Letters to the Editor

Dutch patients with glycogen storage disease type II show common ancestry for the 525delT and del exon 18 mutations

Margreet G E M Ausems, Klara ten Berg, Lodewijk A Sandkuijl, Marian A Kroos, Alfons F J Bardoel, Katerina N Roumelioti, Arnold J J Reuser, Richard Sinke, Cisca Wijmenga

EDITOR—Glycogen storage disease type II (GSD II) is an autosomal recessive lysosomal storage disorder caused by deficiency of acid α -glucosidase. The enzyme deficiency results in intralysosomal accumulation of glycogen in skeletal muscle and in other tissues. There are early and late onset phenotypes which differ with respect to age at onset, extent of organ involvement, and clinical course of the disease.¹ The genotype frequency of GSD II was recently shown to be 1 in 40 000 by mutation screening in the general population, which is higher than previously estimated.^{2,3}

Over 40 different mutations in the acid α -glucosidase (GAA) gene have been reported.⁴ Most mutations are rare and have been found in only a few patients. However, some mutations have been reported in several unrelated patients with defined ethnic origins. The C1935A transversion, frequently found in Chinese patients with infantile GSD II, appears to originate from a common founder.⁵ Other frequent mutations include the R854X mutation in Afro-Americans,⁶ the 2741AG→CAGG insertion in Turkish patients,⁷ and the G925A mutation in European patients.⁸ It remains to be determined whether these frequent mutations represent common descent or result from independent recurrence. The IVS1(−13T→G) mutation is the most frequent mutation in late onset GSD II patients from different ethnic origins.^{9–11}

In The Netherlands, most late onset GSD II patients carry the IVS1(−13T→G) mutation in combination with either the 525delT or the del exon 18 mutation, whereas infantile GSD II patients often show homozygosity or compound heterozygosity for the 525delT and the del exon 18 mutations.¹⁰ The latter mutations are fully deleterious and are associated with complete loss of enzyme activity.^{12,13} The deletion of exon 18 extends from IVS17 to IVS18 and includes the coding sequence of exon 18.

Analysis of the deletion junction showed a direct eight nucleotide repeat sequence flanking the deletion, with one direct repeat included in the deletion and the second direct repeat at the deletion junction.^{14,15} This repeat sequence could be instrumental in the mutation event. So far, the mutation has not been reported in patients of non-white origin. The 525delT mutation has also not been reported in non-white patients, and is relatively rare in "non-Dutch" patients.¹⁶

In order to determine whether the 525delT and del exon 18 mutations represent founder events or independent, de novo mutations, we constructed haplotypes using four single nucleotide polymorphisms (SNPs) in the GAA gene. We used a set of 28 unrelated GSD II patients to determine the extent of haplotype sharing between the individual patients carrying identical mutations. The patient population included 26 white Dutch patients and their parents from 26 families with infantile GSD II, and three white Dutch patients and their parents from two families with adult GSD II. All patients carried at least one frequent mutation (525delT or del exon 18) and had deficient GAA activity, measured in fibroblasts and leucocytes. Genomic DNA was extracted from cultured skin fibroblasts and from peripheral blood cells using standard procedures.¹⁷ Mutation analysis was performed as described previously.¹¹ We analysed four intragenic single nucleotide polymorphisms (SNPs) by PCR amplification, followed by digestion of the PCR product with the appropriate restriction enzyme (table 1). To amplify exons 3, 8, 11, and 17, information was obtained from Martiniuk *et al.*¹⁸ Fragments were electrophoresed on a 2% agarose gel. Genotyping parents of GSD II patients assigned the phase of the alleles. The order of SNPs and mutations was as follows: 525delT - exon 3 SNP - exon 8 SNP - exon 11 SNP - exon 17 SNP - del exon 18.

