Cathepsin D Polymorphism C224T in Childhood-Onset Neurodegenerative Disorders: No Impact for Childhood Dementia

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Abstract

Keywords

- lysosomal dysfunction
- childhood dementia
- neurodegenerative disorders
- cathepsin D polymorphism

Compromised lysosomal functioning has been identified as a major risk factor for neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Furthermore, the association between a defined cathepsin D (*CTSD*) polymorphism and a higher risk of sporadic Alzheimer's disease has been established for particular populations. Here, we analyzed 189 children with rare neurodegenerative disease for carrying the T-allele by polymerase chain reaction-restriction fragment length polymorphism. We found no statistical differences in genotype and allele frequencies between the neurodegenerative group and European descent participants of genetic studies using the Cochran–Armitage's trend test. In contrast to adult-onset neurodegenerative diseases, analysis of clinical datasets of children carrying the T-allele did not demonstrate differences to the general disease group.

Introduction

A couple of hundred different childhood and adolescent-onset neurodegenerative disorders are known—most of them are neurometabolic diseases including lysosomal, mitochondrial, and peroxisomal disorders, as well as inborn errors of amino acid and organic acid metabolism. Clinical signs are multiple intellectual and motor losses of already attained development skills due to generalized dysfunction of the central nervous system.

Compromised lysosomal function has been identified as a major risk factor for neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Recently, Lee et al underlined the pathophysiological relevance of lysosomal dysfunction for Alzheimer's disease by linking presenilin1 mutations

to a lysosomal pH shift and a consecutive dysfunction of lysosomal hydrolases.¹ Parkinson's disease has been associated with heterozygous mutations in the *GBA1* gene coding for the lysosomal enzyme glucocerebrosidase.² Further, the accumulation of gangliosides and autophagic vacuoles has been associated with the intraneuronal accumulation of proteins related to Alzheimer's disease.³

In addition, the association between a defined cathepsin D polymorphism and a higher risk of sporadic Alzheimer's disease has been shown for particular populations. The polymorphism C224T (p.Ala58Val) located in exon 2 of the cathepsin D (*CTSD*) gene was associated with the occurrence of sporadic Alzheimer's disease in the adult German population^{4–6} as well as in Iranian and Ecuadorian patients with sporadic late-onset Alzheimer's disease.^{7,8} In other adult

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populations, for example, the Spanish,⁹ Italian,¹⁰ Korean,¹¹ and North American,¹² this association could not be found.

Mutations in *CTSD* are the primary cause of congenital neuronal ceroid lipofuscinosis (CLN10) with accumulation of sphingolipid activator protein D in all cell types leading to severe neurological deterioration and death, usually before adulthood.¹³ In contrast, the C224T polymorphism of *CTSD* is associated with secondary dysfunction of the lysosomal compartment. These secondary changes of the lysosomal compartment have been described for Alzheimer's, Parkinson's, and Huntington's diseases.¹⁴ Particularly in Alzheimer's disease, it has been demonstrated that the lysosomal hydrolyses are localized in neurons and glial cells close to senile plaques.^{15,16} Furthermore, it has been found that the endosomal-lysosomal system is upregulated in Alzheimer's disease.^{17,18} Animal and tissue culture experiments with lysosomal cathepsin inhibitors demonstrated the induction of A β -containing amyloid precursor protein fragments in neurons.^{19,20}

So far, the pathophysiological role of the *CTSD* C224T polymorphism in regard to the function of lysosomes is uncertain. The theoretical considerations are based on the description from Touitou et al²¹ that the p.Ala58Val substitution in the profragment of cathepsin D is potentially associated with a higher amount of secreted pro-cathepsin D and a decreased maturation of cathepsin D to its active form in a pulse chase experiment in breast cancer cells.

Thus, the aim of this study is to determine the prevalence of the *CTSD* C224T polymorphism in inborn childhood-onset neurodegenerative disorders as well as to determine the relevance of a potential secondary dysfunction of the lysosomal compartment to the phenotypes of neurodegenerative disorders including classical lysosomal storage disorders other than cathepsin D deficiency.

