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ARTICLE

Human α-N-acetylgalactosaminidase (α-NAGA) deficiency: no association with neuroaxonal dystrophy?

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Two new individuals with α-NAGA deficiency are presented. The index patient, 3 years old, has congenital cataract, slight motor retardation and secondary demyelinisation. Screening of his sibs revealed an α-NAGA deficiency in his 7-year-old healthy brother who had no clinical or neurological symptoms. Both sibs are homozygous for the E325K mutation, the same genotype that was found in the most severe form of α-NAGA deficiency presenting as infantile neuroaxonal dystrophy. Thus, at the age of 7 years the same genotype of α-NAGA may present as a 'non-disease' (present healthy case) and can be associated with the vegetative state (the first two patients described with α-NAGA deficiency). The clinical heterogeneity among the 11 known individuals with α-NAGA deficiency is extreme, with a 'non-disease' (two cases) and infantile neuroaxonal dystrophy (two cases) at the opposite sides of the clinical spectrum. The broad spectrum is completed by a very heterogeneous group of patients with various degrees of epilepsy/behavioural difficulties/psychomotor retardation (four patients) and a mild phenotype in adults without overt neurological manifestations who have angiokeratoma and clear vacuolisation in various cell types (three cases). These observations are difficult to reconcile with a straightforward genotype-phenotype correlation and suggest that factors or genes other than α-NAGA contribute to the clinical heterogeneity of the 11 patients with α-NAGA deficiency. *European Journal of Human Genetics* (2001) **9,** 91–96.

Keywords: α-N-Acetylgalactosaminidase; α-NAGA deficiency; Schindler disease; neuroaxonal dystrophy

Introduction

In 1987, two German infants were reported by van Diggelen *et al*[1] with a profound deficiency of the lysosomal enzyme α-N-acetylgalactosaminidase (α-NAGA) and infantile neuroaxonal dystrophy without visceral involvement and dysmorphism.[2] Two years later Kanzaki *et al*[3,4] reported the second independent case of α-NAGA deficiency with an entirely different clinical phenotype. This patient had a late onset disease with slight facial coarseness, disseminated angiokeratoma and mild intellectual impairment (IQ=70) but without neurological symptoms. Unlike the infantile cases, this patient had prominent vacuolisation in all dermal cells, eccrine sweat gland cells, glomerular endothelial cells (but not the epithelial kidney) and also blood lymphocytes.[5] Chabas *et al*[6] reported adult patients of Spanish origin with a similar mild phenotype, including angiokeratoma, slight dysmorphism, lymphoedema and prominent vacuolisation in endothelial cells. A third class of individuals with α-NAGA deficiency was first described by de Jong *et al*:[7] they reported a 3-year-old infant who was apparently normal. At his present age of 8 years, this boy still has no overt clinical symptoms (Drs Jan de Jong and Willy Renier, personal communication). The remaining known cases comprise a heterogeneous

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clinical spectrum with various degrees of epilepsy, behavioural difficulties and psychomotor retardation (in Table 3: patients D2.1, NL1.2, F1.1 and the present index case NL2.2).

All known cases have been discovered through the abnormal urinary oligosaccharides. The major compounds are sialylglycopeptides of the O-glycosidic type with serine or threonine linked to the α-N-acetylgalactosamine (αGalNAc) moieties.[8,9] Mutation analysis of the α-NAGA gene revealed a paradox between genotype and phenotype.[10] At the most severe end of the clinical spectrum (infantile neuroaxonal dystrophy) a homozygous missense mutation E325K was found, with some residual α-NAGA activity. On the other hand, the Spanish patients with a rather mild, late onset disease were homozygous for the nonsense mutation E193X causing the complete absence of α-NAGA-protein.[10] This is unprecedented among the lysosomal storage diseases since the complete absence of enzyme-protein is expected to be associated with the most severe clinical phenotype.

In this study we report two new patients of Moroccan ancestry with α-NAGA deficiency, one of which has no overt clinical symptoms. They have the same genotype (E325K/E325K) as the severe cases with infantile neuroaxonal dystrophy.

