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Genetic Test and Gene Therapy for Krabbe Disease: An Update

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Abstract

Globoid cell leukodystrophy, also known as Krabbe disease, is an inherited metabolic neurodegenerative disease, due to genetic mutation of β-galactocerebrosidase gene. Here we reviewed how the technological advances in gene analysis have enhanced the enrichment of mutation database. Moreover, we focus on the possibility to develop genetic treatments, hoping that the updating of genetic, clinical and biochemical data will improve for an early and efficient diagnosis, until a definitive treatment option will be identified.

Keywords: Globoid cell leukodystrophy; β-galactocerebrosidase; Lysosomal storage disease

Introduction

Leukodystrophies are genetic inheritance disorders of nervous system. They cause progressive neurological disability, both in young [1] and in adult people. The compromised formation of myelin sheath around most of axons in the central nervous system (CNS) or in peripheral nervous system (PNS) is linked to deficiency in oligodendrocytes or Schwann cells, respectively. It is known the myelin sheath is an extension of their plasma membrane which, in lipids-enriched, wraps the axon. The central and peripheral myelin formation is essential for the survival of vertebrates [2].

Most of the genetically determined pathologies are associated with mutations in oligodendrocytes myelin proteins or connexins, the molecular entities forming gap junctions. Similarly, mutations in myelin or gap junction proteins of Schwann cells lead to neuropathies. Krabbe disease (KD), also called Globoid Cell Leukodystrophy (GLD), is a disorder in oligodendrocytes lipid metabolism caused by occurring mutations in β-galactocerebrosidase (GALC) gene [3] or in very rare cases by lack of active saposin A [4]. GALC is located on chromosome 14q31 and encompasses 17 exons. The codified protein is a lysosomal enzyme that catalyzes the degradation of galactose from galactosylceramide and galactosylsphingosine and it acts during myelin formation and turnover. KD patients report impaired activity of GALC and psychosine storage in lysosome and in other compartmets of cell membrane. The result is the disappearance of myelin-generating cells [5]. The storage of non-metabolized products, due to a defect in a hydrolytic enzyme, activator protein, transport protein, or enzyme required for the correct processing of other lysosomal proteins is typical of lysosomal storage diseases (LSDs) [6].

In the last decade, researches about Krabbe disease have been emphasized, as shown by the increased number of published items. Some works are aimed to elucidate pathophysiology, but generally, the field of speculation is more and more addressed to identify and characterize the occurring mutations and to evaluate if a clear genotype/phenotype correlation is possible to realize. Here, we reviewed how the technological advances in gene analysis have enhanced the recent investigations and helped the development of genetic treatments.

Identification of GALC Mutation and Prediction Analysis

GALC gene (MIM#606890; GenBank accession No. NM_000153.3) was mapped to chromosome 14 by multipoint linkage analysis [7,8]. Its linear nucleotide sequence open reading frame is 2,058 nucleotides and has 45.92% GC content. Mutation analysis of the human GALC gene was facilitated by cloning [9] and sequencing of GALC cDNA [10,11]. The Human Gene Mutation Database (http://www.hgmd.org) reports over 130 variants in the GALC gene, which mostly associated with an affected phenotype. Nonsense, missense, small insertion, and small deletion mutations spanning the entire length of the GALC gene have been described. These mutations are increased in quantity in the last period by Reverse-transcription PCR and direct sequencing approach. In 2013, a novel homozygous mutation c.727delT (p.S243QfsX7) was found in a consanguineous Turkish family [12]; four novel GALC gene mutations in two Chinese patients [13].

Considering that the most common mutation for the infantile phenotype is a 30-kb deletion in the homozygous state or in trans with another mutation associated, a combined strategy to further explore the deletion/duplication mutation spectrum of the GALC gene has been developed by Tanner et al.: the sequence analysis is combined with mutation-specific testing for the 30-kb deletion. When two mutations are not found using this approach, despite an established biochemical diagnosis for the patient, a targeted array comparative genomic hybridization (CGH) is performed to look for copy number changes within the GALC gene [14]. In this way, the lacking data on the presence of these types of mutations could be obtained. Moreover, in order to establish the functional relevance of mutations detected, in silico analysis tools are employed, as MutPred [15].

