

Localisation of the gene for glycogen storage disease type 1c by homozygosity mapping to 11q

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Abstract

The microsomal glucose-6-phosphatase (G6Pase) complex regulates the final step in glucose production from glycogenolysis and gluconeogenesis. Glycogen storage disease type 1c (GSD-1c) results from deficient activity of the phosphate/pyrophosphate transporter of this complex and is associated with neutropenia as well as hepatomegaly and hypoglycaemia. Using three affected subjects from a single highly consanguineous family, we have used homozygosity mapping to localise the gene responsible for GSD-1c to a 10.2 cM region on 11q23.3-24.2. The maximum lod score was 3.12. GSD-1c is therefore distinct from GSD-1a, which has been shown previously to be caused by mutations in the G6Pase gene on chromosome 17.

(J Med Genet 1998;35:269-272)

Keywords: glycogen storage disease; neutropenia; phosphate transporter; homozygosity mapping

that performs all the roles of substrate transport and catalysis.

Glycogen storage disease type 1 (GSD-1) is a group of autosomal recessively inherited disorders with an estimated incidence of 1 in 200 000 live births, caused by functional impairment of components of the G6Pase complex. Patients usually present in the first year of life with hepatomegaly, hypoglycaemia, and lactic acidosis.³ Despite improved treatment many of the children grow slowly and develop a wide range of complications.⁴ The condition can be subdivided into four subtypes: GSD-1a, 1b, 1c, and 1d. GSD-1a is caused by mutations in the G6Pase gene on chromosome 17.^{5,6} Patients with defects of T1 have GSD-1b, of T2 have GSD-1c, and of T3 have GSD-1d. The patients with 1b or 1c may have neutropenia and abnormal neutrophil function with recurrent bacterial sepsis and inflammatory bowel disease.⁷ Consequently, these disorders are more severe than type 1a as infection further exacerbates metabolic disturbances.

GSD-1c can be distinguished from 1b by enzymatic studies on liver biopsy material. In GSD-1b, hydrolysis of glucose-6-phosphate in intact microsomal preparations releasing phosphate is reduced and increases to normal when the microsomes are disrupted. In GSD-1c, the phosphate released is within the normal range because of trapping of phosphate within the endoplasmic reticulum. Microsomal disruption increases phosphate production. However, the diagnosis remains difficult and can only be made by in vitro testing of fresh material obtained from liver biopsy. Prenatal diagnosis is not possible. Of the transport proteins, only

Glucose-6-phosphatase (G6Pase) is the enzyme that regulates the final stage in glucose production from glycogen breakdown and gluconeogenesis. Two theories regarding its structural organisation have been proposed. The preferred model is that of a multicomponent translocase catalytic model,¹ with the complex located within the endoplasmic reticulum and consisting of a hydrolytic enzyme and three transport proteins: for glucose-6-phosphate (T1), phosphate and pyrophosphate (T2), and glucose (T3 or GLUT-7) (fig 1). The alternative hypothesis of Schulze *et al*² suggests a single multifunctional membrane channel protein

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Received 26 September 1997
Revised version accepted for
publication 19 November
1997

