

Genotype-Phenotype Correlation in Adult-Onset Acid Maltase Deficiency

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We performed a clinical, biochemical, and genetic study in 16 patients from 11 families with adult-onset acid maltase deficiency. All patients were compound heterozygotes and carried the IVS1(−13T→G) transversion on one allele; the second allele harbored either a deletion of a T at position 525 in exon 2 (7 probands, 64%) or a deletion of exon 18 (1 proband, 9%). Deterioration of handicap was related to age, and decrease in vital capacity to duration of the symptomatic stage. Respiratory insufficiency was never the first manifestation. The levels of activity of serum creatine kinase and of α -glucosidase in peripheral blood cells or muscle were helpful for the diagnosis, but did not have prognostic value. The adult form of acid maltase deficiency appears to be both clinically and genetically rather homogeneous; decrease of α -glucosidase activity is the final common pathway leading to destruction of muscle fibers and progression of muscle weakness over a period of years.

Wokke JHJ, Ausems MGEM, van den Boogaard M-JH, Ippel EF, van Diggelen O, Kroos MA, Boer M, Jennekens FGI, Reuser AJJ, Ploos van Amstel HK. Genotype-phenotype correlation in adult-onset acid maltase deficiency. *Ann Neurol* 1995;38:450–454

Glycogen storage disease type II, acid maltase deficiency (AMD), is caused by deficiency of the lysosomal enzyme acid α -glucosidase (GAA; synonym, acid maltase) [1–3], and is a heterogeneous autosomal recessive disease with three major phenotypes—infantile, juvenile, and adult [2, 3]. Adult-onset AMD may resemble polymyositis or limb-girdle muscular dystrophy [2–6]. Identification of various mutations in the AMD gene could explain differences between and within the phenotypes of AMD [2]. The GAA gene contains 20 ex-

ons and has a length of approximately 20 kb [7, 8]. Mutations in patients with AMD include missense, nonsense, and frameshift mutations [9]. Three mutations seem to be frequent: a deletion of exon 18 (del exon 18) [10, 11], a single base pair deletion (525delT) in exon 2 [12], and the IVS1(−13T→G) transversion in the consensus sequence of the acceptor splice site of intron 1 [13]. We tested patients with adult-onset AMD for these mutations and compared genotype with phenotype.

Patients and Methods

DNA Analysis: Amplification and Mutation Detection

High-molecular-weight DNA was isolated from peripheral blood leukocytes according to established procedures. The patients were specifically tested for the mutations IVS1(−13T→G), 525delT and del exon 18 in the GAA gene using the polymerase chain reaction (PCR). The IVS1(−13T→G) transversion was analyzed with a PCR based on allele-specific amplification. The forward primers used were POM13T (TCCCTGCTGTAGCCCCGCTTT) for the normal sequence and POM13G (CCTGCTGTAGCCC GCTTG) for the mutated allele. As reverse primer, POM13R (TTGTCAGGGGCGCAATCGAA) was used in both reactions. The 525delT mutation was identified by a *DdeI* restriction analysis of the PCR product generated with the forward primer POM525F (AGAGCAGTGCCACAC AGTG) and the reverse primer POM525R (GTGGGTGTC GATGTCCACGC). Del exon 18 was identified using the primers POM17F (TGGTTCCTGAGGACAGCATG) (forward) and POM18R (AGTGGCAGGTAGCCATCGTG) (reverse). The amplification was performed in 33 cycles, each cycle consisting of 1 minute of denaturation at 94°C, 1 minute of annealing at 55°C, and 2 minutes of extension at 72°C, essentially as described elsewhere [14].

Evaluation of Patients

Sixteen adult-onset AMD patients from 11 unrelated families of Dutch Caucasian ancestry, diagnosed between 1982 and 1994, were examined in the summer of 1994 by one of us (J.H.J.W.). We did manual muscle testing and calculated motor sum scores. Handicap was scored using the modified Rankin scale [15]. Vital capacity (VC) was measured in 1994. Three patients (Patients 1, 2, and 6) were only questioned about symptoms and handicap. The following laboratory tests were performed: measurement of blood creatine kinase (CK) activity, electromyography (EMG), electrocardiography (EKG), and measurement of GAA activity in peripheral blood lymphocytes and leukocytes using glycogen as substrate at pH 4, and in muscle with the methylumbelliferyl substrate [16]. Skeletal muscle biopsy specimens from 11 patients were prepared for light microscopy using routine methods for histology and histochemistry of cryostat sections. In 1994 residual activity of GAA was measured in 9 biopsy specimens.

