European Journal of Human Genetics (2000) 8, 557–560

www.nature.com/ejhg

SHORT REPORT

Mutation detection in the aspartoacylase gene in 17 patients with Canavan disease: four new mutations in the non-Jewish population

Erik A Sistermans[1], René FM de Coo[1,4], Hetty M van Beerendonk[1], Bwee Tien Poll-The[2], Wim J Kleijer[3] and Bernard A van Oost[1,5]

[1]Department of Human Genetics, University Hospital, Nijmegen; [2]Department of Metabolic Disorders, Wilhelmina Children's Hospital, Utrecht Medical Centre; [3]Department of Clinical Genetics, University Hospital, Rotterdam, The Netherlands

Canavan disease is a severe progressive autosomal recessive disorder, which is characterised by spongy degeneration of the brain. The disease is caused by mutations in the aspartoacylase gene. Two different mutations were reported on 98% of the alleles of Ashkenazi Jewish patients, in which population the disease is highly prevalent. In non-Jewish patients of European origin, one mutation (914C > A) is found in 50% of the alleles, the other alleles representing all kinds of different mutations. We here describe the results of the mutation analysis in 17 European, non-Jewish patients. Ten different mutations were found, of which four had not been described before (H21P, A57T, R168H, P181T). A deletion of exon 4, which until now had only been described once, was revealed in all five alleles of Turkish origin tested, indicating that this is a founder effect in the Turkish population. *European Journal of Human Genetics* (2000) **8**, 557–560.

Keywords: Canavan disease; aspartoacylase; mutation detection; *N*-acetylaspartic acid; prenatal diagnosis

Introduction

Canavan disease (OMIM 271900) is an autosomal recessive lethal neurodegenerative disorder characterised by spongy degeneration of the brain. The most common clinical features are hypotonia, megalencephaly, severe mental retardation, optic atrophy and early death. The disease is caused by a deficiency of aspartoacylase (ASPA), an enzyme that catalyses the hydrolysis of *N*-acetylaspartic acid (NAA) into aspartate and acetate.[1] The cloning of the *ASPA* gene (Genbank S67156) allowed mutation analysis for Canavan disease.[2] To date, more than 20 different mutations in the *ASPA* gene have been reported, including two Ashkenazi Jewish (98% of alleles) and one Pan-European (50%) founder mutation. In the non-Jewish population the other alleles consist of different mutations spread throughout the gene.[3–5]

Canavan disease is diagnosed by measuring the elevated levels of NAA in urine or cerebro–spinal fluid, or enzymatically in cultured skin fibroblasts.[6] Both methods, however, have drawbacks as there is a wide variation in the amount of NAA excreted and because the enzyme assay is complex and very sensitive to assay conditions. Furthermore, prenatal diagnosis on the basis of an enzymatic assay on aminocytes and chorion villi has been reported unreliable.[7] Therefore, preference should be given to molecular techniques for performing reliable prenatal diagnosis.

Materials and methods

Samples from 17 families reported to be from non-Jewish European descent were analysed. All patients were diagnosed by the demonstration of increased levels of NAA in urine. All patients were sporadic cases, except for the monozygotic twins of family 1, and the two brothers of family 16. In the latter family the patients had died and the parents were analysed in order to allow carrier analysis for their daughter. Genomic DNA was isolated by a simple salt-precipitation

Correspondence: Erik A Sistermans, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel: +31 24 36 13 799; Fax: +31 24 36 16 658; E-mail: E.Sistermans@antrg.azn.nl
[4]Current address: Department of Child Neurology, University Hospital, Rotterdam, The Netherlands
[5]Current address: Department of Clinical Sciences of Companion Animals, Utrecht, The Netherlands
Received 7 September 1999; revised 18 January 2000; accepted 21 January 2000

method.[8] Total RNA was isolated using RNAzol™ (CAMPRO Scientific, Veenendaal, The Netherlands). cDNA was generated with Superscript™ II Rnase H reverse transcriptase (Life Technologies, Breda, The Netherlands) using random sequence hexadeoxynucleotides (Promega, Leiden, The Netherlands).

The 914C > A mutation was analysed as described[9] although the forward primer was replaced by 632F (5'TTAACTCTTGATGGGAAGACGATC3') to allow the identification of the mutation at the DNA level. The same fragment was used for the analysis of the 854A > C mutation, which causes gain of both an *Eag*I and a *Not*I site. The 693C/T > A mutation, which results in gain of an *Mse*I site, was assayed at the DNA level by amplifying exon 5 with the primers 4F and 3R (see below).