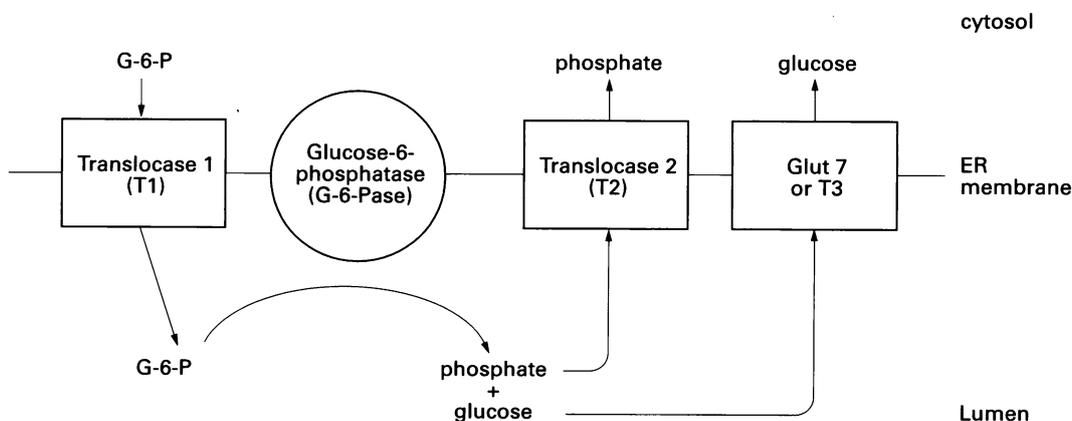


Figure 1 A diagrammatic representation of the multicomponent translocase model of the glucose-6-phosphatase complex as proposed by Arion *et al*.¹ Glucose-6-phosphate (G6P) is transported into the lumen of the ER by interaction with translocase 1 (T1). Glucose-6-phosphatase interacts with G6P and produces phosphate and glucose, which are then transported from the lumen by the translocases T2 and T3, respectively.

the gene for the glucose transporter has been cloned.⁸ In one patient with GSD-1c studied, no mutations were found within the G6Pase gene.⁹

We have used homozygosity mapping to map the gene causing GSD-1c by studying three patients from a highly consanguineous family and found linkage to 11q23.3-24.2. This confirms that GSD-1c is a disorder genetically distinct from GSD-1a.

Materials and methods

Three children from a single inbred family with GSD-1c attending the metabolic clinic at Great Ormond Street Hospital for Children, London, UK were studied (fig 2). Two sibs are the product of first cousin parents and the final patient is their cousin who is the offspring of the brother and sister of the other parents (that is, double first cousins). Two of them (patients IV.3 and IV.5) had liver biopsy proven GSD-1c (table 1) and all were neutropenic (polymorphonucleocyte count $<0.5 \times 10^9/l$). The liver biopsy of patient IV.5 was also analysed by Dr A Burchell (Dundee, UK). This showed normal G6Pase enzyme activity (V_{max} 0.3 $\mu\text{mol}/\text{min}/\text{mg}$) and abnormally high K_m (6

mmol/l), using glucose-6-phosphate as a substrate in intact microsomes. Using pyrophosphate as the substrate, V_{max} was zero. The two sibs had associated inflammatory bowel disease and have been treated with recombinant granulocyte colony stimulating factor (GCSF) at doses of 3-5 $\mu\text{g}/\text{kg}/\text{day}$ for the past six years. As needle biopsies were used, the glycogen content was not assayed, but all samples had marked glycogen accumulation shown histologically by periodic acid Schiff staining and normal G6Pase activity histochemically.

Genomic DNA was extracted from samples obtained from the affected children and their parents. The initial strategy was to screen all members for mutations in the G6Pase gene with single strand conformational polymorphism (SSCP) analysis.⁹

HOMOZYGOSITY MAPPING

With the highly inbred pedigree that we were studying, we estimated that on average the homozygous region, inherited identical by descent in the family and containing the gene responsible for GSD-1c, should be approximately 16 cM in size (L A Sandkuijl, personal communication). We therefore planned to