Patients and methods

Patients/case report

Case 1 (the proband NL2.2) is the 3-year-old son of consanguineous Moroccan parents with bloodgroup O. His birthweight was 4550 g and his birth was complicated by a humerus fracture. At the age of 4 weeks, strange eye movements were noticed and ophthalmoscopy showed congenital bilateral cataract. General metabolic screening of 24 h urine showed normal excretion patterns of aminoacids, organic acids, purines and pyrimidines, mucopolysaccharides, but an abnormal oligosaccharide pattern suggestive for α-NAGA deficiency. The pattern was comparable to that of the first patients with α-NAGA deficiency.[11] At the age of 12 months his neuromotor development was slightly delayed, which became more prominent in the next 2 years. At 3 years old he was unable to walk without support but hand co-ordination and speech development was normal. NMR of the brain showed diffuse white matter abnormalities and the white matter in capsula interna and corpus callosum appeared to be swollen. There was a secondary, symmetrical demyelinisation. Histopathological studies could not be performed.

Case 2 (NL2.1) was discovered by screening of the healthy family members. He is the 7-year-old brother of the proband NL2.2 and also had bloodgroup O. His urinary oligosaccharide pattern was essentially the same as that of his affected brother. At his present age of 7 years he has no clinical or neurological symptoms.

Oligosaccharide screening

Urinary oligosaccharides were analysed qualitatively after desalting, using thin-layer chromatography on silicagel followed by orcinol staining.[12]

Cell culture and enzyme assays

Skin fibroblasts were cultured according to routine procedures. α-N-acetylgalactosaminidase (αNAGA) activity in leukocytes and fibroblasts was assayed as described.[10]

DNA amplification and sequencing

DNA was isolated from cultured fibroblasts or leukocytes and PCR amplification and sequencing was performed as described.[10] Restriction enzyme digestions (*Taq*I, Boehringer) were done according to the instructions of the manufacturer.

Results

The index patient (NL2.2) was found by routine urinary screening for oligosaccharides, showing the characteristic pattern of α-NAGA deficiency. The α-NAGA activity of the proband and the other members of his family are shown in Table 1. The index case and his 7-year-old healthy brother (NL2.1) had undetectable α-NAGA activity in leukocytes and a profound deficiency in fibroblasts. The parents had α-NAGA activity consistent with heterozygosity.

Mutation analysis of the nine exons of the α-NAGA gene, including all flanking intron regions, revealed the G11005A mutation (E325K) in both patients (Table 2). This mutation destroys a *Taq*I restriction site and its presence could be confirmed by *Taq*I digestion of the 351-bp long PCR products of exon 8. The digestion of the normal PCR product into fragments of 174 and 177 bp was not observed in the PCR products of the mutant alleles (Figure 1). Homozygosity for the E325K mutation in the patients was confirmed by analysing DNA from the parents: both were heterozygous for the E325K mutation (Figure 1). The entire sequenced parts of the α-NAGA gene (sense as well as antisense strand) were identical in both patients and a control (with the exception of the mutations). The sequence was also identical to our previously published data[10] on four α-NAGA patients and two controls, which differed from the published sequence of the

Table 1 α-NAGA activity in leukocytes and fibroblasts from the proband, his sibs and parents

	α=NAGA activity (nmol/h/mg)	
	Leukocytes	*Fibroblasts*
NL2.2 (proband)	0.0	1.9
NL2.1 (healthy sib)	0.0	1.0
Third sib (heterozygote)	7.8	
Father	5.4	
Mother	7.4	
Control range	6–21	54–160
Mean control	13	102

Table 2 The mutations in all patients with α-NAGA deficiency

Patient	*Nucleotide change*[a] *(exon/exon)*	*Protein change*[a]	*Reference mutations*
D1.1	G11005A (8/8)	E325K/E325K	Wang *et al*[15]
D1.2	G11005A (8/8)	E325K/E325K	Wang *et al*[15]
D2.1	G11005A (8/8)	E325K/E325K	Keulemans *et al*[10]
E1.1	G5371T (5/5)	E193X/E193X	Keulemans *et al*[10]
E1.2	G5371T (5/5)	E193X/E193X	Keulemans *et al*[10]
F1.1	not published	E325K/E367K	Simonaro *et al*[16]
J1.1	C11017T (8/8)	R329W/R329W	Wang *et al*[17]
NL1.1	C4969G/G11005A (4/8)	S160C/E325K	Keulemans *et al*[10]
NL1.2	C4969G/G11005A (4/8)	S160C/E325K	Keulemans *et al*[10]
NL2.1	G11005A (8/8)	E325K/E325K	present study
NL2.2	G11005A (8/8)	E325K/E325K	present study

[a]Nucleotide and aminoacid numbering is according to Yamauchi *et al*[18] and EMBL database accession no. M59199 (Wang and Desnick). The exons in which the mutation was found are shown in brackets.

