RESEARCH BRIEF

Profile of Patients With Von Gierke Disease From India

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Molecular diagnosis of Von Gierke disease is possible by mutation analysis of *G6PC* gene. *GSD type 1a cases account* for 20 % of glycogenoses in our center. We diagnosed ten unrelated patients with glycogen storage disease based on clinical, biochemical and histopathology investigations. Mutation analysis was done by sequencing the *G6PC* gene. Two unrelated patients were found to be homozygous for a novel mutation c.355 C>G (p.H119D). They were born to non-consanguineous parents from Karnataka. This suggests founder effect. Mutation detection confirms the diagnosis and assists in counseling and prenatal diagnosis.

Key words: Glycogen storage disease type 1a, *G6PC, India, Von Gierke disease.*

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Glycogen storage disease (GSD) type 1a (von Gierke disease) (OMIM 232200) is an inborn error of metabolism caused by the deficiency of glucose-6-phosphatase-α in liver and kidney. Children present with short stature, failure to thrive, hepatomegaly and symptoms of early morning hypoglycemia such as drowsiness and seizures. Biochemical features include fasting hypoglycemia, lactic acidosis, hypertriglyceridemia and hyperuricemia [1]. Confirmation by biochemical method needs enzyme assay on liver biopsy. Without differentiation between GSD types 1a, 1b and 3 may not be always possible. The *G6PC* gene comprises of five exons and sequencing the gene is known to reliably diagnose GSD type 1a with 100% sensitivity and specificity [2]. We sequenced this gene in cases with clinical, biochemical and histopathology features of GSD type 1a to provide molecular diagnosis, genetic counseling and prenatal diagnosis for the affected families.

METHODS

Patients and their families were enrolled in this study after obtaining an informed consent. The clinical history, examination and investigation findings were recorded for the patients. Essential clinical inclusion criteria included hepatomegaly with glycogen accumulation demonstrated in liver histopathology, and additional criteria were failure to thrive, early morning seizures, fasting hypoglycemia, hyper-triglyceridemia, lactic acidosis and hyperuricemia. Patients with elevated serum creatine phosphokinase levels (more than 250 IU/l) were excluded since that suggested a diagnosis of GSD type 3. Five mL of blood was collected from the patients and their family members for the mutation analysis. DNA was isolated from peripheral blood by standard salt extraction method. The five coding exons and the exon-intron boundaries were amplified by polymerase chain reaction (PCR) using previously published primers [3]. PCR steps were as previously described [4]. Sanger sequencing was performed using ABI BigDye Terminator mix and automated sequencing was performed on ABI3100 (Applied Biosystems, Foster city CA). This study was approved by the institute's Ethics Committee.

RESULTS

Nine patients with glycogen storage disease were evaluated during the study period, and the tenth case was reviewed postmortem. Their clinical and biochemical features are summarized in ***Table* 1**. Patient number 1 and 7 were diagnosed as GSD type 3 (elevated total serum creatine phosphokinase levels) and excluded from the study. Bidirectional sequencing analysis for patient number 3 and 6 showed that they were homozygous for a novel substitution mutation c.355C>G (p.H119D) (substitution of cytosine by guanine) (missense mutation leading to substitution of histidine by aspartic acid). Parents of patient number 6 were found to be heterozygous for the same mutation. Samples from parents of patient number 3 were unavailable for

WHAT THIS STUDY ADDS?

- GSD type 1a accounted for 2 out of our 10 cases of clinically suspected liver glycogenoses.

analysis. Both the affected patients were referred from Karnataka and were unrelated. They belonged to Hindu families, both being products of non-consanguineous marriages. Haplotype analysis to determine whether this mutation could have originated from a common founder for both the families could not be performed as parents of patient 3 were unavailable for analysis. No mutations were found in the other five patients. Parents of the tenth case were consanguineous. No mutations in *G6PC* gene were detected in them.

The mutation p.H119D was found to abolish the restriction site of endonuclease *Eae*I similar to a previously reported mutation *viz* p. H119L [5]. Approximately 500 ng of PCR product of exon 3 from control individuals, affected patients and their parents was digested by 0.5 U of enzyme (concentration 5 U/ microliter). Digested control PCR products showed two bands sized 100 base-pairs (bp) and 200 bp, patients having mutation p.H119D showed only one band sized 300 bp and carrier parents showed three bands sized 300 bp, 200 bp and 100 bp on 2 % agarose gel electrophoresis.