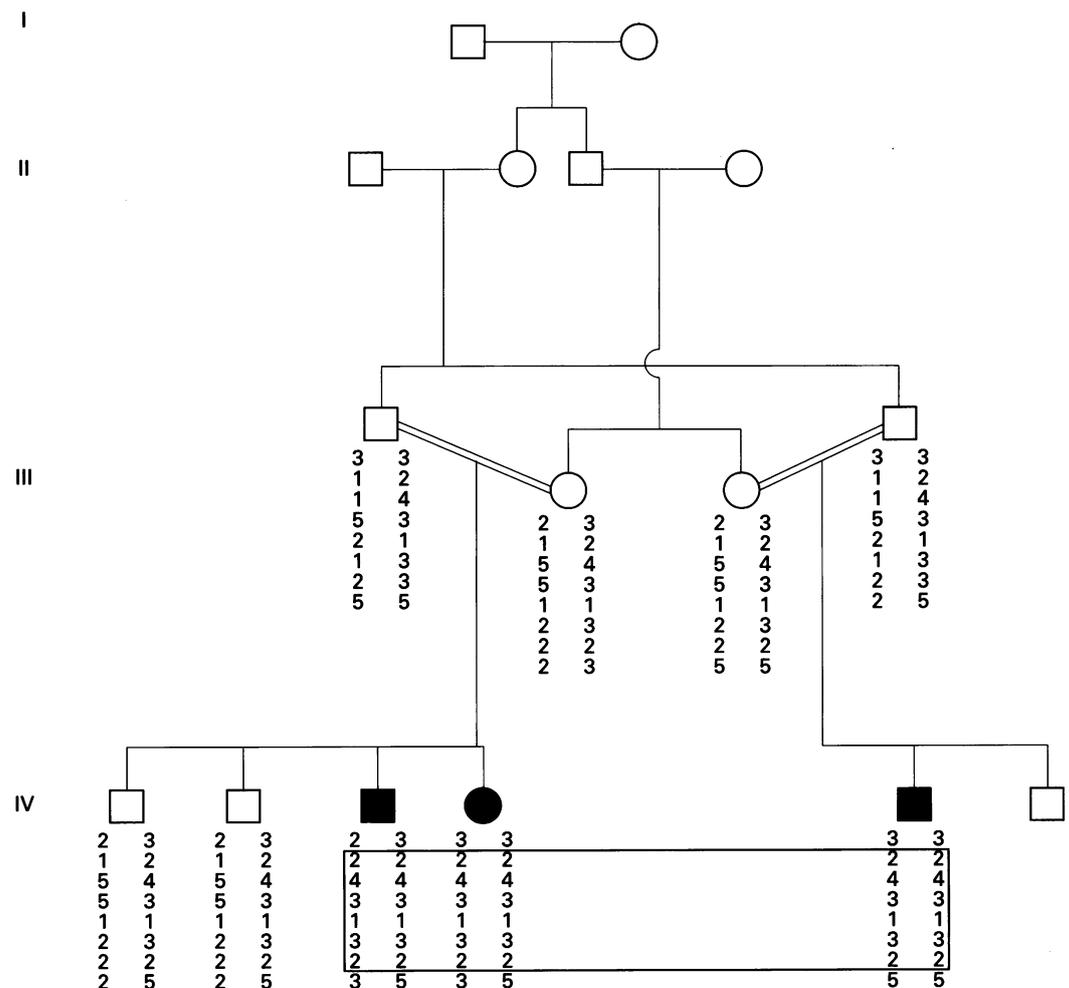


Figure 2 Pedigree of GSD-1c family with haplotype for markers used on chromosome 11q23-24. Markers and loci are, from the top downwards: GATA23E06 (D11S1998), AFM220xh6 (D11S924), CROT-1 (D11S994), AFM220yb6 (D11S925), GATA64D03 (D11S4464), GATA140F03 (D11S4958), AFM240ye1 (D11S933), AFM248waf5 (D11S934). Marker order is uncertain in the cases of GATA64D03 and GATA140F03, and AFM220xh6 and CROT-1, where there are no recombinations recorded. The boxed area shows the region that is inherited homozygously by descent, limited proximally by a recombination in IV.3 and distally by a recombination higher up the pedigree inherited by III.2.

Table 1 Liver biopsy enzymology data on patients studied

	G6Pase activity (intact microsome)	G6Pase activity (disrupted microsome)	G6Pase activity (reference) *
Pt IV.3	4.0 $\mu\text{mol Pi/min/g}$ liver	9.5 $\mu\text{mol Pi/min/g}$ liver	2.6–13.9 $\mu\text{mol Pi/min/g}$ liver
Pt IV.5	109.6 $\mu\text{mol Pi/min/g}$ protein	128.7 $\mu\text{mol Pi/min/g}$ protein	34–166 $\mu\text{mol Pi/min/g}$ protein

*The reference ranges (which represent "normal" values) and patient values differ because two methods have been used. Until 1988 microsomes were disrupted by freezing and the results expressed in terms of wet weight of liver. After 1988 microsomes were disrupted by the addition of histone and the results expressed in terms of the protein content. Patient IV.3 was tested before 1988, while patient IV.5 has results from after that date.

screen with a series of markers to enable the identification of a number of regions with unusual sharing. These areas would be saturated with more closely spaced markers to determine regions of homozygosity as opposed to single marker homozygosity, which would clearly occur in this pedigree.