We estimated allele frequencies by direct counting of chromosomes. It has been shown in several studies¹⁹ that inclusion of genotypes from incomplete families, or the inclusion of reconstructed genotypes, may introduce serious bias into the estimation of allele and haplotype frequencies. Therefore, in the analysis of linkage disequilibrium and the estimation of

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**Department of
Medical Genetics,
University Medical
Centre Utrecht, PO
Box 85090, 3508 AB
Utrecht, The
Netherlands**
M G E M Ausems
K ten Berg
L A Sandkuijl
F J Bardoel
K N Roumelioti
R Sinke
C Wijmenga

**Department of Clinical
Genetics, Erasmus
University, Academic
Hospital Rotterdam,
The Netherlands**
L A Sandkuijl
M A Kroos
A J J Reuser

Correspondence to:
Dr Ausems,
M.G.E.M.Ausems@dmg.
azu.nl

Table 1 Analysis of SNPs within the GAA gene

	Exon 3 SNP	Exon 8 SNP	Exon 11 SNP	Exon 17 SNP
Polymorphism	C642T	A1203G	A1581G	A2338G
Restriction enzyme	DdeI	BsrI	BanII	BsrI
Major allele	389 bp	186, 175, 45, 24 bp	442 bp	317 bp
Minor allele	310, 79 bp	361, 45, 24 bp	123, 319 bp	246, 71 bp

Table 2 Allele frequencies in wild type and mutant chromosomes

A	Exon 3 SNP*			Exon 8 SNP*			Exon 11 SNP*			Exon 17 SNP*		
	N	525	d18	N	525	d18	N	525	d18	N	525	d18
1	0.24			0.81	1.0	1.0	0.74		0.82	0.22		0.08
2	0.76	1.0	1.0	0.19			0.26	1.0	0.18	0.78	1.0	0.92
No	51	24	12	52	23	13	41	17	11	50	22	13

A denotes the different alleles; allele 1 represents the major allele and allele 2 the minor allele (see table 4).

N represents wild type chromosomes; 525 represents chromosomes containing the 525delT mutation; d18 represents chromosomes containing the del exon 18 mutation.

No denotes the number of individual chromosomes counted.

Table 3 Distribution of the core haplotypes constructed from exon 3, 8, and 11 SNPs

Haplotype	Wild type	525delT	Del exon 18	Other mutations*
Minor-minor-minor	0	0	0	0
Minor-minor-major	8	0	0	2
Minor-major-minor	8	14	0	0
Minor-major-major	10	0	7	4
Major-minor-minor	0	0	0	0
Major-minor-major	0	0	0	0
Major-major-minor	0	0	0	0
Major-major-major	8	0	0	3

*The chromosomes not bearing a 525delT or del exon 18 mutation.

haplotype frequencies, only families for which DNA was available for genotyping from the patient and from both parents were included. Genotypes for a given polymorphic marker in a given family were only included in the statistical analysis if completely unambiguous results had been obtained for that marker in all available DNA samples in that family. As a result of this rigorous constraint, the total number of scorable haplotypes was not identical for all marker combinations. The statistical significance of allelic association between various polymorphisms on wild type chromosomes was assessed using Fisher's exact test. Haplotype frequencies were determined using the EM algorithm, as implemented in the EH program.²⁰ Table 2 summarises the frequencies of the alleles observed for the SNPs in wild type chromosomes (n=52) and the panel of 525delT (n=24) and del exon 18 (n=14) chromosomes.

Wild type chromosomes

The chromosomes that were not transmitted by parents to their affected children were considered as wild type chromosomes. A pairwise analysis of SNPs on non-transmitted (wild type) chromosomes showed close to significant evidence for linkage disequilibrium between two pairs of adjacent polymorphisms: exon 3 and exon 8 SNPs ($p=0.06$, with complete absence of the major-minor haplotype), exon 8 and exon 11 SNPs ($p=0.09$, with complete absence of the minor-minor haplotype), but no disequilibrium for one pair, exon 11 and exon 17 SNPs ($p=0.52$).

In the estimation of haplotype frequencies for the combination of the exon 3, exon 8, and exon 11 SNPs, only four of the eight possible haplotypes were observed (table 3), with highly significant statistical evidence for linkage disequilibrium ($p=0.002$).