Statistical Analysis

The motor sum scores of upper and lower limbs were compared using the nonparametric Wilcoxon matched-pairs

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Received Mar 23, 1995, and in revised form Jun 7. Accepted for publication Jun 8, 1995.

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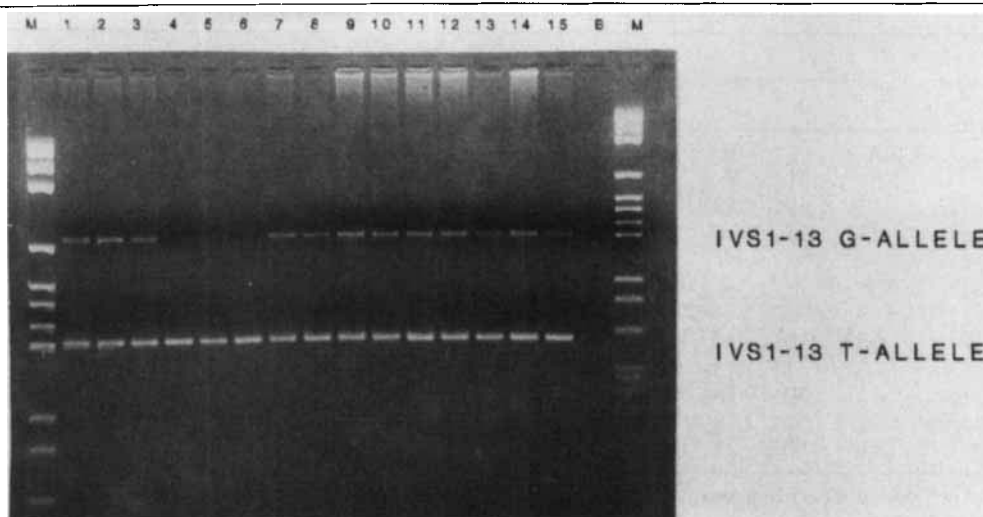


Fig 1. Agarose gel electrophoresis of the IVS1(-13T→G) allele-specific polymerase chain reaction (PCR) products of 12 patients with adult-onset acid maltase deficiency (AMD) (lanes 1–3 and 7–15). Lanes 4 to 6 show the products of infantile AMD patients which served as controls. The PCR products for the two alleles are approximately of the same size (351 bp for the T allele, 349 for the AMD G allele). The two PCRs underwent electrophoresis consecutively within a period of 1 hour. The molecular weight marker (M) for the T allele is shown on the left, for the G allele on the right.

signed rank test and the *p* value was calculated as a two-tailed test. In patients with various degrees of handicap, we tested differences of age, CK activity, and residual activity of GAA using analysis of variance (ANOVA) and Student's *t* test. These tests were also used to assess differences between duration of disease and VC.

Results

DNA Analysis

Patients of 11 families with adult-onset AMD were screened for three mutations: IVS1(-13T→G) [13], 525delT [12], and del exon 18 [10, 11]. Genetic testing for the IVS1(-13T→G) transversion identified all patients with adult AMD as compound heterozygotes as they had amplification of both the normal T allele and the AMD G allele (Fig. 1). Seven patients had 525delT as second mutation; in 1 proband del exon 18 was detected. Three patients did not show either the 525delT or the del exon 18. To prove that indeed the mutations are located on different alleles, we followed the segregation of the gene defects in the families. Figure 2 depicts an example of the analyses for two compound heterozygotes: IVS1(-13T→G); 525delT, respectively, IVS1(-13T→G); del exon 18. Analysis of the parents of all patients who were compound heterozygotes for the above mutations (Table; Patients 1–10, 15, and 16) showed that the mutations were on separate alleles.

