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ORIGINAL COMMUNICATION

Genetic variants of methionine metabolism and X-ALD phenotype generation: results of a new study sample

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Abstract X-linked adrenoleukodystrophy (X-ALD) is the most common inherited leukodystrophy. Nevertheless, no genotype–phenotype correlation has been established so far. Unidentified modifier genes or other cofactors are suspected to modulate phenotype and prognosis. We recently described polymorphisms of methionine metabolism as possible disease modifiers in X-ALD. To retest these findings, we analyzed 172 new DNA samples of X-ALD patients from different populations (France, Germany, USA, China) by genotyping eight genetic variants of methionine metabolism, including DHFR c.594+59del19bp, CBS c.844_855ins68, MTR c.2756A>G, MTHFR c.677C>T and c.1298A>C, MTRR c.60A>G, RFC1 c.80G>A, and Tc2 c.776C>G. We compared three X-ALD phenotypes: childhood-onset cerebral demyelinating inflammatory type (CCALD; $n = 82$), adulthood onset with focal cerebral demyelination (ACALD; $n = 38$), and adulthood onset without cerebral demyelination (AMN; $n = 52$). The association of genotypes and phenotypes was analyzed with univariate two-sided Pearson's χ^2. In the comparison between AMN and CCALD, the G allele of Tc2 c.776C>G was associated with X-ALD phenotypes ($\chi^2 = 6.1$; $P = 0.048$). The prevalence of the GG genotype of Tc2 c.776C>G was higher in patients with CNS demyelination compared to those without CNS demyelination ($\chi^2 = 4.42$; $P = 0.036$). The GG genotype was also more frequent in CCALD compared to AMN ($\chi^2 = 4.7$; $P = 0.031$). The other polymorphisms did not show any significant associations in this study sample. Whereas the influence of other polymorphisms of methionine metabolism was not confirmed, the present study supports the previously made observation that the Tc2 genotype contributes to X-ALD phenotype generation.

Keywords Leukoencephalopathy · Neurogenetics · Association studies in genetics · Leukodystrophies · Peroxisomes · Metabolic disease (inherited)

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Introduction

X-linked adrenoleukodystrophy (X-ALD, OMIM #300100) is caused by defects of the *ABCD1* gene on chromosome Xq28, resulting in an impairment of peroxisomal beta oxidation and the accumulation of saturated very long chain fatty acids (VLCFA). The minimum frequency of hemizygotes plus heterozygotes is 1:16,800 in the United States, suggesting that X-ALD is the most common inherited leukodystrophy [3].

X-ALD is characterized by a highly variable clinical spectrum. It ranges from cerebral demyelinating, inflammatory childhood phenotypes associated with a poor prognosis (CCALD), to milder adulthood disease with focal demyelination in the central nervous system (ACALD), to adulthood forms without central nervous system demyelination and considerably slower symptom progression (AMN). The appearance of cerebral demyelination is crucial to the phenotype and prognosis of X-ALD as well as therapy for it. The development of the phenotype cannot be predicted by biochemical factors like levels of VLCFA. In addition, no genotype/phenotype correlation has been established [13]. The clinical course can range widely, even within closely related patients. Thus, as yet unidentified modifier genes or other cofactors are likely to modulate the phenotypic variation and disease severity [5–8].

One possible X-ALD disease modifier may be methionine metabolism. Methionine metabolism plays a crucial role in DNA methylation and in providing methyl groups necessary for building brain myelination. It is also involved in the generation of glutathione that is necessary for defense against oxidative stress and in the regulation of inflammatory processes [15]. The metabolism exhibits marked interindividual variety due to the existence of functionally relevant polymorphisms and differences in vitamin availability, which are important cofactors in methionine metabolism (vitamin B12, B6, folic acid). We have previously reported that polymorphisms of methionine metabolism are associated with the clinical phenotype of X-ALD. The insertion allele of cystathionine beta-synthase (CBS) c.844_845ins68 was significantly more common in AMN patients than in the cerebral demyelinating forms CCALD and ACALD in a sample of 86 Dutch and German patients [12]. In addition to that, the GG genotype of transcobalamin 2 (Tc2) c.776C>G was more common in ACALD than in AMN, and a combined risk genotype was more common in ACALD than in AMN. The combined risk genotype was defined as the presence of at least one of the following genotypes: transcobalamin 2 (Tc2) c.776C>G (GG), methylenetetrahydrofolate reductase (MTHFR) c.677C>T (TT), or methionine synthase (MTR) c.2756A>G (AG/GG) [9]. Here we studied the genotypes of 172 additional X-ALD patients to retest our previous data.

