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Mutation analysis of the chromosome 14q24.3 dihydrolipoyl succinyltransferase (DLST) gene in patients with early-onset Alzheimer disease

Marc Cruts[a], Hubert Backhovens[a], Geert Van Gassen[a], Jessie Theuns[a], Sheng-Yue Wang[a], Anita Wehnert[a], Cornelia M. van Duijn[b], Thomas Karlsson[c], Albert Hofman[b], Rolf Adolfsson[c], Jean-Jacques Martin[d], Christine Van Broeckhoven[a,*]

[a]*Laboratory of Neurogenetics, Born-Bunge Foundation, University of Antwerp (UIA), Department of Biochemistry, Universiteitsplein 1, B-2610 Antwerpen, Belgium*

[b]*Department of Epidemiology and Biostatistics, Erasmus University Medical School, 3000 DR Rotterdam, The Netherlands*

[c]*Department of Psychiatry, University of Umeå, S-901 85 Umeå, Sweden*

[d]*Laboratory of Neuropathology, Born-Bunge Foundation, University of Antwerp (UIA), Department of Medicine, B-2610 Antwerpen, Belgium*

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Abstract

Linkage analysis studies have indicated that the chromosome band 14q24.3 harbours a major gene for familial early-onset Alzheimer's disease (AD). Recently we localized the chromosome 14 AD gene (AD3) in the 6.4 cM interval between the markers D14S289 and D14S61. We mapped the gene encoding dihydrolipoyl succinyltransferase (DLST), the E2k component of human α-ketoglutarate dehydrogenase complex (KGDHC), in the AD3 candidate region using yeast artificial chromosomes (YACs). The DLST gene is a candidate for the AD3 gene since deficiencies in KGDHC activity have been observed in brain tissue and fibroblasts of AD patients. The 15 exons and the promoter region of the DLST gene were analysed for mutations in chromosome 14 linked AD cases and in two series of unrelated early-onset AD cases (onset age < 55 years). Sequence variations in intronic sequences (introns 3, 5 and 10) or silent mutations in exonic sequences (exons 8 and 14) were identified. However, no AD related mutations were observed, suggesting that the DLST gene is not the chromosome 14 AD3 gene.

Keywords: Alzheimer disease; Alpha-ketoglutarate dehydrogenase complex; KGDHC; E2k component; Dihydrolipoyl succinyltransferase gene; DLST; chromosome 14; Mutation analysis; Polymorphisms

A genome-wide search has resulted in the localization of a gene for familial early-onset Alzheimer's disease (AD) on chromosome 14q24.3 in an interval of 22.7 cM between the markers D14S52 and D14S53 [19]. Chromosome 14 linkage of early-onset AD was also found independently by three other groups [16,21,22]. Since these initial chromosome 14 linkage reports many other investigators have reported linkage to chromosome 14 in their families [4,8,15,23], indicating that the chromosome 14 gene is a major gene for familial early-onset AD. More recently, we were able to reduce substantially the size of the candidate region for the chromosome 14 AD gene (AD3) based on informative recombinants in the two Belgian chromosome 14 linked early-onset AD families AD/A and AD/B [7]. The AD3 was localized between the markers D14S289 and D14S61, a region of 6.4 cM on the integrated genetic map of chromosome 14 [3]. Also, we constructed a physical map of the AD3 candidate region consisting of overlapping yeast artificial chromosomes (YACs) and estimated that the size of the AD3 candidate region is between 2 and 6 Mb [5,7]. The YAC contig map was used to map known genes that were previously assigned to chromosome 14 using different mapping methods. Three genes mapped within the AD3 candidate region, i.e. the cellular oncogene c-*fos* (FOS), the transforming growth factor β3 gene (TGFB3) and the dihydrolipoyl succinyltransferase gene (DLST) [5,7]. FOS has been excluded as the AD3 gene since sequence analyses

* Corresponding author. Tel. +32 3 8202601; fax: +32 3 8202541.

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Table 1
Primer sequences used in the SSCP analysis and sequencing of DLST