Gene Technology Applied to KD Diagnosis

The normal range for GALC activity is between 0.60 and 3.29 nmol/h/mg protein and, generally, GALC mutation flows into lower or lost activity of lysosomal enzyme. The measure of GALC activity is performed by biochemical assay using radioactive [16] or fluorescent

[17] galactosylceramide substrate in blood leukocytes or in cultured skin fibroblasts.

It was demonstrated that GALC activity in newborn dried blood spots is a highly sensitive test, even when samples have been stored for many years [18]. However, a serious diagnostic problem is an overlap in GALC activity ranges between the control groups and carriers when multiple polymorphic changes occur in the GALC gene. It was reported a case of a high residual activity of GALC obtained in leukocytes and fibroblasts of a KD patient. This parameter was misinformative for the diagnosis. Thus, the sequencing analysis of the GALC gene has been recommended to confirm the diagnosis in problematic patients that show discrepancies or doubts between biochemical results and clinical phenotype [19]. In the reported case, the failure of enzyme activity determination could be related to different factors. First of all, the enzyme mutation may preserve the ability to hydrolyze the fluorogenic substrate, or the tested samples contained not substrate-specific enzymes that can be involved in the degradation of the artificial substrate. Fortunately, this case seems to be isolate, and the biochemical assays for GALC activity remain sensitive methods to KD diagnosis. The gene sequencing should be associated when the clinical picture, the family history and the biochemical information are hard to synchronize, due to the rarity of disease.

Moreover, genetic screening is a particularly useful diagnostic tool in families when parents are known carriers. In these cases, PCR-based tests are designed to check for a specific mutation and are employed in prenatal diagnosis by using amniotic or chorionic villus cells [20].

Gene Technology in Therapy

The possibility of GALC enzyme deficiency correction is under investigation by using cDNA vectors. Different studies were performed in the mouse model of KD, the twitcher mouse. Multiple injections-intracerebroventricularly, intracerebellarly, and intravenously-of AAVrh10-GALC was reported useful to optimize delivery and overall cross-correction: high enzyme activity was achieved in the brain and cerebellum, and moderate to high activity was detected in the spinal cord and the sciatic nerve of mice. Furthermore, treated newborn mice successfully lived up to 8 months despite the 40 days of untreated animals [21]. More recently, the same group demonstrated that mice receiving a single intravenous injection of AAVrh10-GALC at PND10 had no tremor and continued to gain weight until a few weeks before they died. An extend life span up to 20-25 days respect to untreated mice was reported [22].

Functional GALC gene was transferred in the brain of twitcher newborn mice by lentiviral vector (LV) with a proficient transduction of proliferating and post-mitotic oligodendroglia, a relevant target cell type in GLD [23].

Moreover, the mammalian artificial chromosomes (MACs) have been studied as alternatives to viral vectors for gene therapy applications. Among them, the satellite DNA-based artificial chromosome expression vehicle (ACE) has been described as a versatile platform for ex vivo gene therapy strategies for KD. ACEs-treated twitcher mice carrying a therapeutic gene lived longer than untreated counterparts [24,25]. This gene therapy method is called combined mammalian artificial chromosome-stem cell therapy. In 2014, a novel method to load multiple genes onto the ACEs by using two selectable marker genes was described to target the treatment of more complex disorders [26].

A potential treatment for Krabbe disease is the transplantation of hematopoietic stem cells (HSCT). HSCT treats the disease by introducing donor-derived GALC-positive cells of hematopoietic origin; the enzyme produced and secreted by the donor cells can then be endocytosed by neighbouring cells via cross-correction. Numerous studies in humans and animal models have shown varying degrees of benefit with HSCT. Benefits are reported in patients with infantile-and juvenile-[27-29] and adult-onset [30] disease, when treated prior to the development of neurological symptoms.

Conclusion

Krabbe disease is a rare autosomal recessive leukodystrophy. Still now, a primary relevance is addressed to identify mutations and clarify their correlation with clinical aspects.

The genetic techniques are the only reliable method to enrich the GALC pathogenic mutation database, while a deeper information exchange and social communications are needed to increase public awareness of Krabbe disease all over the world.

Finally, we would like to emphasize that gene analysis should be more linked to biochemical and clinical data in order to facilitate the diagnosis of this rare and difficult neurological diseases, and give support to patients and their own family.

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