The primer sets used for preparing the four overlapping fragments used for single-strand conformation polymorphism (SSCP) analysis and sequencing are:

1F 5'CTTTGATCTCTCTTCTGAATTGCA3';

1R 5'CAGGTCACAGTCAATATATCTGGTAC3';

2F 5'CAGGGCTGGAGGTAAAACCA3';

2R 5'GCATATTTGAGGGAAGGATG3';

3F 5'CATTACATTAAGACTTCTCTGGCT3';

3R 5'CCTGCAGATTAGGATGG3';

4F 5'AAGAATTTCCTCCCTGCGCC3';

4R 5'GTAAGACACCGTGTAAGATGTAAGCT3'.

SSCP analysis was performed on a GenePhor electrophoresis unit using the accompanying GeneGel Excel 12.5/24 kit. Fixation and staining (5') was performed in a Hoefer™ automated gel stainer using the PlusOne DNA silver staining kit (Amersham Pharmacia Biotech, Roosendaal, The Netherlands).

Sequencing of the fragments was performed using a dye terminator sequencing kit (PE-ABI, Nieuwerkerk a/d IJssel, The Netherlands). Samples were analysed on an ABI 373 automatic sequencer (PE-ABI).

The 62A > C mutation results in gain of a *Bst*NI site and loss of an *Nco*I site. The 169G > A mutation was analysed using primer 1F and primer 1647R (5'CAATATATCTGGTACACTTCTTCACAG3') which introduces an *Alu*I site which is disrupted by the mutation. PCR with primers HASP3B[4] and 1425F (5'CCTCAAATATGCGACCACGC3') creates an *Mlu*I site which is disrupted by the 503G > A mutation. Mutation 541C > A, results in gain of an *Rsa*I site.

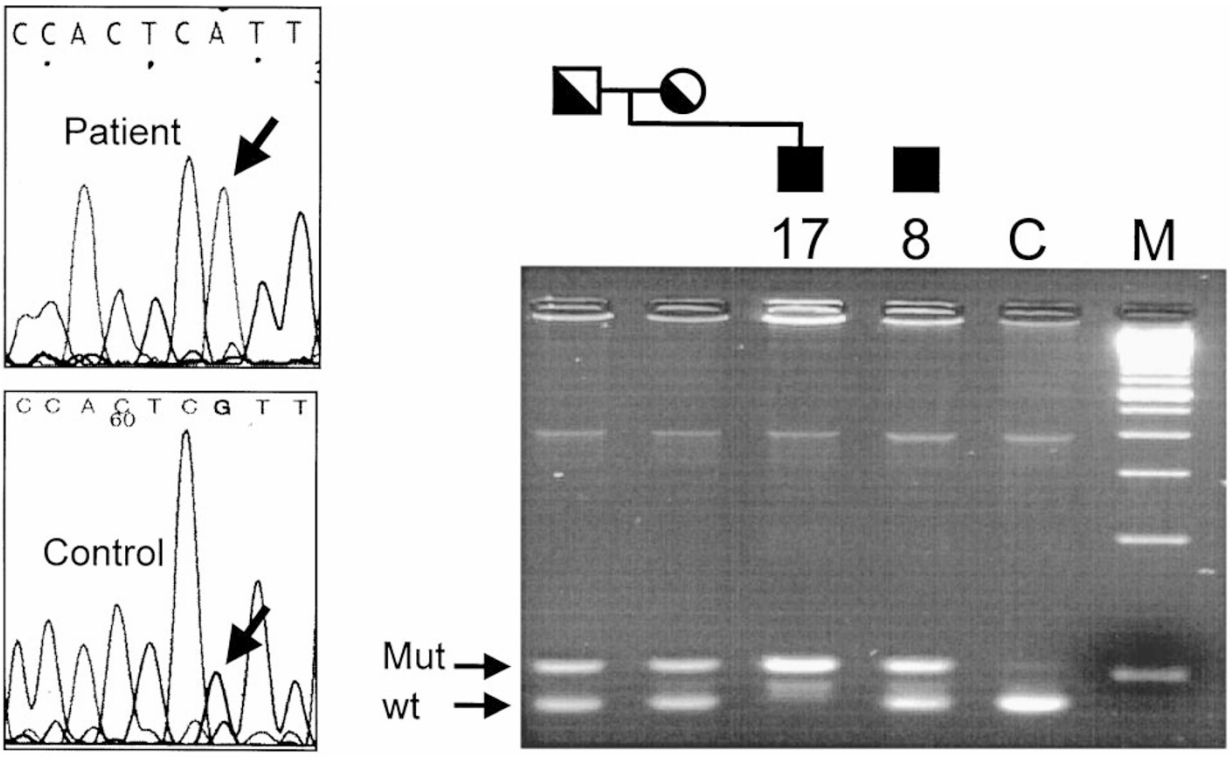

Figure 1 Mutation 503G - > A (R168H). Results of the sequence analysis are shown for patient 17, who is homozygote for this mutation (left panels). Digestion with *Mlu*I revealed that both parents of patient 17 were heterozygous carriers of this mutation, as was patient 8: C; control sample: M; 100bp marker, arrows indicate the wild-type and mutant bands.