Mutant chromosomes

Given the observed strong linkage disequilibrium between the exon 3, 8, and 11 SNPs, and the absence of linkage disequilibrium with

Table 4 Distribution of extended 525delT and del exon 18 haplotypes among Dutch GSD II patients

Exon 3	Exon 8	Exon 11	Exon 17	No
525delT				
Minor	Major	Minor	Minor	16
Minor	Major	NI	Minor	5
Minor	Major	NI	ND	1
Minor	Major	NI	NI	1
Minor	NI	Minor	Minor	1
Del exon 18				
Minor	Major	Major	Minor	6
NI	Major	Major	Minor	2
Minor	NI	ND	Minor	1
Minor	Major	ND	Minor	1
Minor	Major	NI	ND	1
Minor	Major	Minor	Minor	2
Minor	Major	Major	Major	1

NI: genotyping was not informative.

ND: genotyping was not carried out.

No: number of chromosomes.

other polymorphisms on wild type chromosomes, we first evaluated these three polymorphisms as a core haplotype on mutant chromosomes. When the patients' chromosomes were divided into three groups, the 525delT, del exon 18, and other mutations, respectively, a single core haplotype was found on all 525delT chromosomes ($p<10^{-8}$), and a single different core haplotype was found on all del exon 18 chromosomes ($p=0.0003$), but no obvious shared haplotypes on the remaining mutant chromosomes (table 3). These data are consistent with a common founder for each of the two common mutations separately.

Origin of 525delT and del exon 18 chromosomes

Table 4 shows extended haplotypes of chromosomes bearing the 525delT and del exon 18 mutations, including the exon 17 SNP. This list also includes haplotypes from patients that could be unequivocally reconstructed from incomplete families. Such haplotypes were not included in the statistical analysis of core haplotypes because the inclusion of reconstructed haplotypes introduces bias.

Although wild type chromosomes did not show linkage disequilibrium between the exon 11 and exon 17 SNP, all of the 525delT chromosomes shared the exon 3 (minor) - exon 8 (major) - exon 11 (minor) - exon 17 (minor) haplotype. These data further support common ancestry for the 525delT mutation. The majority of the del exon 18 chromosomes share the exon 3 (minor) - exon 8 (major) - exon 11 (major) - exon 17 (minor) haplotype. The data in table 3 suggest the presence of only one haplotype for the del exon 18 chromosomes. While data from unequivocal scoring chromosomes were consistent with the presence of one core haplotype, the additional chromosomes listed in table 4 showed two other haplotypes. Three chromosomes which differ for the intra-genic haplotype were excluded from the statistical analysis owing to incompleteness of the families. These data further support common ancestry for the majority of the del exon 18 chromosomes.

In the present study, we performed haplotype analysis in GSD II patients with the 525delT and del exon 18 mutations. The results show that both the 525delT and del

exon 18 mutations in Dutch GSD II patients originate from common founders. This conclusion is mainly based on the observation of strong allelic association between SNPs within the GAA gene and the two mutations (table 3).

We selected, in our initial analysis, a subset of three SNPs which showed significant evidence for linkage disequilibrium with each other, identifying a total of four core haplotypes. Both the 525delT and the del exon 18 mutations were in complete association with one of these core haplotypes. In further analyses we included one additional SNP to construct extended haplotypes. The 525delT mutation has never been reported in non-white patients and is relatively rare outside The Netherlands.¹⁶ It cannot be excluded that other, non-Dutch, white patients carry the same haplotype, since Dutch immigrants may have introduced the mutation into Canada and the USA, for instance. It will be interesting to determine whether patients with a 525delT mutation observed outside The Netherlands share the same haplotype.

Although the majority of the del exon 18 chromosomes shared an identical haplotype, three of the chromosomes carried a different haplotype (table 4). The presence of at least three different haplotypes with an identical mutation suggests that the repeat sequence could have been instrumental in several independent mutational events. Remarkably, the del exon 18 mutation is rarely observed outside The Netherlands. It will be interesting to investigate the repeat sequence in other ethnic groups, with further studies in non-white GSD II patients.

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