Materials and methods

Patients

We analyzed 172 DNA samples: 34 DNA samples from China (CCALD: $n = 21$; ACALD: $n = 9$; AMN: $n = 4$), 110 samples from France (CCALD: $n = 50$; ACALD: $n = 25$; AMN: $n = 35$), 8 DNA samples from Germany (ACALD: $n = 4$; AMN: $n = 4$), and 20 samples from the USA (CCALD: $n = 11$; AMN: $n = 9$). Patients from China were of Han ethnicity; patients from France, Germany and USA were of Caucasian origin. All patients or their trustees have given written informed consent. The study was approved by the local ethical committees.

Genotyping

Genomic DNA prepared from peripheral leukocytes was used for genotyping by PCR amplification and restriction analysis of eight genetic variants of methionine metabolism, including the intronic deletion dihydrofolate reductase (DHFR) c.594+59del19bp (affecting the transcript level; GenBank NM_000791.3), the splice alteration cystathionine beta-synthase (CBS) c.844_855ins68 (affecting the transcript level; GenBank S78267.1), and the missense mutations (i.e., leading to amino acid exchanges) methionine synthase (MTR) c.2756A>G (p.D919G; rs1805087), methylenetetrahydrofolate reductase (MTHFR) c.677C>T (p.A222V; rs1801133) and c.1298A>C (p.E429A; rs1801131), methionine synthase reductase MTRR c.60A>G (p.I49M), reduced folate carrier 1 (RFC1) c.80G>A (p.R27H; rs1051266), and transcobalamin 2 (Tc2) c.776C>G (p.P259R; rs1801198) [10].

Statistics

The association of genotypes and phenotypes was analyzed with univariate two-sided Pearson's χ^2 tests. As this is a confirmatory study, we did not correct for multiple testing.

Results

Analyses with two degrees of freedom (all genotypes) revealed an association of the G allele of Tc2 c.776C>G with the X-ALD phenotype in the comparison between AMN and CCALD ($\chi^2 = 6.09$; $P = 0.048$), but not in the comparison between AMN and the pooled demyelinating phenotypes CCALD+ACALD, $\chi^2 = 4.74$; $P = 0.093$. The other polymorphisms did not reveal significant associations in this study sample (Table 1). When only the Caucasian patients without the patients from China or subgroups divided by populations were analyzed, the differences in the frequencies of the Tc2 genotype among different X-ALD phenotypes were not significant (not shown). The prevalence of the GG genotype of Tc2 c.776C>G was higher in patients with CNS demyelination than in those without CNS demyelination ($\chi^2 = 4.42$; $P = 0.036$). Additionally, the GG genotype was more frequent in patients with the most severe phenotype, CCALD, than in patients with AMN ($\chi^2 = 4.71$; $P = 0.031$) (Table 2).

Table 1 Result of all polymorphisms analyzed and X-ALD phenotypes: CCALD ($n = 82$), ACALD ($n = 38$), and AMN ($n = 52$)

Tc2 c.776C>G	CC	CG	GG	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.19	0.42	0.39	$P = 0.048$	$P = 0.093$
ACALD	0.34	0.32	0.34		
AMN	0.35	0.44	0.21		
MTR c.2756A>G	AA	AG	GG	CCALD vs. AMN	CCALD/ACALD vs. AMN
CCALD	0.72	0.25	0.03	$P = 0.364$	$P = 0.393$
ACALD	0.78	0.22	0		
AMN	0.69	0.31	0		
MTHFR c.677C>T	CC	CT	TT	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.42	0.39	0.19	$P = 0.940$	$P = 0.970$
ACALD	0.31	0.53	0.16		
AMN	0.40	0.42	0.18		
MTHFR c.1298A>C	AA	AC	CC	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.59	0.33	0.08	$P = 0.721$	$P = 0.964$
ACALD	0.42	0.53	0.05		
AMN	0.51	0.41	0.08		
CBS c.844_845ins68	dd	di	ii	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.89	0.11	0	$P = 0.659$	$P = 0.989$
ACALD	0.89	0.11	0		
AMN	0.89	0.11	0		
MTRR c.60A>G	AA	AG	GG	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.14	0.42	0.44	$P = 0.226$	$P = 0.502$
ACALD	0.24	0.54	0.22		
AMN	0.15	0.57	0.28		
RFC1 c.80G>A	GG	AG	AA	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.31	0.47	0.22	$P = 0.948$	$P = 0.822$
ACALD	0.27	0.43	0.30		
AMN	0.33	0.46	0.21		
DHFR c.594+59del19bp	dd	di	ii	CCALD vs. AMN	CCALD+ACALD vs. AMN
CCALD	0.14	0.51	0.35	$P = 0.253$	$P = 0.238$
ACALD	0.12	0.54	0.34		
AMN	0.06	0.48	0.46		