Sets of markers (6 and 6A, Marshfield, Wisconsin) consisting of dinucleotide, trinucleotide, and tetranucleotide repeats and spaced at 2–20 cM intervals throughout the human genome were used for initial screening.¹⁰ Both fluorescent and radioactive protocols were used. DNA (30 ng) extracted from blood samples from the subjects within the pedigree was amplified using either fluorescently labelled primer map pairs or incorporating ³²P labelled dCTP in a standard PCR protocol. The products of the reaction were separated by electrophoresis on a polyacrylamide gradient gel and analysed by scanning fluorescent densitometry or by autoradiography.

Regions of the genome where the initial series of markers were spaced further than 10 cM apart were screened with further markers to reduce these distances and to increase the possibility of identifying homozygous areas/markers.

Apart from chromosome 17, on which the gene responsible for GSD-1a has been localised,⁹ there were no other obvious candidate areas to start mapping. Thus, a series of microsatellite markers spanning all chromosomes was used to trace the segregation of the gene for GSD-1c. Areas homozygous for the initial set of markers were investigated further using more closely spaced markers.

Results

GLUCOSE-6-PHOSPHATASE MUTATIONS

SSCP analysis of the glucose-6-phosphatase gene in affected subjects and their parents yielded band patterns consistent with controls, suggesting that mutations in this gene were unlikely to be responsible for the disorder.

MAPPING

Analysis of the subsequent genome wide scan showed eight areas of homozygosity on seven chromosomes (chromosomes 2, 4, 8, 10, 11, 14, and 16), together with isolated markers for which the affected subjects were homozygous. On further screening with more closely spaced markers, seven of the areas of homozygosity were shown to be insignificant with heterozygosity in adjacent or close markers. In particular, on chromosome 17, no area of homozygosity was found. However, closer screening of one

area on chromosome 11 showed homozygosity, which became even more apparent on screening with markers which saturated the region (fig 2). This area of homozygosity was found to span 10.2 cM (from 140.9–151.1 cM), equivalent to a chromosomal location of 11q23.3–11q24.2.

Multipoint (six point) analysis was performed using the GENEHUNTER program.¹¹ The disease was modelled as a rare recessive trait ($q=0.002$) with complete penetrance. Distances between markers were obtained from Marshfield (<http://www.marshmed.org/genetics>). Using marker frequencies from this family and eight other ethnically matched subjects, the multipoint lod score was 2.82. Using publicly available marker frequencies, the multipoint lod score increased to 3.12.

Discussion

The concept of homozygosity mapping was devised by Lander and Botstein.¹² It takes advantage of the increased risk of recessively inherited disorders occurring in families in which the parents are closely related. The method allows rare recessive traits to be mapped by linkage analysis by examining relatively few affected subjects. This method has already been used to map the genes responsible for alkaptonuria,¹³ Friedreich's ataxia,¹⁴ merosin negative congenital muscular dystrophy,¹⁵ and familial persistent hyperinsulinaemic hypoglycaemia of infancy.¹⁶

We have used this method to map the gene for GSD-1c to 11q23.3–24.2. The clinical and pathological features of GSD-1 have been recognised for over 65 years¹⁷ and it was one of the first enzyme deficiencies recognised to be associated with a disease.¹⁸

There has been conflicting evidence about the molecular organisation of the G6Pase complex. Molecular studies⁹ suggest that the multi-component translocase catalytic model¹ is the most likely, despite kinetic studies supporting the single, multifunctional, conformational model.¹⁹ Mapping of the GSD-1c gene to an area distant from the G6Pase gene provides overwhelming evidence for a separate protein being essential for the transport of phosphate out of the endoplasmic reticulum and the disorder being genetically distinct from GSD-1a. Whether GSD-1b maps to the same locus as 1c is unknown.

Recently there has been some debate about what lod scores should be accepted to represent evidence for linkage. Even adopting conservative estimates for allele frequencies, the lod score in this single family is in excess of 2.7, which must be taken to be strong evidence that the deleterious gene lies within chromosome 11q. Studying yet more families affected with GSD-1c would be expected to increase this lod score further, and may reduce the size of the interval. There are no obvious candidate human genes in this region of chromosome 11, so the approach for cloning the gene will rely on finding recombinant events in other families followed by positional cloning.

We would like to thank the families for taking part in the study. We would like to thank Mr A Whitfield, Institute of Child Health, London, UK, and Dr A Burchell, Dundee, UK for the enzyme data. We would also like to thank Professor Tim Bishop for his help and advice. This work was supported in part by a grant from Action Research.

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