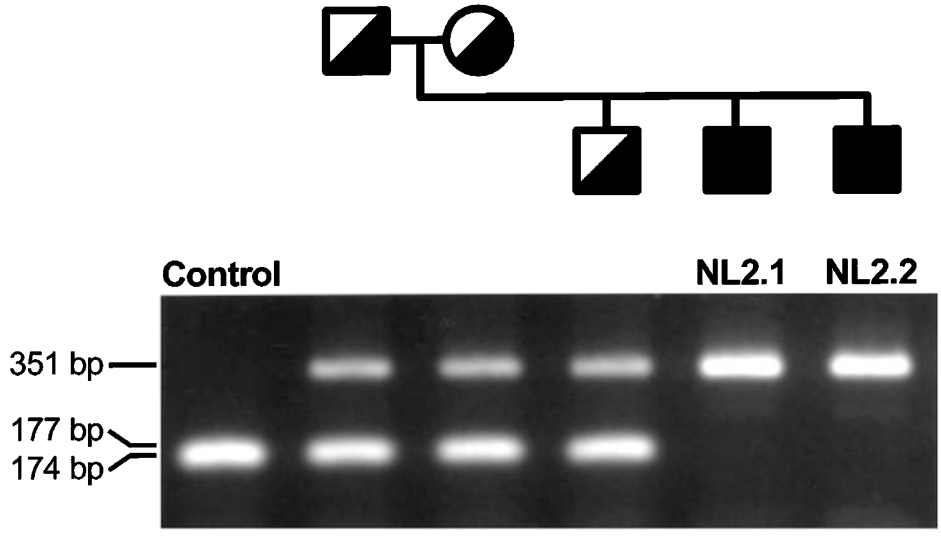

Figure 1 Restriction enzyme analysis of PCR amplified α-NAGA exons 8. *Taq*I digests of exon 8 of both patients with α-NAGA deficiency (NL2.1 and NL2.2), their parents and their brother.

α-NAGA gene (Wang and Desnick, EMBL database accession no. M59199) at 13 nucleotides.[10]

Discussion

α-NAGA deficiency is one of the rarest and probably most heterogeneous lysosomal storage disorders. At present, only 11 patients are known from seven families of German (D), Japanese (J), Dutch (NL1), Spanish (E), French/Italian/Albanian (F) and Moroccan (NL2) descent. This includes the most recently discovered cases reported here (summary in Table 3). Clinical variability is common in lysosomal storage disorders but overlapping signs and symptoms among subtypes is the rule. In α-NAGA deficiency, however, infantile and adult patients have no obvious overlap of signs and symptoms and with each new patient the clinical variability appears to broaden. One of the present cases (NL2.2) has added cataract to this list and the recently discovered French case[13] had yet another phenotype with intellectual impairment and severe autistiform behavioural problems (patient F1.1).

In a previous paper[10] some of us proposed that α-NAGA deficiency is not a single disease entity. This was based on the

Table 3 Summary of all patients with α-NAGA deficiency

	Infantile α-NAGA deficiency								*Adult α-NAGA deficiency*		
	D1.1	*D1.2*	*D2.1*	*NL1.1*	*NL1.2*	*F1.1*	*NL2.1*	*NL2.2*	*J1.1*	*E1.1*	*E1.2*
Age											
Age at onset	1 year	6 months	7 months	1 year	not yet	3 years	not yet	1 month	28 years	14 years	n.i.
Present age [age in 1996]	17 years	16 years	†1.5 years	9 years	8 years	10 years	7 years	3 years	[51 years]	[42 years]	[38 years]
Neurological signs											
Convulsions during fever	+	+	+	+	–	–	–	–	–	–	–
Epilepsy	+++	+++	+	–	–	–	–	–	–	–	–
Psychomotor retardation	+++	+++	?	+	–	±	–	±	–	–	–
Hypotonia	+++	+++	?	–	–	–	–	–	–	–	–
Physical signs											
Skeletal abnormalities	–	–	–	–	–	–	–	–	–	–	–
Dysmorphism	–	–	–	–	–	–	–	–	±	±	?
Cataract	–	–	–	–	–	–	–	+	–	–	–
Organomegaly	–	–	–	–	–	–	–	–	–	–	–
Angiokeratoma	–	–	–	–	–	–	–	–	+++	++	+
Lymphoedema	–	–	–	–	–	–	–	–	–	+++	+++
Histology											
Vacuolisation	–	–	–	–	–	–	–	–	++	++	++
Neuroaxonal dystrophy	+++	+++	n.i.	–	–	–	–	–	–	–	–
α-GalNAc lectin staining	++++	+++	++++	+++	n.i.	n.i.	n.i.	n.i.	n.i.	+++	+++
Mutations	– – – homozygous E325K – – –			E325K/S160C		E325K E367K	homozygous E325K		R329W R329W	homozygous E193X	