Clinical Abnormalities

At the initial examination, 3 young adults had no symptoms or signs; 2 had an affected sibling and 1 was referred for hyper-CK-emia. The age at onset of complaints in the other 13 patients varied between 17 and 45 years (mean, 31 years). The presenting symptoms were abnormal fatigue in 5 patients and proximal weakness of the legs in 8. The standard neurological examination at the time of diagnosis revealed normal findings in 4 patients with complaints. In the others we found symmetrical proximal weakness of the flexor muscles of the hips and knees. After a follow-up period of 1 to 13 years (mean, 6 years), 2 patients remained asymptomatic. Deterioration of proximal muscle weakness always occurred gradually except in Patient 14, who at the age of 39 had acute respiratory insufficiency during a respiratory tract infection. At the end of the follow-up period, weakness was found in 11 of 13 patients; the motor sum scores of the upper limbs ranged between 24 and 30 (median, 28) and those of the lower limbs between 21 and 30 (median, 24; *p* < 0.03). The mean age of 4 patients with handicap grade 0 or 1 on the modified Rankin scale was 22 ± 4 years; of 6 patients with grade 2, 40 ± 10 years; and of 6 patients with grade 3 or 4, 47 ± 7 years (see Table). The difference between the younger and the two older groups was significant (*p* < 0.005). VC was decreased in 11 (77%) of 14 examined patients. After a symptomatic stage of 1 to 5 years, mean VC was $96 \pm 6\%$; after 6 to 10 years, mean VC was $69 \pm 14\%$; and after 11 to 15 years, mean VC was $53 \pm 25\%$. The difference between the first and the other two groups was significant (*p* < 0.005).

Laboratory Analysis

CK elevation ranged between 1.5 and 15 times and was not related to severity of handicap. The EKG only

	Patient No.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pedigree	A	A	B	C	D	E	E	F	F	G	H	H	I	J	K	K
Sex	F	M	F	F	M	F	M	F	F	M	F	F	M	M	F	F
Age at diagnosis (yr)	18	19	52	24	19	41	41	24	36	45	33	24	15	39	47	42
Age at evaluation (yr)	23	21	58	37	21	49	50	28	40	46	37	33	19	45	58	49
525delT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IVS1(-13T→G)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Del exon 18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Vital capacity (%)	ND	101	30	100	92	ND	49	103	80	84	87	90	92	30	64	69
Rankin score	2	1	4	2	0	2	3	0	3	2	2	3	1	2	3	3
GAA in leukocytes ^a	42	50	36	20	51	21	55	48	41	43	53	59	14	36	33	45
GAA in lymphocytes ^a	41	51	53	ND	36	ND	39	52	44	40	32	31	2	39	26	15
GAA in muscle ^a	11	ND	21	ND	ND	ND	21	ND	ND	9	ND	30	7	16	9	4

^aα-Glucosidase (GAA) values as measured in peripheral blood leukocytes, lymphocytes, and muscle; values given are percentage of lower limits in healthy controls.

ND = not done.

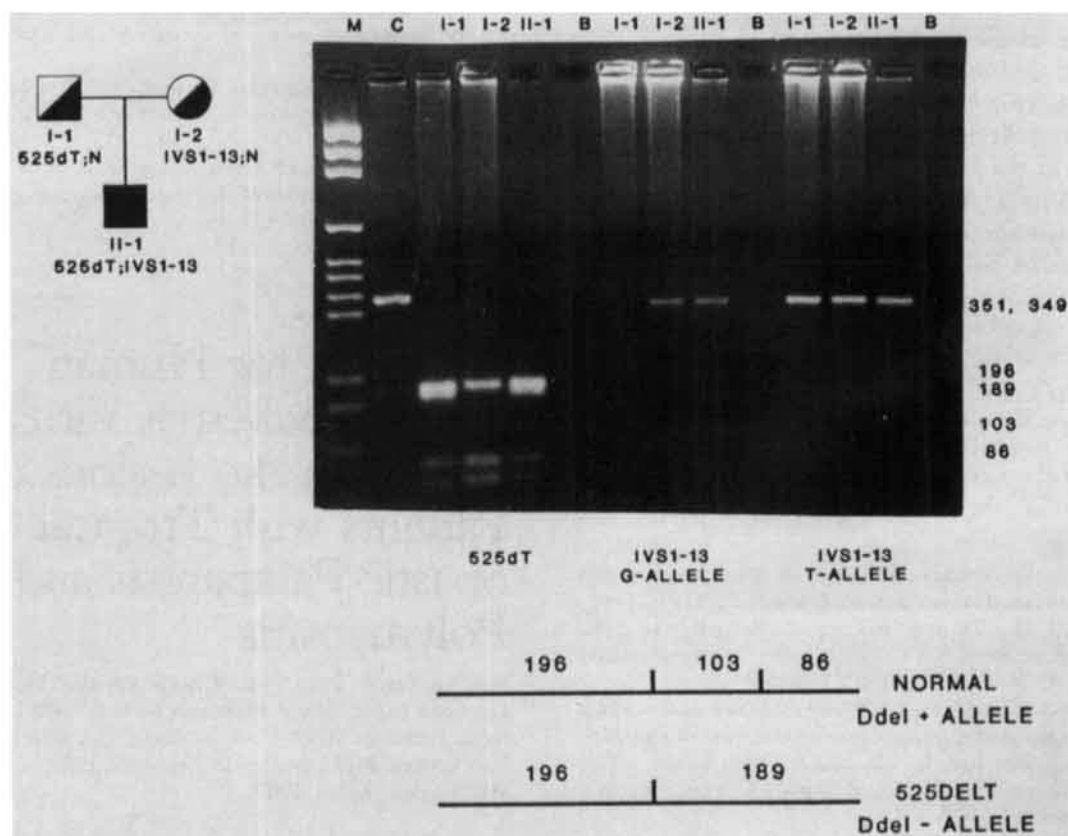
was abnormal in Patient 10 who had had a myocardial infarction at the age of 43 years. EMG showed myopathic changes in 8 of 13 patients; 1 of 6 patients had a normal EMG including analysis of erector spinal muscles. Skeletal muscle biopsy specimens were histologically and histochemically normal in 3 patients but in all specimens GAA activity was decreased (values between 4 and 30% [see Table]). GAA activity in peripheral blood cells was decreased in all patients.

