The GALT rush: High carrier frequency of an unusual deletion mutation of the GALT gene in the Ashkenazi population

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Classic galactosemia is an autosomal recessive disorder of galactose metabolism manifesting in the first weeks of life following exposure to a milk-based diet. Despite the benefit of avoidance of lactose, many patients suffer from long-term complications including neurological deficits and ovarian failure. To date, over 230 mutations have been described in the GALT gene resulting in galactosemia. Recently, an unusual mutation was characterized causing a 5.5 kb deletion, with a relatively high carrier rate in subjects of Ashkenazi Jewish (AJ) descent. The aim of this study was to estimate the carrier frequency of this mutation in the AJ population in Israel. For this purpose we developed a high-throughput methodology to genotype both normal and deleted alleles using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer and Multiplex PCR.

DNA samples of 760 anonymous AJ subjects were submitted for analysis, subsequently detecting six individuals heterozygous for the GALT deletion mutation, giving a carrier frequency of 1 in 127 (0.79%). Based on these results, we suggest that the method described here provides a basis for genetic screening and prenatal counseling and can potentially reduce the morbidity and mortality associated with delayed diagnosis of galactosemia in this patient population.

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1. Introduction

Classic galactosemia is an autosomal recessive disorder (OMIM 230400) of galactose metabolism manifesting in the first few days of life. However, despite avoidance of lactose, many patients with classical galactosemia suffer from long-term complications including verbal apraxia, cognitive and developmental delays and primary or premature ovarian failure [1–3].

Mutation in the GALT gene is the most common cause of galactosemia and over 230 variations have been reported to date [4], of which the most prevalent among Caucasian populations is Q188R [5]. Loss of galactose-1-phosphate uridylyl transferase (GALT) enzymatic activity prevents the conversion of galactose-1-phosphate (Gal-1-P) and UDP-glucose into glucose-1-phosphate and UDP-galactose. This causes the accumulation of Gal-1-P in various organs leading to the clinical signs and symptoms described earlier [6].

In contrast to the United States, where all states routinely include galactosemia in all newborn screening programs, this is not the case in Israel.

Recently, an unusual deletion mutation of the GALT gene detectable by Southern analysis was characterized [7]. PCR walking strategy has enabled the mapping of the mutation breakpoints, unraveling a non-contiguous 5.5 kb deletion which includes most of the gene, retaining only a short internal segment. A common ethnic origin of several unrelated patients with this mutation was Ashkenazi Jews. Examination of this mutation in Ashkenazi patients diagnosed
with galactosemia and followed at our center showed that 7 out of 8 Ashkenazi alleles had the mutation.

The aim of this study was to estimate the carrier frequency of this mutation in the Ashkenazi Jewish population in Israel. For this purpose we have developed a high-throughput methodology to genotype both normal and deleted alleles using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer. Our results provide a basis for prenatal counseling for this population.

2. Materials and methods

Analysis of the GALT deletion in the Ashkenazi population.

2.1. Subjects

760 DNA samples from random non-related anonymous subjects of Ashkenazi Jewish descent were available for molecular diagnosis at the metabolic disease unit, Sheba Medical Center, Tel-Hashomer.

2.2. Genotyping methodology

GALT mutated and normal alleles were genotyped using a chip-based matrix-assisted laser desorption-time-of-flight (MALDI-TOF) mass spectrometer (Sequenom, San Diego, CA). Polymerase chain reaction (PCR) and extension reactions were designed using MassARRAY® software (Sequenom). We sought to find the carrier frequency of specific mutations in the Ashkenazi population using this advanced method.

PCR amplifications were carried out in standard 384-well plates, a 5 μl final volume containing 10 ng of template DNA, 0.15 U of Taq polymerase (HotStarTaq, Qiagen, Valencia, CA, USA), 0.5 mM of each dNTP, 100 nmol of each primer, 1.625 mM MgCl2 and 1.25× HotStar buffer. The following primers were used: for the mutated allele

F: 5′-ACGTTGGATGAGCTTCTGAAGACAGTGCCC-3′ and for the normal allele

R: 5′-GTGTGATTTCCCCACCCACAGGG-3′. The primers were designed to give rise to amplicons of different length allowing the detection of both the deleted and normal alleles. PCR conditions were: 4 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 5 s at 52 °C and 5 s at 72 °C. Following this step, 3 μg MassEXTEND® cleanup resin (Sequenom, San Diego, CA) and 25 μl of Double-distilled water (DDW) were added to remove extraneous salts. Samsung nanodispenser was used to apply 15 nl of extension products from each well of the sample plate onto the SpectroChip. Mass spectra were recorded on a Bruker Biflex MALDI-TOF mass spectrometer operated in the linear mode, and were analyzed by MassARRAY Typer software (Sequenom, San Diego, CA).

