giant cell hepatitis, 3 had inadequate liver tissue for evaluation, and 4 with inadequate clinical data were not available for follow-up. Thus, 17 children (9 boys) were diagnosed as having GSD based on liver histology with compatible clinical and biochemical features. In none had liver tissue enzyme or glycogen levels been estimated. Fatty change, nuclear hyperglycogenation and fibrosis were graded on liver biopsy.

Guidelines of the institute Ethics Committee for the protection of human subjects were followed. Consent was obtained from each patient’s parent after full explanation of the purpose, nature and risks of all procedures used.

**Mutation analysis**

The three commonest GSD 1a mutations and their frequency worldwide are R83C mutation in exon 2 (33%), Q347X mutation in exon 5 (14%), and G727T splice site mutation in exon 5 (11%). All 17 GSD patients were tested for these mutations using restriction fragment length polymorphism (RFLP). In brief, DNA (100 ng) extracted from blood using a QIAamp DNA Midi Blood kit (Qiagen, Hilden, Germany) was amplified in 100 µL volume containing 40 pmol of each specific primer, 2 U of Taq polymerase and 1.5 mmol/L MgCl₂ in a thermal cycler.

For R83C mutation, DNA corresponding to a part of GSD gene exon 1 was amplified using PCR (primers: 5’-GCATTGCATTCAATGCC and 5’-TCCACTCAGCTTCTGGTCT with 35 cycles of 94°-60°-72°C for 30 s each). The 192-bp amplicon (10 µL) was digested using 0.4 U Taa I, and run on 1.5% agarose; the R83C mutation yields two fragments (133 bp and 59 bp) whereas the wild type DNA, which lacks the restriction site, yields only one (192-bp) band.

For detection of Q347X mutation, a 647-bp fragment corresponding to GSD exon 5 was amplified using specific primers (5’-CTTCCTATCTCTACAGTCA and 5’-TCACTCTGCTCCAATACC) and 35 amplification cycles (94°-58°-72°C for 1 min each). The amplicon (10 µL) was digested with 0.4 U XmaJ1 and run on
Detection of G727T gene mutation involved PCR of a part of exon 5 of GSD gene using one usual primer (5’-TGCTTTCTTCACCTCAGGCA) and one mutagenic primer 5’-AATCCGATGCGAAGCTGTA); in the latter, the nucleotide followed by an asterisk is not present in the wild type. The 123-bp amplicon (10 µL) was digested with 10 U Rsa1. The digested fragments were separated on 7.5% PAGE. The wild type yields two fragments (104 bp and 19 bp) unlike the G727T mutation, which lacks the restriction site and therefore generates only a 123-bp fragment. Complete digestion using the restriction enzyme protocol was verified using DNA fragments known to have the restriction sites. Positive controls from patients known to have these mutations were not used.

Results

The median age at onset was 15 (range 0-46) months (Table). Seven children were born of second-degree consanguineous marriages. None of the patients had another affected family member. The major manifestations were: symptoms of hypoglycemia (n=9; convulsions, eye rolling, unconsciousness or hypotonia), hepatomegaly (8), delayed milestones (4) and splenomegaly (1). Thirteen (76%) children had growth failure (height <5th centile).

The laboratory abnormalities detected were hypoglycemia (blood sugar <50 mg/dL) in 8 children, increased serum cholesterol (>200 mg/dL) and triglycerides (>200 mg/dL) in six, and ketonuria in two. Liver biopsy (Fig 1) showed mosaic pattern with abundant glycogen deposition, which showed diastase-sensitive periodic acid-Schiff staining. One child had cirrhosis and two others had moderate fibrosis. The Table shows the degree of fatty change, nuclear hyperglycogenation and fibrosis on biopsy.

None of the patients showed R83C, Q347X or G727T mutation. Typical gels of R83C and G727T mutation RFLP are shown in Fig 2.
None of our patients showed the common mutations described in GSD type 1a.

Proper and early diagnosis of GSD is essential as dietary treatment with raw cornstarch and frequent high-starch feeds can prevent progression of the disease.9 Therefore, there is a need for facilities for enzyme estimation of liver tissue and sequencing of GSD genes in order to better understand the frequency and nature of the disease in India.

References

Correspondence to: Professor Koshy, Department of Gastroenterology. Fax: (80) 2553 0070. E-mail: koshyabe@yahoo.com

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