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ARTICLE

Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling

MGEM Ausems[1], J Verbiest[2,3], MMP Hermans[2,3], MA Kroos[2], FA Beemer[1], JHJ Wokke[4], LA Sandkuijl[1,2], AJJ Reuser[2] and AT van der Ploeg[3]

[1]*Department of Medical Genetics, University Medical Center, Utrecht*
[2]*Department of Clinical Genetics, Erasmus University, Academic Hospital, Rotterdam*
[3]*Department of Pediatrics, Sophia Children's Hospital, Rotterdam*
[4]*Department of Neurology, University Medical Center, Utrecht, The Netherlands*

Glycogen storage disease type II (GSD II) is an autosomal recessive myopathy. Early and late-onset phenotypes are distinguished – infantile, juvenile and adult. Three mutations in the acid α-glucosidase gene are common in the Dutch patient population: IVS1(-13T→G), 525delT and delexon18. 63% of Dutch GSD II patients carry one or two of these mutations, and the genotype–phenotype correlation is known. To determine the frequency of GSD II, we have screened an unselected sample of neonates for the occurrence of these three mutations. Based on the calculated carrier frequencies, the predicted frequency of the disease is 1 in 40 000 divided by 1 in 138 000 for infantile GSD II and 1 in 57 000 for adult GSD II. This is about two to four times higher than previously suggested, which is a reason to become more familiar with the presentation of GSD II in its different clinical forms and to adjust the risk assessment for genetic counselling.

Keywords: glycogenosis; Pompe; carrier frequency; genotype frequency

Introduction

Glycogen storage disease type II (GSD II), acid maltase deficiency or Pompe disease, is an autosomal recessive disease caused by deficiency of the lysosomal enzyme acid α-glucosidase.[1] There are three major phenotypes (infantile, juvenile and adult). The infantile form is characterised by generalised hypotonia and cardiomyopathy. Death occurs within the first two years of life due to cardiorespiratory failure. Late-onset GSD II is clinically very heterogeneous, and presents at juvenile age to late adulthood as a slowly progressive proximal myopathy.[2]

The prospect of enzyme therapy for GSD II highlights the immediate need for accurate figures on the frequency of the disease.[3,4] The figure of 1 in 100 000 normally quoted is not based on solid data.[1,5] Given present knowledge of the molecular genetics of GSD II,[1,2,6] and the genotype–phenotype correlation,[7,8] we now have the tools to update the meagre and aging data on the frequency of the disease. In order to determine the frequency of GSD II we screened a random sample of newborns for three frequent mutations in the acid α-glucosidase gene. These mutations are IVS1(-13T→G), leading to late-onset GSD II,[7,9,10] and two completely deleterious mutations 525delT and

Correspondence: MGEM Ausems, Department of Medical Genetics, University Medical Center Utrecht, PO Box 85090, 3508 AB Utrecht, The Netherlands. Tel: 030 2504000; Fax: 030 2503801; E-mail: MG.E.M.Ausems@dmg.azu.nl
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delexon18.[11–14] In total these mutations comprise 63% of the disease-related alleles in the Dutch patient population.[8] The screening was performed on DNA extracted from over 3000 Guthrie cards from Dutch neonates.

Materials and Methods

Sample Collection

Anonymous Guthrie cards were collected from the central screening laboratory in the Netherlands. The cards were chosen at random from all provinces in proportion to the number of live births in each province.

Mutation Analysis

The extraction of DNA from the blood spots on the Guthrie cards was according to the method of Walsh *et al*[15] with slight modifications. The PCR amplification was performed in a final volume of 25 μl containing 10 pmol of each of the appropriate primers, 250 μM of each dNTP, 1 mM MgCl2, 10% DMSO, 1 unit of Taq polymerase (Gibco-BRL, Rockville, Maryland, USA) in 1 × PCR buffer (Gibco-BRL, Rockville, Maryland, USA). The PCR was accomplished in 40 cycles (94°C, 30 s; 59°C, 1 min; 72°C, 30 s) preceded by 2 min at 94°C and followed by 10 min at 72°C. The primers used for the detection of the IVS1(-13T→G) mutation were: forward 5' TGCTGCAGTGCCAGCCGCGGTTGATGTCT and reverse 5' AGTGCAGCGGTTGCAAGGACACGGAGG; of the 525delT mutation forward 5' GAACCTGAGCTCCTCTGAAATGGGCTAC and reverse 5' CCCACCCTTGTGAGGGTGC; and of the delexon18 mutation forward 5' TGGTTCCTGAGGACACAGCATG and reverse 5' AGTGGCAGGTAGCCATCGGTG. The IVS1(-13T→G) and 525delT mutation were detected by MboII and DdeI digestion of the PCR product, respectively, and electrophoresis on a 3% metaphor-agarose gel. The del exon18 mutation was detected by fragment size analysis on a 1.5% agarose gel.

Statistics

The carrier frequencies of the mild mutation (IVS1 (-13T→G)) and of the two severe mutations (525delT and delexon18) we screened for were directly calculated from the observed number of carriers and the total number of tested Guthrie cards. The overall carrier frequency of severe mutations was calculated by extrapolation using the allele frequencies of the 525delT and delexon18 mutation in 67 Dutch patients with GSD II as determined in our previous study.[8] Genotype frequencies were calculated assuming Hardy-Weinberg equilibrium.