2.3. Multiplex PCR

The GALT deletion positive samples, as determined by the SEQUENOM MassArray, were reconfirmed by multiplex PCR assay using primer pair flanking the deletion (F: 5′-AGTACCAGGGAGAATTAAATTGAGATT-3′ and R: 5′-GTTGATTCCCCACCCACAGG-3′) and a third primer within the deletion (5′-CATACTCTCTGGCCTGG-3′). The primers were designed to give rise to amplicons of different length allowing the detection of both the deleted and normal alleles. PCR conditions were: 4 min at 95 °C, followed by 35 cycles of 45 s at 95 °C, 60 s at 59 °C, 45 s at 72 °C, and a final extension at 72 °C for 10 min.

PCR products were resolved by electrophoresis in 2% agarose gel and visualized on UV-transilluminator after ethidium bromide staining.

3. Results

The allele frequency of the GALT deletion was estimated using MALDI-TOF mass spectrometry method designed to detect both mutated and normal alleles. We developed a multiplex assay based on two sets of primers for the amplification of the GALT alleles, followed by primer extension reaction, generating extension products that differ in their mass values allowing us to distinguish between homozygous, heterozygous, and wild type (WT) subjects (Fig. 1).

Analysis of 760 DNA samples from individuals of Ashkenazi descent detected six heterozygous for the GALT deletion mutation.
and North American Caucasians (1:30,000 to 1:60,000). The one per 64,500 live births, similar to the carrier rate seen in Europeans Ashkenazi Jews is 1:127 indicating a galactosemia incidence of at least based high-throughput assay that can be used for large-scale analysis. For this purpose, we have developed a novel MALDI-TOF and therefore led us to investigate the mutation carrier frequency in this population. For this purpose, we have developed a novel MALDI-TOF
diagnosed with galactosemia suggests a putative founder mutation
identi
its carriers to appear as homozygotes for the genotype of their other
diagnosis of the deleted allele in all our Ashkenazi patients
demonstrating the possible genotypes: 475-bp product was obtained only if a deletion allele was present, whereas the 330-bp product was obtained only if a normal allele was present.

4. Discussion
The GALT deletion mutation analyzed in our study has been previously reported in several patients of Ashkenazi Jewish descent [7]. Molecular genotyping with simple hybridization methods for allel-specific mutations commonly used for genetic diagnosis of galactosemia following positive newborn screening would miss this deletion, causing its carriers to appear as homozygotes for the genotype of their other allele, thus resulting in possible misinterpretation and misdiagnosis [8].

The recent finding of the aforementioned deletion along with the identification of the deleted allele in all our Ashkenazi patients diagnosed with galactosemia suggests a putative founder mutation and therefore led us to investigate the mutation carrier frequency in this population. For this purpose, we have developed a novel MALDI-TOF based high-throughput assay that can be used for large-scale analysis.

Our data shows that the carrier rate of the deletion mutation in Ashkenazi Jews is 1:127 indicating a galactosemia incidence of at least one per 64,500 live births, similar to the carrier rate seen in Europeans and North American Caucasians (1:30,000 to 1:60,000) [1,9,10]. The heterozygote frequency found in other Ashkenazi Jewish disorders, namely Bloom syndrome [11], Maple syrup urine disease (MSUD) [12] and Mucolipidosis IV [13] of which population screening is currently available, resembles the heterozygote frequency found in our Ashkenazi Jewish cohort.

In view of the life altering complications galactosemia patients, even under strict diet, are prone to develop [1,2], and the relatively high carrier frequency of the GALT mutation, population screening and prenatal counseling for this disorder should be considered in the future. Analysis by MALDI-TOF mass spectrometry (when large numbers of samples are screened) as well as by multiplex PCR assay (for the day-to-day prenatal screening) described here can serve as an efficient and reliable tool for this purpose.

Notably, our results have led to an appeal to the Israeli Ministry of Health and subsequent approval for implementation of the method described here in population screening for this disease in people of Ashkenazi Jewish descent. Analysis of the initial experience with this prenatal screening during the first eight months of its use at our center shows that of 808 subjects of Ashkenazi origin screened, 5 carriers of the deletion mutation were detected, thus indicating a carrier frequency of 1:162 (0.62%).

References