DISCUSSION

The above results show that GSD type 1a accounted for 20% (2/10 cases) of liver glycogenoses in our case series. The prevalence of GSD type 1a amongst liver glycogenoses in India is very similar to that observed in other studies [1,6]. Sequence analysis of *G6PC* detects mutations in up to 100% of affected individuals in some homogeneous populations but in mixed populations (*eg*, in the US) detection rate is approximately 94% because both mutations could not be detected in some individuals with clinically and enzymatically confirmed GSD type [7]. This could be because deletion mutations of an exon(s) or a whole gene are unlikely to be detected by sequence analysis. There remains a possibility that the cases with no detectable *G6PC* mutations were likely to be another type of GSD (type 3 or type 1b) since those genes (*AGL* and *G6PT*, respectively) were not analyzed [8].

To date 54 missense, 10 nonsense, 17 insertion/ deletion and 3 splicing mutations have been identified in the *G6PC* gene [2]. A previously known mutation p.H119L (histidine replaced by leucine or C.356A>T) disrupts one of the four crucial catalytic sites of the *G6PC* enzyme and leads to null enzyme activity [2]. The mutation p.H119D can also be predicted to lead to null enzyme activity. A previous study has demonstrated c.150_151delGT; a 2 base pair deletion in two unrelated Indian families in UK [9]. This mutation was not demonstrated in our study.

TABLE1 CLINICAL AND BIOCHEMICAL FEATURES, AND RESULT OF MUTATION ANALYSIS IN PATIENTS

SN	*Age Yrs*	*Sex*	*LS cm*	*TG mg/dL*	*UA mg/dL*	*Lac*	*SG PT*	*CPK IU/L*	*LB*	*Sequence change*
1	5	M	12	220	6.6	N	140	1400	G	EX
2	1.2	F	8	360	7.8	NA	50	140	G	N
3	1	F	9	450	6.7	E	50	200	G	H119D.H119D
4	4	M	10	500	NA	NA	200	120	G	N
5	4	F	14	240	5.2	NA	50	140	G	N
6	1	F	9	2262	7.1	E	61	120	G	H119D/H119D
7	6	F	8	880	5.8	N	440	550	G	EX
8	3	F	10	450	9	E	387	140	G	N
9	0.5	M	10	550	8.2	E	220	125	G	N
10	0.5	M	8	800	10.4	E	150	110	G	CN

(SN: Patient ID, M: male, F: female, LS: Liver span, TG: serum triglycerides, UA: serum uric acid, Lac: serum lactate, NA: not available; E: elevated; N: Normal, SGPT: serum glutamate pyruvate transaminase (IU/L), CPK: serum total creatine phosphokinase, LB: liver biopsy; G: glycogen accumulation, EX: excluded from sequencing analysis, CN: parents detected not to be carriers of any G6PC mutation. All patients had fasting hypoglycemia. None of these cases had neutropenia)

It is expected that the mutations in Indian patients may be different from those described in other populations. Knowing the frequent mutations in a given population helps to provide a rapid cost effective way to dignose these patients in a clinical setting. However, till data on more patients is available, sequencing the whole gene is the only option.

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REFERENCES

1. Chen YT. The glycogenoses. *In*: Rudolph AM (editing). Rudolph Pediatrics. Norwalk. CT, Appleton and Lange. 1991. p.331.
2. Chou JY, Mansfield BC. Mutations in the Glucose-6-phosphatase-α gene that cause type 1a glycogen storage disease. Hum Mutat. 2008;29:921-30.
3. Ki CS, Han SH, Kim HJ, Lee SG, Kim EJ, Kim JW, *et al*. Mutation spectrum of the glucose-6-phosphatase gene and its implication in molecular diagnosis of Korean patients with glycogen storage disease type Ia. Clinical Genetics. 2004;65:487–9.
4. Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY. Mutations in the glucose-6- phosphatase gene that cause glycogen storage disease type 1a. Science. 1993;262:580-3.
5. Wu MC, Tsai F, Lee CC, Tsai CH, Wu JY. A novel missense mutation (H119L) identified in a Taiwan Chinese family with glycogen storage disease Ia (Von Gierke disease). Hum Mutat. 2000;16:447.
6. Hers HG, Van Hoof F, De Barsy T. Glycogen storage diseases. *In*: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Basis of Inherited Disease 6th ed., New York, McGraw-Hill. 1989. p. 425.
7. Seydewitz HH, Matern D. Molecular genetic analysis of 40 patients with glycogen storage disease type Ia: 100% mutation detection rate and 5 novel mutations. Hum Mutat. 2000;15:115–6.
8. Bali DS, Chen YT, Goldstein JL. Glycogen Storage Disease Type I. 2006 [updated 2010 Dec 23]. *In:* Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993. Available from http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=gsd1
9. Meaney C, Cranston T, Lee P, Genet S. A common 2 bp deletion mutation in the glucose-6-phosphatase gene in Indian patients with glycogen storage disease type 1a. J Inherit Metab Dis. 2001;24:517-8.