Gene 526 (2013) 176–181

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Apolipoprotein E genotype and LRP1 polymorphisms in patients with different clinical types of metachromatic leukodystrophy

Agnieszka Ługowska [a,*], Małgorzata Musielak [a], Ewa Jamroz [b], Antoni Pyrkosz [c], Tomasz Kmieć [d], Anna Tylki-Szymańska [e], Małgorzata Bednarska-Makaruk [a]

[a] Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland
[b] Department of Child Neurology, Medical University of Silesia, Katowice, Poland
[c] Department of General and Molecular Biology and Genetics, Medical University of Silesia, Katowice, Poland
[d] Department of Neurology, The Children's Memorial Health Institute, Warsaw, Poland
[e] Department of Metabolic Diseases, The Children's Memorial Health Institute, Warsaw, Poland

ARTICLE INFO

Article history:
Accepted 10 May 2013
Available online 20 May 2013

Keywords:
Metachromatic leukodystrophy
Apolipoprotein E
LDL receptor-related protein
Lysosomal storage disease

ABSTRACT

Metachromatic leukodystrophy (MLD) is a severe, neurodegenerative, metabolic disease which is caused by deficient activity of arylsulfatase A (ARSA). Sulfatides and other substrates of ARSA are stored in central and peripheral nervous systems, and in some other organs. Accumulated sulfatides are especially toxic to oligodendrocytes and Schwann cells leading to progressive demyelination. The kind of apolipoprotein E (apoE) isoform is of essential significance for the modulation of sulfatide quantity in the brain as apoE4 contains more sulfatides than apoE3. Taking into consideration the fact that apoE4 leads to the loss of sulfatides in CSF of Alzheimer's disease patients, we examined if apoE isoforms display any impact on clinical outcome in patients with different forms of MLD in whom sulfatides accumulate. The significant association of age at the onset of MLD symptoms with *APOE ε2/ε3/ε4* and *LRP1* c.766C>T polymorphisms was shown in multivariate stepwise regression analysis, in which other factors known to affect age at onset of the disease, i.e. clinical type of MLD, family connection of the patient and sex were also analyzed. As expected, the clinical type of MLD explained about 80% of the variance of the dependent variable. The impact of both polymorphisms on age of onset of the disease was considerably lower: 2.0% in the case of *APOE* polymorphism and 1.0% in the case of *LRP1* polymorphism. Thus, the clinical outcome in MLD patients is related principally to the genotype of the *ARSA* gene, while the polymorphisms in the *APOE* and *LRP1* genes are only slightly modifying factors.

1. Introduction

Metachromatic leukodystrophy (MLD) is a severe, neurodegenerative, metabolic disease which is caused by deficient activity of a lysosomal hydrolase–arylsulfatase A (ARSA; EC 3.1.6.8). Sulfatides and other substrates of ARSA are stored in central and peripheral nervous systems, and in some other organs like kidneys and gallbladder. Accumulated sulfatides are especially toxic to oligodendrocytes and Schwann cells leading to progressive demyelination.

Based on the age at onset and clinical outcome of the disease, three main MLD types are distinguished: I) late infantile type (0–2 yr., severe), II) juvenile type (4–6 yr.) and late juvenile (8–17 yr.), and III) adult type (in adults, mild).

All forms of MLD are inherited in an autosomal recessive trait and are caused by mutations in the *ARSA* gene coding for the protein of ARSA.

Human apolipoprotein E (apoE) is a 34 kDa protein whose sequence is coded by the gene *APOE* localized on chromosome 19. Thanks to its ability of interaction with the LDL receptor and LRP1 protein (LDL receptor-related protein), apoE plays a major role in transport and metabolism of plasma cholesterol and triglycerides. ApoE is engaged in the redistribution of cholesterol and phospholipids in the central nervous system, where *APOE* gene is expressed mainly in astrocytes and microglia (Grehan et al., 2001). Apart from its function as a lipid transporter, apoE participates also in pathologic processes like Alzheimer's disease (AD) interacting with beta-amyloid (Aβ) (Bales et al., 2002). ApoE polymorphisms were also shown to be associated with age at onset or clinical severity in some other neurodegenerative disorders (Chapman et al., 2001).

Three isoforms of apoE are distinguished in human beings, which differ in the amino acid composition at the positions 112 and 158 of this protein. The most frequent is apoE3 (Cys112, Arg158), then — apoE4

Abbreviations: MLD, metachromatic leukodystrophy; ARSA, arylsulfatase A; apoE, apolipoprotein E; LRP1 protein, LDL receptor-related protein; AD, Alzheimer's disease; Aβ, beta-amyloid.

* Corresponding author at: Department of Genetics, Institute of Psychiatry and Neurology, Al. Sobieskiego 9, 02-957 Warsaw, Poland. Tel.: +48 22 4582 610; fax: +48 22 858 91 69.
E-mail address: alugipin@yahoo.com (A. Ługowska).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.05.009

(Arg112, Arg158), and the least frequent is apoE2 (Cys112, Cys158). These polymorphic variants of apoE are encoded by three alleles of the same genomic locus. It has been observed that in neurons cultured in the presence of Aβ, the addition of exogenic apoE4 caused the leakage of lysosomes and activation of apoptotic processes, whereas the presence of apoE3 showed protective properties (Ji et al., 2002). Additionally, apoE4 had higher influence on the cell membrane instability than apoE3 (Ji et al., 2006).