Region	Primer	Position	Sequence	Size
Promoter	E2k-3	−649 to −629	AGTTTGGCTGGAATTTGCGTT	462
	E2k-4	−188 to −210	GAGGTGGTTCTCCTGAAGGTGTA	
	E2k-52	−645 to −627	TGGCTGGAATTTGCGTTGG	(Sequencing primer)
Exon 1	E2k-19	−71 to −55	AGTGGTTCGCTCCGAAC	223
	E2k-20	151–135	AACAGACTCCTCACCCG	
Exon 2	E2k-21	543–565	GTTGCATTTACTTCGGAGTTACG	310
	E2k-22	852–831	CGTGATTATGTAGCATTGCGTC	
Exon 3	E2k-23	3540–3559	TATATCCGTTGCCGTTGATC	231
	E2k-24	3770–3744	CTTTATGTCTTTAAATAATAACCCGTG	
Exon 4	E2k-25	7116–7134	AAATGTGAGTGGTTCGCCT	280
	E2k-26	7395–7376	TGACAGATTCTGCAAACGCT	
Exon 5	E2k-27	7250–7273	TTTATGGGAAGAGCAACAATTGAT	306
	E2k-30	7494–7475	ACTCCAGGCTGGGAACAGAG	
Exon 6	E2k-5	7847–7870	CATATGTACTTTCTGCTGGCCCTG	220
	E2k-6	8066–8043	AAGCCCATGAAAAGCTGGTACGAA	
Intron 6	E2k-17	8669–8692	GAAGTGAATCTTCACAGCTAGCGA	207
	E2k-18	8875–8856	GGCAGGTGGAGCCATATCGT	
Exon 7	E2k-7	8995–9016	GTGATCCGCCTGCCTTGGCATC	296
	E2k-8	9292–9267	GAGGGAACACTGGAGACCTTGACCAC	
Exon 8	E2k-9	10793–10815	GCTGATACCCAGTAGACATTCGT	334
	E2k-10	11126–11102	CCTTTGAAGGAACATATTAGACGAG	
Exon 9	E2k-15	11327–11353	CTTGAAGATTAAGTAACAGTACATATG	266
	E2k-16	11592–11569	CATTCAAAGGTAGGCTACACCACA	
Exon 10	E2k-31	12293–12312	ATCTACTCGTGGCAAGCCGT	266
	E2k-32	12558–12537	ATAAGGAGTGGGGCAAGTTCGT	
Exon 11	E2k-33	16360–16385	CACCTAACTTGTGTATATGGATTGAG	285
	E2k-34	16644–16622	GGAGCAGGTCTGTGCTCATACAC	
Exon 12	E2k-35	17939–17964	ATATTACCTCATTAGTCTTGGCCTTC	339
	E2k-36	18277–18252	CTAGAATACCTTCATTACTCAGTGTG	
Exon 13	E2k-37	18293–18317	GGTTGAATTCAAGGGAGTTGTTAAC	260
	E2k-38	18552–18530	ACTTGACTTTCCATGAGCACTGC	
Exon 14	E2k-39	19056–19071	TCGGCCTCCCAAAGCG	288
	E2k-40	19343–19319	CCACTAGGACCTTAGAAGTGACGGT	
	E2k-51	19108–19132	GGGGTGGCTTAATATTTCAATTATG	(Sequencing primer)
Exon 15	E2k-11	20134–20159	AATACTGTAAATATGCAGAGCGTAAC	255
	E2k-12	20388–20370	TCTGGGATCCTCTACCGCT	
	E2k-13	20282–20300	TGCGGCCCATGATGTACGT	237
	E2k-14	20518–20496	ACAGCATGTGTCTGCCTGTCACC	

The position of the primers is according to the numbering of Nakano et al. [17] starting at exon 1. The size of the PCR products is indicated in bp. The PCR amplification of 200 ng genomic DNA was performed in a total volume of 25 μl according to standard procedures. The PCR sequencing was performed as described in the text. In case of the promoter region and exon 14, internal primers were designed for sequencing.

did not reveal AD3 related mutations in patients belonging to different chromosome 14 linked early-onset AD families [2,6,18].

The DLST gene which codes for the E2k component of the mitochondrial α-ketoglutarate dehydrogenase complex (KGDHC), is a candidate for the AD3 gene because reductions in KGDHC activity have been observed in AD patients. KGDHC is a multicomponent mitochondrial enzyme complex that catalyses the rate-limiting step of oxidative decarboxylation of α-ketoglutarate to succinyl-CoA in the tricarboxylic acid cycle. A defect of this enzyme complex could explain the energy metabolism defects observed in brain and fibroblasts of AD patients [13,20]. In AD brain the neuronal degeneration may be explained by excitotoxicity of glutamate, a brain neurotoxin accumulating because of the KGDHC deficiency [13]. In cultured fibroblasts of both AD and chromosome 14 linked AD patients, immunoblotting identified an abnormality of the E2k component of KGHDC [20]. The DLST gene coding for E2k, was cloned and mapped to chromosome 14q24.3 by somatic cell hybrid mapping and in situ hybridisation [1]. We used the YAC contig map to localize the DLST gene in the AD3 candidate region between the linked markers D14S43 and D14S284 [5,7].

In this study we performed a mutation analysis of the DLST gene in patients with proven chromosome 14 linked AD and in patients belonging to two series of unrelated AD cases with onset of AD below or at age 55 years. The cut-off age of 55 years was chosen because in the majority of the chromosome 14 linked families the

mean age at onset is before this age [9]. The AD patients with chromosome 14 linked AD are AD/A-V43 and AD/B-IV15 belonging to families AD/A and AD/B [22], and two patients of each family FAD1, FAD2 and FAD4 [21], namely AG7647A, AG6849A, AG8526, AG8562A, AG8109A and AG7877 of which cell lines were obtained through the NIA cell repository. The two series of early-onset AD patients contained 21 patients with a mean onset age of 50.2 ± 1.9 years (range 47–55 years) obtained in a population-based study of early-onset AD in the area of Rotterdam, The Netherlands [11], and 20 patients with mean onset age of 51.2 ± 3.3 years (range 45–55 years) clinically ascertained at the University Hospital in Umeå, Sweden (Adolfsson et al., unpublished data). Each of the patients received a diagnosis of presenile dementia that fulfilled the NINCDS-ADRDA criteria of probable AD [14]. Also, mutation analysis of exons 16 and 17 of the amyloid precursor protein (APP) gene at chromosome 21q21.2, using both single stranded conformational polymorphism (SSCP) analysis and direct PCR sequencing had not revealed any mutations in these patients ([24]; Adolfsson et al., unpublished data).