Methods

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

The origin of the tested DNA was from stored diagnostic DNA, dry blood spot cards, and fibroblasts cultures. Overall, we analyzed 189 patient samples from children with neurodegenerative disease for bearing the T-allele by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

A 328 bp fragment of exon 2 containing the dbSNP rs17571:C > T was amplified by polymerase chain reaction using oligonucleotide primers (forward: 5'-tttcagggtgacaggcaggagtttgg-3' and reverse: 5'-agcagggtgaagccctactactacg-3') and the HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The optimized PCR protocol started with an initial heat activation step at 95°C for 15 minutes. The three-step amplification program with 32 cycles included 30 seconds at 95°C, 30 seconds at 63°C, and 25 seconds at 72°C, followed by a final extension period of 10 minutes at 72°C.

The amplicon was digested with the restriction endonuclease Mwo I (New England Biolabs, Frankfurt, Germany) and products were analyzed on a 1.5% agarose gel. The rs17571 C-allele was

associated with distinctive fragments of 162 and 82 bp, whereas the T-allele yielded a fragment of 237 bp.

Population Data and Statistical Methods

The established genotype and allele frequencies were compared with different datasets of whole exome sequencing cohorts, in which European descent was separately stated online at dbSNP (dbSNP:rs17571C > T). These are shown in detail in the ClinSeq: A Large-Scale Medical Sequencing Clinical Research Pilot Study (<https://www.genome.gov/20519355/clinseq-a-large-scale-medical-sequencing-clinical-research-pilot-study/>), the ESP: NHLBI Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), and the ExAC: Exome Aggregation Consortium (<http://exac.broadinstitute.org/>), at which the datasets of the first described cohorts ClinSeq and ESP are included. The population includes 36,313 participants (72,626 alleles) of European descent. Unfortunately, clinical information about the participants were very cursory but were predominantly patients with cardiovascular disease. Nevertheless, the inclusion of individuals with monogenic neurodegenerative diseases can be largely excluded by the age of the individuals.

The allele frequencies between the different collectives and the Göttinger cohort were compared by the Cochran-Armitage's trend test for genetic analyses,²² using the functions of the package "coin" for the statistic software R.²³ The significance level was set to $\alpha = 5\%$.

Results and Discussion

This study describes the first analysis of the *CTSD* polymorphism C224T in pediatric patients. A total of 189 children with very rare inborn neurodegenerative disease diagnosed by genetic or enzymatic testing were analyzed.

► **Table 1** lists the patients' genotypes and allele frequencies of the Göttinger cohort determined by PCR-RFLP. The genotypes of the compared populations as well as their *p*-values for their comparison with the Göttinger cohort are listed in ► **Table 2**.

The observed allele frequencies were 0.9153 for C-allele and 0.0847 for T-allele. These allele frequencies were very similar to almost all compared populations, yielding a test result between $p = 0.72$ and $p = 0.91$. Simply, the Finish population has an allele frequency significantly different from the Göttinger cohort and the other European populations (► **Fig. 1**). This is in accordance to the finding from the ExAC that classified the Finns as a population of their own because they are so distinctive in terms of their genotype.²⁴ Therefore, it is not remarkable that this genetic group differs also in the analyzed cathepsin D polymorphism.

The allele frequency for the T-allele of each subgroup varies from 0 to 50% depending on the number of individuals in the respective subgroup. As a result of the small case size, no statement can be made about the subgroups. The list of subgroups and their allele frequencies in ► **Table 1** are purely informative. In conclusion, the prevalence of the cathepsin D polymorphism C224T in our whole cohort of children with inborn neurodegenerative disease was comparable to the genetic background of European. This remains true, despite

Table 1 Diagnoses, genotypes, and allele frequencies of C224T polymorphism in a Göttinger pediatric cohort of childhood-onset neurodegenerative diseases