n.i.=not investigated. References of clinical report – D1: van Diggelen *et al*[1], Schindler *et al*[2]; D2: Keulemans *et al*[10]; NL1: de Jong *et al*[7]; NL2: present study; E1: Chabas *et al*[6]; F1: Gay *et al*[13]; J1: Kanzanki *et al*.[4]

following observations on the eight patients known at that time:

(1) No vacuolisation of cells was observed in the most severely affected patients (D1.1 and D1.2). On the other hand, this hallmark of lysosomal storage disorders was present in the mildly affected late onset patients (J1.1, E1.1 and E1.2).
(2) A homozygous stop mutation (E193X) was found in the mild, Spanish patients (E1.1 an E1.2). It represents in essence as a null-allele and therefore should be associated with the most severe form of α-NAGA deficiency.
(3) Since the discovery of the first patients with α-NAGA in 1987, the association of α-NAGA deficiency and infantile neuroaxonal dystrophy (Seitelberger) has not been found again.

The present cases (NL2) strongly support the notion that α-NAGA deficiency is not a single disease entity. Both cases are homozygous E325K, the same genotype as the most severe German cases (D1). Thus, at the age of 7 years this genotype of α-NAGA may present as a 'non-disease' (present healthy case NL2.1) and can be associated with the vegetative state (D1 patients). This indicates that factors other than α-NAGA contribute to the phenotypic variation.

The bloodgroup A determinant, containing an αGalNAc moiety, has been considered as a contributing factor to the phenotype. This is very unlikely because the less affected sib of the D1 family had blood group A,[11] whereas both NL2 cases have blood group O and a different phenotype. It is unknown whether crisis situations (eg infections) may lead to exposure to high amounts of αGalNAc containing material and subsequent damage to the patients. There is no correlation between severity of the disease and the intensity of the abnormal oligosaccharides in urine. Comparison of the patterns of several urine samples from the proband NL2.2, his healthy sib and the two severe cases (D1) did not show significant differences.

The simplest explanation for the extreme variation in phenotypes would be that the severe infantile patients (D1) have a 'double disease': neuroaxonal dystrophy *in addition* to α-NAGA deficiency, without causal relationship. Accidental occurrence of two independent monogenic diseases in one patient is not as uncommon as one would think, particularly in consanguineous families (the parents of the D1 patients are consanguineous). For example, in the Clinical Genetics Centres of Rotterdam, Amsterdam and Leiden, seven cases are known. Also, one of the first patients with *β*-mannosidosis had mucopolysaccharidosis type III A as a second disease.[14]

A complete α-NAGA deficiency would cause a mild, late onset disease with angiokeratoma as manifested in patient J1.1 and the Spanish patients (E1).

The Dutch patient NL1.2, present age 8 years, is still without overt signs and symptoms (Drs Jan de Jong and Willy Renier, personal communication). Together with the present Dutch case NL2.1, they could be regarded as preclinical cases of α-NAGA deficiency which were detected by screening family members of the respective index cases. In this view, the infantile index cases are in fact also preclinical cases which were 'accidentally' detected during a routine urinary screening for lysosomal storage disorders. Further follow-up of these cases is important, but the future discovery of considerable numbers of new patients with α-NAGA deficiency will eventually establish which phenotypes are solely caused by α-NAGA deficiency and which other factors/genes play a role in other phenotypes. Meanwhile mutation analysis for infantile neuroaxonal dystrophy (Seitelberger disease) and characterisation of knock-out mice with α-NAGA deficiency should shed new light on the genotype-phenotype correlation in α-NAGA deficiency.

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