A support interval (SI) was constructed for the extrapolated carrier frequency of severe mutations (q_{se}), taking into account both the sampling error in the estimated frequency of the two severe mutations tested (525delT and delexon 18) (q_{st}) and the sampling error in the estimated contribution (q_{st}/q_{se}) of these two mutations to the entire group of all severe mutations found in patients with the infantile form of GSD II. This support interval included all values of q_{se} for which –2 times the natural logarithm of the likelihood fell within 3.84 units of the maximum likelihood. It corresponds approximately to a 95% confidence interval (CI).

Results

We screened 3043 Guthrie cards for all three mutations (IVS1(-13T→G), 525delT and delexon18). Analyses of these cards revealed a total number of 31 mutations. Thus the calculated carrier frequency of the three mutations was 1/100 (95% CI: 1/69–1/144). PCR amplification was not always successful for the different gene fragments. In practice, the mild IVS1(-13T→G) mutation was detected in 20 of 3075 samples, leading to a carrier frequency of 1/154 (95% CI: 1/100–1/251). The severe mutations, 525delT and del exon18, were detected in 7 of 3123 and 4 of 3128 samples, respectively. The calculated carrier frequency of the known severe mutations together was 1/284 (95% CI: 1/159–1/568). Since the 525delT and del exon18 mutation constitute together 65% of all severe mutations,[8] the extrapolated overall carrier frequency of severe mutations is 1/187 (95% SI: 1/105–1/368), from which it is assumed that the unknown mutations in patients with

Table 1

A Allele frequency and carrier frequency of mild and severe GSD II mutations

Mutation	*Carriers/ Samples tested*	*Allele frequency*	*Carrier frequency*
IVS1 (-13T→G)	20/3075	$q_m = 0.003252$	1 in 154
525delT	7/3123	$q_{st} = 0.001761$	1 in 284
delexon 18	4/3128		
extrapolated 'severe'		$q_{se} = 0.002693$	1 in 187
extrapolated 'all'		$q_e = 0.005946$	1 in 85

B Predicted frequency of GSD II based on the Hardy-Weinberg equilibrium

		95% CI	
Infantile GSD II	1/138 000	1/43 169–1/536 485	(q_{se}^2)
Adult GSD II	1/57 000	1/27 734–1/128 255	($2\times q_{se}\times q_m$)
Infantile plus adult GSD II	1/40 000	1/17 622–1/100 073	($q_e^2 - q_m^2$)[a]

q_m: allele frequency of the mild IVS1(-13T→G) mutation
q_{st}: allele frequency of the two severe mutations tested (525delT and delexon18)
q_{se}: extrapolated allele frequency of severe mutations
q_e: extrapolated allele frequency of all GSD II mutations (eg the mild IVS1(-13T→G) mutation and all severe mutations)
[a]predicted frequency of homozygosity for the IVS1 (-13T→G) mutation (q_m^2) is not included because this genotype probably does not give rise to clinical symptoms[9,10,16].

Table 2 Risk estimates for having a child affected with infantile or adult GSD II

Status of the parent	Risk of having an affected child		
	Infantile GSD II	Adult GSD II	Total
Carrier of IVS1(-13T→G)	0	1/745	1/745
Adult GSD II patient	1/745	1/337	1/232
Carrier of 525delT or delexon 18	1/745	1/615	1/337

Risk estimates for infantile and adult GSD II in offspring of a carrier and an unrelated individual depend on the mutation status of the carrier parent.

infantile GSD II are always severe. The overall carrier frequency for all GSD II mutations is 1/85 (95% SI: 1/59–1/125). Based on these figures, we calculated the frequency of infantile and adult GSD II separately. The frequency of infantile GSD II is 1/138 000 (95% CI: 1/43 169–1/536 482), and of late-onset, adult GSD II 1/57.000 (95% CI: 1/27 734–1/128 255). The combined frequency of infantile and adult GSD II is 1/40 000 (95% CI: 1/17 622–1/100 073). See also Table 1.

Discussion

The results from this study predict a combined frequency of infantile and adult GSD II of 1/40 000 in the Netherlands. This we consider to be a minimum figure because the unknown mild and severe mutations in adult GSD II patients and the mutations in juvenile GSD II patients are not included in our extrapolations. Therefore the actual frequency of the disease will be higher.

A recent study of randomly selected normal individuals from the New York area of various ethnic origins indicated a carrier frequency of about 1 in 100, based on testing for seven different mutations.[17] These mutations constitute only 29% of the chromosomes from 74 GSD II patients, and therefore the extrapolations are considerably less accurate than ours, but coincidentally the estimated frequency of GSD II in the New York study was also 1 in 40 000. Thus, the overall frequency of GSD II is about two to four times higher than previously estimated. This has practical implications for genetic counselling (see Table 2 for risk estimates) and suggests that the disease is underdiagnosed. A reason for this could be that adult GSD II escapes diagnosis when the clinician is not familiar with the various clinical presentations of this rare disease. Alternatively, it is not excluded that individuals with a GSD II genotype may remain asymptomatic. To this end further studies are needed to determine the penetrance of the adult GSD II genotype.

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