First we examined all 15 exons of the DLST gene for mutations by SSCP analysis. For this purpose intronic primers flanking each exon were designed based on the published genomic sequence of the DLST gene [17]. For the larger exon 15, two overlapping primer sets were used. The sequences of each primer pair used in the SSCP analysis are illustrated in Table 1. Each exon was PCR amplified under standard conditions and the PCR products were heat denatured and separated by electrophoresis at room temperature on a 1 × Hydrolink MDE gel (J.T. Baker, Phillipsburg, USA) in the presence or absence of 10% glycerol. The electrophoresis conditions of 13 h at 800 V allowed the analysis in one experiment of both the heteroduplex and SSCP. The DNA fragments were visualized by silver-staining [10]. Clearly altered SSCP patterns were detected when exons 5 and 11 were analysed in the presence of glycerol and exons 8 and 14 in the absence of glycerol. The change in the SSCP pattern of exons 5, 8, 11 and 14 was the same in each case, suggesting that the same sequence variation was present. Further, the altered SSCP patterns of exons 5, 8, 11 and 14 were present in some but not all of the chromosome 14 linked AD and early-onset AD patients. Also, since for each of the exons 5, 8, 11 and 14, homozygotes were detected for each SSCP allele, the data suggested that the sequence variations in these exons are polymorphisms and not AD related mutations. No polymorphic variations were detected for the $(GTT)_5$ repeat located in intron 6 [17], when genomic DNA of the 49 AD patients was PCR amplified using primers E2k-17 and E2k-18 (Table 1).

The sequences of exons 5, 8, 11 and 14 were determined after PCR amplification of genomic DNA of patients AD/A-V43 and AD/B-IV15, and one escapee, AD/A-V30 and AD/B-IV5, of each of the two Belgian chromosome 14 linked families AD/A and AD/B [12]. An escapee was defined as a healthy individual of whom the current age was at least two standard deviations beyond the mean age at onset of 35 years in the family. For sequencing, the PCR products of six identical PCR reactions were pooled and the PCR products were purified by the Spinbind Purification System of FMC (Rockland, USA). The sequences of both strands were determined with the fluorescent T7 terminator system of Applied Biosystems (Foster City, USA) and were analysed on an automated DNA sequencer model 373A of Applied Biosystems (Foster City, USA). In each case a mutation was found that could explain the altered bands in the SSCP analysis (Table 2). Two of the mutations are intronic sequence variations, a diallelic $(T)_n$-repeat in intron 5 and a transition of A to G in intron 10. The mutations in exons 8 and 14 are silent mutations at third codon bases at codons 192 and 366, respectively. None of the mutations were AD specific since they were found in both the AD patients and escapees, confirming our previous observation that the altered SSCP patterns represent DLST polymorphisms. The allele frequencies for each DLST polymorphism were calculated from the SSCP results obtained in the whole population of 49 AD patients and were 0.57/0.43 for the intron 5 polymorphism, 0.81/0.19 for the exon 8 polymorphism, 0.46/0.54 for the intron 10 polymorphism and 0.44/0.56 for the exon 14 polymorphism.

Table 2
DLST polymorphisms

Primers	Location of polymorphism	Nucleotide position	Type of polymorphism
E2k-25, E2k-26	Intron 3	7160	C insertion
E2k-27, E2k-30	Intron 5	7461	$(T)_n$ repeat
E2k-9, E2k-10	Exon 8	11044	G to A transition
E2k-31, E2k-32	Intron 10	16439	G to A transition
E2k-39, E2k-40	Exon 14	19183	C to T transition

The position of the polymorphisms is according to the numbering of Nakano et al. [17] starting at exon 1.

Linkage analysis in families AD/A and AD/B using the exon 14 DLST polymorphism showed complete segregation of DLST with AD. Consequently we sequenced all other 11 exons and the promoter region of DLST (Table 1). No AD related sequence differences were observed. However, one more polymorphism was detected in intron 3 where a C insertion was observed in patient AD/A-V43 and escapee AD/B-IV5 (Table 2).

In conclusion, the mutation analysis of DLST in chromosome 14 linked AD patients and patients with early-

onset of AD before or at age 55 years has not revealed mutations that are AD related. In total, five polymorphic sequence variations were detected, three in, respectively, introns 3, 5 and 10, and two in, respectively, exons 8 and 14. The latter two polymorphisms, although in coding sequences, do not cause a change at the protein level since they are both silent mutations at third codon bases. The absence of AD related mutations in the coding region and the promoter of DLST excludes this gene as candidate for AD3.

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