	n	Genotype						Allele frequency	
		CC	CT	TT	C	T			
Lysosomal disorders									
Metachromatic leukodystrophy	19	16	84.2%	3	15.8%	0	0.0%	0.921	0.079
Multiple sulfatase deficiency	1	0	0.0%	1	100.0%	0	0.0%	0.500	0.500
Globoid cell leukodystrophy (Krabbe's disease)	8	7	87.5%	1	12.5%	0	0.0%	0.938	0.063
GM1 gangliosidosis	5	5	100.0%	0	0.0%	0	0.0%	1.000	0.000
GM2 gangliosidosis	7	7	100.0%	0	0.0%	0	0.0%	1.000	0.000
Fabry disease	4	4	100.0%	0	0.0%	0	0.0%	1.000	0.000
Mucopolysaccharidoses	16	11	68.8%	5	31.3%	0	0.0%	0.844	0.156
Neuronal ceroid lipofuscinoses	77	65	84.4%	12	15.6%	0	0.0%	0.922	0.078
Mitochondrial disorders									
Mitochondrial complex II deficiency	2	1	50.0%	0	0.0%	1	50.0%	0.500	0.500
Miscellaneous									
Alexander's disease	6	5	83.3%	1	16.7%	0	0.0%	0.917	0.083
Megalencephalic leukoencephalopathy with subcortical cysts	7	5	71.4%	2	28.6%	0	0.0%	0.857	0.143
Vanishing white matter	32	28	87.5%	3	9.4%	1	3.1%	0.922	0.078
Aicardi–Goutières' syndrome	1	1	100.0%	0	0.0%	0	0.0%	1.000	0.000
Leukoencephalopathy with involvement of brain stem and spinal cord and elevated white matter lactate	4	4	100.0%	0	0.0%	0	0.0%	1.000	0.000
Total	189	159	84.1%	28	14.8%	2	1.1%	0.9153	0.0847

Table 2 Comparison of genotypes and allele frequencies of C224T polymorphism in populations of European descent and the Göttinger pediatric cohort

Cohort			Genotype						Allele frequency		Difference to Göttinger cohort (<i>p</i> -Value)	
		<i>n</i>	CC		CT		TT		C	T		
ClinSeq ^a			659	560	0.8498	94	0.1426	5	0.0076	0.9211	0.0789	0.72
ESP ^b			4,299	3,646	0.8481	609	0.1417	44	0.0102	0.9189	0.0811	0.81
ExAc1 ^c	European (non-Finnish)	33,022	27,783	0.8413	4993	0.1512	246	0.0075	0.9169	0.0831	0.91	
ExAc2 ^c	European (Finnish)	3,291	2,976	0.9043	308	0.0936	7	0.0021	0.9511	0.0489	< 0.01	
ExAc3 ^c	Total	36,313	30,759	0.8471	5301	0.1460	253	0.0069	0.92	0.08	0.74	
GÖ ^d			189	159	0.8413	28	0.1481	2	0.0106	0.9153	0.0847	–

Note: Populations with European descent (adults). *n* = count of participants.

^aClinSeq: A Large-Scale Medical Sequencing Clinical Research Pilot Study (<https://www.genome.gov/20519355/clinseq-a-largescale-medical-sequencing-clinical-research-pilot-study/>).

^bESP: NHLBI Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>).

^cExAC: Exome Aggregation Consortium (<http://exac.broadinstitute.org/>).

^dGÖ: Göttinger pediatric cohort.

Fig. 1 This vertical-bar chart illustrates the similarity of genotypes in populations of European descent and the Göttinger pediatric cohort.

the size of the cohorts, which limits the detection of very small differences. Such small differences would in any case have no significance for the majority of children in our collective group.

We further analyzed available clinical datasets from six patients with the polymorphism in heterozygote C/T (five patients) or homozygote T/T (one patient) form. The diagnosis of these children was neuronal ceroid lipofuscinosis in three patients, metachromatic leukodystrophy in two patients, and globoid cell leukodystrophy in one patient, respectively. An analysis of the age of onset and disease progression in these patients did not demonstrate obvious differences from the general disease group. Thus, in contrast to adult-onset neurodegenerative diseases, the polymorphism did not seem to influence the natural disease course for this pediatric patient group.

Conclusion

We conclude that, in contrast to adult-onset neurodegenerative diseases, a potential subtle affection in lysosomal function by *CTSD* C224T polymorphism (p.Ala58Val) is probably not significant for the neurodegeneration in childhood-onset neurodegenerative diseases. Nevertheless, longer observation periods are needed to thoroughly investigate a potential influence of this cathepsin D polymorphism on disease onset and progression in childhood neurologic disorders.

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