Discussion

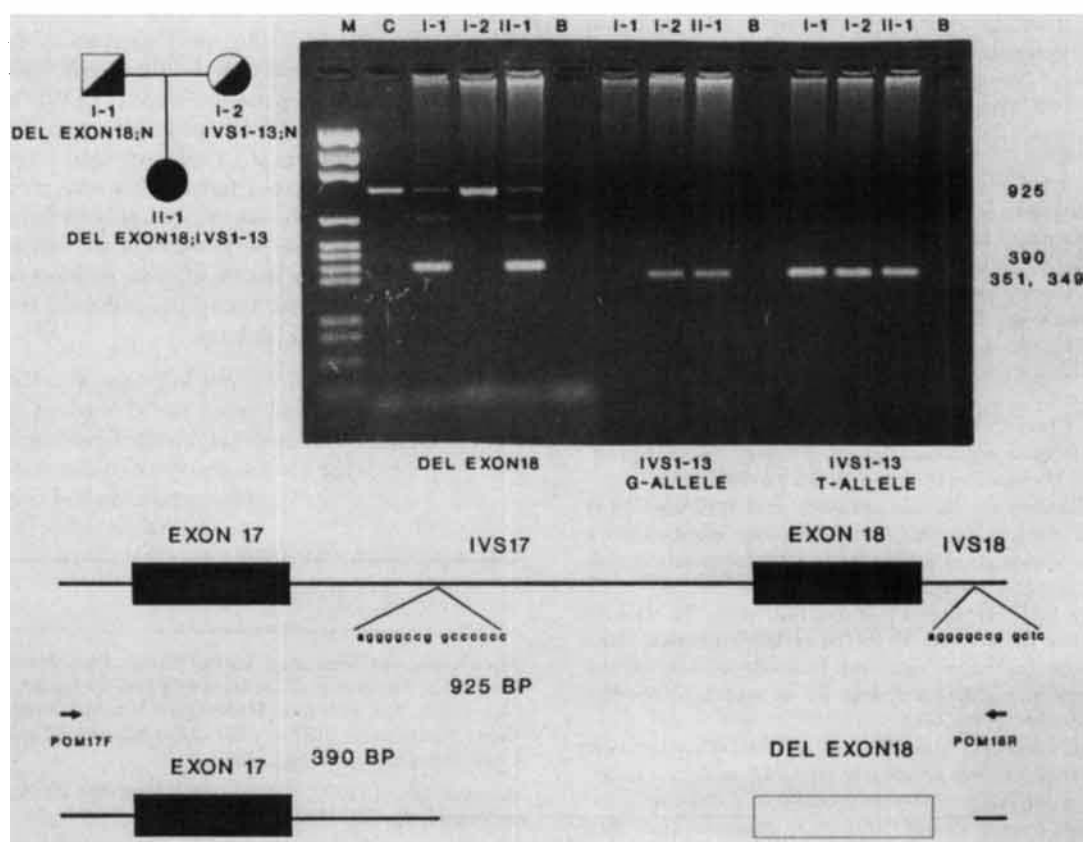
All 11 probands and their affected siblings were compound heterozygotes; they carried the IVS1(-13T→G) mutation on one allele; the second allele harbored either 525delT (7 probands, 64%) or del exon 18 (1 proband, 9%). In 3 probands (27%), the second mutation has not been identified. We could identify mutations in 19 (86%) of the 22 alleles. Huie and coworkers [13] detected the IVS1(-13T→G) transversion on one allele in 28 of 41 patients with adult-onset AMD. In the present study we found an allele frequency of 0.5 among probands. The 525delT was found in 7 of 11 probands (allele frequency, 0.32). This frameshift mutation recently was described in 3 patients with AMD [12]. The del exon 18 mutation that we found in 1 proband is reported mostly in patients with infantile-onset AMD [10, 11]. The 525delT mutation has been associated with complete absence of GAA. Expression studies of the mutant complementary DNA in SV 40 transformed monkey kidney cells showed that the 525delT deletion prohibits the formation of mature enzyme [12]. Del exon 18 is also a disastrous mutation, that is, associated with complete loss of enzyme activity (Ausems et al, unpublished data, 1995). The IVS1(-13T→G) mutation is associated with aberrant splicing of the pre-messenger RNA