Table 2 Tc2 c.776-GG genotype and X-ALD phenotypes: CCALD ($n = 82$), ACALD ($n = 38$), and AMN ($n = 52$)

Tc2 c.776C > G	CC/CG	GG	Pure AMN vs. CCALD	Pure AMN vs. CCALD + ALMN
CCALD	0.61	0.39	$P = 0.031$	$P = 0.036$
ACALD	0.66	0.34		
AMN	0.79	0.21		

Discussion

In the present study consisting of 172 patients with CCALD, ACALD and AMN, we found that the G allele of the Tc2 c.776C>G polymorphism is associated with the occurrence of CNS demyelination in X-ALD patients. The G allele of Tc2 c.776C>G leads to the amino acid substitution p.P259R that seems to affect the affinity of the transcobalamin 2 protein to vitamin B12 and the ability to transport vitamin B12 into tissues [1, 2, 4]. Vitamin B12 is necessary to synthesize *S*-adenosylmethionine, which serves as methyl group donor for CNS myelination. Accordingly, vitamin B12 deficiency can lead to demyelination of the central nervous system in otherwise healthy individuals [16].

Methotrexate is a widely used chemotherapeutic drug that may lead to neurotoxic side effects accompanied by cerebral white matter changes. Methotrexate reduces the vitamin B12-dependent synthesis of *S*-adenosylmethionine

in the central nervous system [14]. In a series of 68 patients treated with methotrexate, the G allele and the GG genotype of Tc2 c.776C>G were associated with the occurrence of white matter changes in the brain [11]. We conclude that it is unlikely that the GG genotype of Tc2 c.776C>G is the main determinant of the different X-ALD phenotypes, but this observation may serve as an indicator of a role of the methionine metabolism in X-ALD disease progression in general. We speculate that the influence of the GG genotype of Tc2 c.776C>G on the functional availability of vitamin B12 and SAM synthesis may dispose X-ALD patients to demyelination of the central nervous system.

In a previous study with a sample of 86 Dutch and German patients, we observed an association of X-ALD phenotypes with additional genetic variants of the methionine metabolism [9, 12]. In the actual sample we only confirmed the association of the G allele of the polymorphism Tc2 c.776C>G with the occurrence of CNS demyelination in X-ALD patients, whereas the other previous observations were not confirmed. We cannot completely exclude differences between the previous and the actual patient samples that might have contributed to the differences in the results. Dietary influences concerning the uptake of amino acids or vitamins may speculatively modify effects of the analyzed polymorphisms on X-ALD phenotype generation, and dietary conditions might have differed between the old and the new patient sample. However, we conclude that only the influence of the Tc2 polymorphism is validated by the results of the present study, whereas the other previously reported findings must be assumed to be false positives so long as they are not confirmed in an additional X-ALD patient sample.

These data are limited, as the association is only weakly significant. When corrected for multiple testing or analyzed in subgroups divided up by populations or ethnicity, the results were not significant. However, this study was of a confirmative nature, and as the results concerning the Tc2 variant confirmed our previous observations in an independent sample of X-ALD patients, we consider the influence of the Tc2 c.776C>G variant on phenotype generation in X-ALD to be confirmed.

An examination of the effect of experimentally induced vitamin B12, folate and SAM depletion in addition to vitamin B12, folate and SAM substitution on the phenotype of X-ALD mouse models appears to be warranted in order to estimate whether clinical studies investigating the effect of vitamin or SAM substitution on the clinical course of X-ALD are justified.

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