Sulfatides represent a class of sulfated galactocerebrosides, differing in composition of fatty acids. These compounds intermediate in many of biological processes e.g. regulation of cell growth, protein transport, signal transduction, cell adhesion, neuronal plasticity, and morphogenesis. Sulfatides are a major compound of the myelin sheath of axons (Vos et al., 1994). They are stored in massive quantities in MLD but their loss was observed in the early stages of AD (Han et al., 2002). Sulfatides are almost exclusively synthesized in oligodendrocytes and in Schwann cells. Alternatively, sulfatides can be imported into neurons on the way dependent on receptors involved in the endocytosis of lipoproteins. Sulfatides are localized in the HDL-like lipoproteins containing apoE and present in the cerebro-spinal fluid. ApoE lipoproteins released by astrocytes 'collect' sulfatides from the myelin surface most probably by the mechanism of 'kiss-and-run' and eventually enter the neurons by means of LDL and LRP receptors (Cheng et al., 2010). The kind of apoE isoform is of essential significance for the modulation of sulfatide quantity in the brain as apoE4 contains more sulfatides than apoE3 (Han et al., 2003).

Although a genotype–phenotype correlation for MLD causing mutations has been proposed, it is not very strict and especially in later onset forms of MLD the clinical picture of the disease may vary between patients with the same genotype. Taking into consideration the fact that apoE4 leads to the loss of sulfatides in CSF of AD patients (Han et al., 2003), it is interesting to study if apoE isoforms display any impact on clinical outcome in patients with different forms of MLD in whom sulfatides accumulate due to the lack of ARSA activity.

Gene *LRP1* is situated on the long arm of chromosome 12 (12q13–14). It is composed of 89 exons and it codes for LRP1 protein consisting of 4544 amino acids.

LDL-receptor related protein (LRP1) is a multifunctional protein that binds over 30 different ligands, among which are apolipoprotein E, lipoprotein lipase, and alfa-2-macroglobulin. LRP1 plays an important role in the metabolism of the lipoproteins rich in triglycerides and containing apoE. LRP1 is also called 'remnant receptor', since it mediates in collecting the remnants of chylomicrons and VLDL by hepatocytes from the blood stream (Havel and Kane, 2001). LRP1 is synthesized in atherosclerotic plaques mainly by macrophages and myocytes of smooth muscles. LRP1 is also involved in the catabolism of proteases and their inhibitors, and synthesis and degradation of beta-amyloid in the brain. LRP1 and its ligands were present in senile plaques in the brains of patients with Alzheimer's disease (Hyman et al., 2000). Apart from the transporter function LRP1 intermediates in signaling processes in the nervous system cells as well as in cells of the blood vessel wall (Herz and Strickland, 2001).

In the *LRP1* gene a few polymorphic sites have been identified. The main impact on the risk of Alzheimer's disease has polymorphism c.766 C>T (p.D100D) in exon 3 and the tetranucleotide polymorphism [TTTC]n located in the 5′ flanking region of the *LRP1* gene. These polymorphisms do not cause changes in the amino acid sequence of the polypeptide chain of LRP1.

The aim of this study was to identify the apoE polymorphisms as well as the polymorphism c.766C>T in the *LRP1* gene in Polish patients with different types of metachromatic leukodystrophy, and to establish a correlation of obtained results with the phenotype presented by patients.

2. Materials and methods

This study was reviewed and approved by the Bioethics Committee at the Institute of Psychiatry and Neurology. The material consisted of DNA samples received from 62 Polish patients with different types of MLD: 21 persons with late infantile form, 20 — with juvenile (early and late juvenile together) form, and 21 adults. Among them there were 7 pairs of sibling. The diagnosis of MLD was confirmed biochemically by the measurement of ARSA activity in isolated blood leukocytes and, if it was possible, by determination of sulfatide excretion in urine sediment. Screening for the most frequent mutations in the *ARSA* gene, i.e., c.459+1G>A, p.P426L. p.I179S was performed, followed by sequencing analysis of the *ARSA* gene as described earlier (Ługowska et al., 2010).

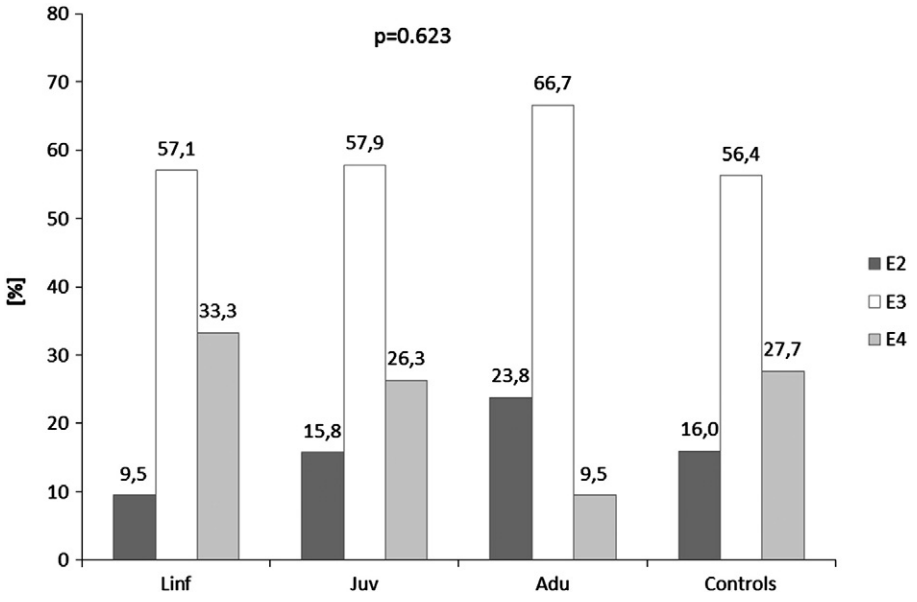

Fig. 1. *APOE* genotypes in patients with different forms of metachromatic leukodystrophy. E2 — ε2/2 and ε2/3 genotypes; E3 — ε3/3 genotype; E4 — ε4/3 genotype. ε4/2 genotype was excluded from the analysis.