Results

All patients were first analysed for the common pan-European 914C > A mutation. This mutation was found in 17 of the 34 alleles tested (50%), seven patients being homozygous and three being heterozygous. Of the common Jewish mutations only the 693C > A mutation was found on one allele of patient 10.

DNA samples of the 10 patients that were not homozygous for the 914C > A mutation were further analysed. Two patients were pre-screened by SSCP analysis followed by sequence analysis. This approach revealed 169G > A (A57T) and 244–245insA mutations in patient 12 and a 62A > C (H21P) mutation in patient 14.

DNA samples of the remaining eight patients were directly sequenced, revealing four more mutations. A 541C > A (P181T) transversion was found in both patients from family 1. Mutation 503G > A (R168H) was present heterozygous in the English patient and homozygous in a Belgian patient (Figure 1), whereas 79G > A (G27R) was found in patient 9. Finally an in-frame deletion of exon 4 was detected on all five alleles of Turkish origin tested.

In two patients (8, 13) where only one mutation was found, the mutation was homozygous at the cDNA level but heterozygous at the DNA level. Analysis of the 3' half of the cDNA of these patients revealed a faint band of increased length, not present in control cDNA (Figure 2). It was impossible to sequence the longer fragment, as it could not be purified at sufficiently high levels. Neither could the underlying mutation be identified at the DNA level, as the intronic sequences are unknown. A summary of the results is given in Table 1.

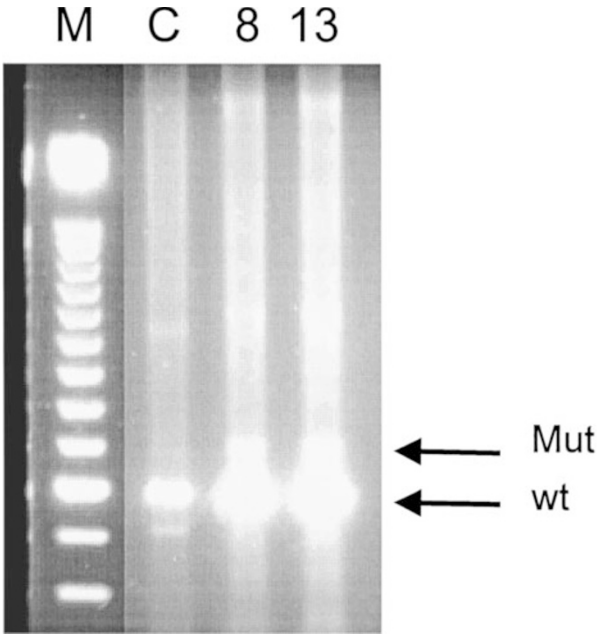

Figure 2 Putative splicing mutation in patients 8 and 13. In these patients a discrepancy was seen between sequencing results on cDNA (homozygous mutations) and DNA (heterozygous mutations). Generation of a 3' cDNA fragment comprising exons 3–6 revealed a faint larger fragment: C; control sample: M; 100bp marker, arrows indicate the wild-type and mutant bands.

Discussion

The mutational spectrum within the non-Jewish population is more complex than in the Ashkenazi Jewish population. (An overview of all known mutations is available at the Human Gene Mutation Database, http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html). Although the frequency of the common 914C > A mutation (50%) in the 17 patients was

Table 1 Overview of the results of the mutation analysis in 17 patients with Canavan disease. In those cases where the parents could be tested to prove that the mutations were located on different alleles, the paternal mutation is indicated as mutation 1, the maternal mutation as mutation 2. Mutations that have not been described before are in italics