(mRNA): The exon 2 sequence is skipped from the GAA mRNA [13]. However, Huie and coworkers [13] showed that still some correctly spliced mRNA is present. Our studies confirmed this finding that the IVS1(-13T→G) mutation can be considered as mild, as our patients all had a significant level of GAA activity in peripheral blood cells. The phenotype of adult-onset AMD can be explained by the genotype, for example, the combination of one deleterious mutation with the milder mutation IVS1(-13T→G). The residual enzyme hypothesis may explain major differences between infantile and adult forms of AMD, but not within adult-onset AMD [2]. The conclusion of adult-onset AMD being a mild, homogeneous limb-girdle dystrophy-like syndrome with very slow progression is favored by two arguments: First, young adults had either no symptoms or only abnormal fatigue, but were not handicapped, whereas older patients were more or less severely incapacitated. Second, during the follow-up period all patients showed deterioration. Respiratory insufficiency was never the first manifestation in

Fig 2. Confirmation that the mutations IVS1(-13T→G) and del exon 18 in Proband K and the IVS1(-13T→G) and 525delT in Proband D are located on separate alleles. (A) The pedigree and the agarose gel electrophoresis for Proband K and her parents (C = normal polymerase chain reaction fragment without Ddel). (B) The analysis for Proband D (C = normal control). At the bottom of each panel, a schematic representation of the mutation analysis is indicated. The 525delT disrupts a Ddel site giving rise to an additional fragment of 189 bp (vertical bars represent the recognition site of Ddel). Del exon 18 is indicated by the appearance of a fragment with a reduced length of 390; the normal fragment has a length of 925 bp. The additional fragment between the two fragments is caused by heteroduplex formation between the opposite strands of the two alleles.



A



B

previously asymptomatic patients. This relatively mild appearance of the adult form of AMD is remarkable and can be explained by earlier referral of patients. In our department, AMD is diagnosed yearly in 1 or 2 new patients. At present the frequency of the various mutations in the Dutch population is not known, but as 7 (64%) of 11 families showed the same combination of mutations, adult-onset AMD appears to be genetically rather homogeneous in the Netherlands.

This study was supported by de Stichting Willem H. Kröger and the Prinses Beatrix Fonds.

We are grateful to P. Liam Oey, MD, for helpful discussions of the EMG findings, Gerard H. Jansen, MD, who revised the muscle biopsies, Mr Henk Veldman who advised on statistical methods, and Miss Birgitta Kraan for her expert secretarial assistance.

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A Search for Human T-Cell Leukemia Virus Type I in the Lesions of Patients with Tropical Spastic Paraparesis and Polymyositis

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We searched for the presence of human T-cell leukemia virus type I (HTLV-I) sequences in central nervous system and muscle lesions of 3 patients with tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) and 3 patients with HTLV-I-associated polymyositis. Proviral DNA coding for the Tax protein was found by polymerase chain reaction amplification in DNA extracted from lesions of every patient with TSP/HAM or HTLV-I-associated polymyositis. In contrast, viral RNA was found occasionally by *in situ* hybridization in muscle lesions of some patients with polymyositis, but was never found in central nervous system lesions of TSP/HAM patients.

Tangy F, Vernant J-C, Coscoy L, Ossondo M, Bellance R, Zaninovic V, Cartier L, Brahic M, Ozden S. A search for human T-cell leukemia virus type I in the lesions of patients with tropical spastic paraparesis and polymyositis. *Ann Neurol* 1995;38:454–460

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Received Jan 31, 1995, and in revised form Apr 24. Accepted for publication Apr 25, 1995.

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