Patient	*Mutation 1 DNA*	*Mutation 2 DNA*	*Mutation 1 protein*	*Mutation 2 protein*	*Parents tested*	*Ethnic background*
1	*541C->A*	del exon 4	*Pro181Thr*	193del36	yes	Dutch/Turkish
2	del exon 4	del exon 4	193del36	193del36	yes	Turkish
3	914C->A	914C->A	Ala305Glu	Ala305Glu	yes	Dutch
4	914C->A	914C->A	Ala305Glu	Ala305Glu	no	Czech
5	del exon 4	del exon 4	193del36	193del36	no	Turkish
6	914C->A	914C->A	Ala305Glu	Ala305Glu	no	Czech
7	914C->A	914C->A	Ala305Glu	Ala305Glu	no	Czech
8	*503G->A*	*splicing*	*Arg168His*	*unknown*	no	British
9	914C->A	79G->A	Ala305Glu	Gly27Arg	yes	Dutch
10	693C->A	–	Tyr231Ter	–	father	South African
11	914C->A	914C->A	Ala305Glu	Ala305Glu	yes	Dutch
12	244–245insA	*169G->A*	*Frameshift*	*Ala57Thr*	no	German
13	914C->A	*splicing*	Ala305Glu	*unknown*	no	Czech
14	*62A->C*	914C->A	*His21Pro*	Ala305Glu	yes	Dutch
15	914C->A	914C->A	Ala305Glu	Ala305Glu	yes	Belgian
16	914C->A	914C->A	Ala305Glu	Ala305Glu	yes	Dutch
17	*503G->A*	*503G->A*	*Arg168His*	*Arg168His*	yes	Belgian

comparable with earlier studies,[3–5] differences were observed between countries of origin. It was found on 88% of the eight Czech alleles but was absent in all five alleles of Turkish origin tested. These alleles all carried an in-frame deletion of exon 4 which has been described before in a Turkish patient born of consanguineous patients.[3] The results presented here assign this deletion as a common mutation in the Turkish population.

Three more recurrent mutations were identified, 79G > A,[4] 244–245insA[5] and 503G > A which has not been reported before, but was found on three independent alleles. These mutations should be screened for when molecular analysis is performed in non-Jewish patients.

Not much is known about the structural properties of aspartoacylase, making it difficult to predict the effect of the new mutations (H21P, A57T, R168H, P181T) on the enzymatic activity. However, all residues involved are highly conserved between the unrelated species human, bovine, and *Cynechocystis sp.*, H21, A57 and P181 even in *Prochlorococcus marinus*. Furthermore, the mutations were not found in 200 control alleles. Finally, most mutations involve non-conserved amino-acid changes, except for 503G > A. However, this mutation affects the same residue as the R168C mutation which renders the protein completely inactive.[4] Of high interest is the 62A > C mutation as it affects the His 21 residue, which is an invariable residue in the first esterase consensus sequence present in the protein. This residue was predicted to be part of a Ser, His, Glu triad that forms the catalytic domain.[3] In analogy with the common Jewish 854A > C mutation that involves the Glu residue of this triad, it can be predicted that substitution of the His residue will also result in inactivation of ASPA.

In conclusion, mutations found in Canavan disease patients not only differ between Jewish and non-Jewish patients, but also within the non-Jewish population. Prior knowledge of the ethnic background of a patient should therefore be obtained before mutation analysis is initiated.

References

1 Matalon R, Michals K, Sebasta D, Deanching M, Gashkoff P, Casanova J: Aspartoacylase deficiency in *N*-acetylaspartic aciduria in patients with Canavan disease. *Am J Med Genet* 1988; **29**: 463–471.
2 Kaul R, Gao GP, Balamurugan K, Matalon R: Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. *Nat Genet* 1993; **5**: 118–123.
3 Shaag A, Anikster Y, Christensen E *et al*: The molecular basis of Canavan (Aspartoacylase deficiency) disease in European non-Jewish patients. *Am J Hum Genet* 1995; **57**: 572–580.
4 Kaul R, Gao GP, Matalon R *et al*: Identification and expression of eight novel mutations among non-Jewish patients with Canavan disease. *Am J Hum Genet* 1996; **59**: 95–102.
5 Elpeleg ON, Shaag A: The spectrum of mutations of the aspartoacylase gene in Canavan disease in non-Jewish patients. *J Inher Metab Dis* 1999; **22**: 531–534.
6 Matalon R, Kaul R, Michals K: Canavan disease: Biochemical and molecular studies. *J Inher Metab Dis* 1993; **16**: 744–752.
7 Matalon R, Michals K, Gashkoff P, Kaul R: Prenatal diagnosis of Canavan disease. *J Inher Metab Dis* 1992; **15**: 392–394.
8 Millar SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
9 Kaul R, Gao GP, Aloya M *et al*: Canavan disease: Mutations among Jewish and Non-Jewish patients. *Am J Hum Genet* 1994; **55**: 34–41.