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Brief Communication

Founder mutation causing infantile GM1-gangliosidosis in the Gypsy population

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Abstract

The Gypsies are a trans-national founder population of Asian descent, whose genetic heritage is still incompletely characterized. Here, we describe the first founder mutation leading to a lysosomal storage disorder in this population: R59H in *GLB1*, which causes infantile GM1-gangliosidosis. The R59H carrier rate is ~2% in the general Gypsy population and ~10% in the Rudari sub-isolate. Haplotype analysis suggests that the Gypsy diaspora may have contributed to the spread of this mutation to South America.

Keywords: Infantile GM1-gangliosidosis; β-Galactosidase (GLB1) gene; Gypsy population; Founder mutations; Genetic diagnosis

Introduction

The Roma/Gypsies are a young founder population of Asian origins nowadays scattered across the world, whose demographic history has contributed to strong drift effects and a high frequency of autosomal recessive disease-causing mutations [1]. Previous studies of inborn errors of metabolism have focused on disorders covered by mass newborn screening: phenylketonuria and medium chain acyl-coenzyme A dehydrogenase deficiency, caused by mutations "imported" from the surrounding populations (reviewed in [2]), and galactokinase deficiency, where a unique ancestral mutation accounts for Gypsy patients across Europe [3]. Here, we report on the first lysosomal storage disorder found to occur at high frequency in this population: infantile GM1-gangliosidosis (MIM # 230500), where deficiency of acid β-galactosidase (EC 3.2.1.23) leads to accumulation of glycosaminoglycans and glycopeptides in visceral organs, and of GM1-ganglioside in neural tissue [4]. The infantile form is characterized by early onset, massive nervous system involvement, skeletal dysplasias, visceromegaly, and rapid progression to death [5]. The global incidence of infantile GM1-gangliosidosis is estimated at 1:100,000–300,000, with higher frequencies observed in the Maltese Islands (1:3700) and southern Brazil (1:17,000) [6,7]. This study was prompted by the observed over-representation of Gypsy patients among cases of infantile GM1-gangliosidosis diagnosed in Bulgaria over 25 years (expected 2–3, observed 11 out of 25 cases), suggesting an ancestral mutation and founder effect.

Subjects and methods

The study included 12 affected Gypsy families, 11 residing in Bulgaria and one in Italy [8,9], none of which were aware of any relatedness. The total number of affected children was 15, including two prenatally

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diagnosed fetuses. Informed parental consent was obtained from all families. The study complies with the ethical guidelines of the institutions involved.

The age at the time of referral ranged between 3 and 9 months. Affected children presented with facial dysmorphism (100%), retarded psychomotor development (100%), hepatosplenomegaly (100%), muscle hypotonia (82%), and ocular abnormalities, including a cherry red spot (55%). Bone abnormalities and limited joint mobility were documented in five cases. Two patients had skin abnormalities. Two others, referred by a paediatric cardiology clinic, had hypertrophic cardiomyopathy. The age at death, known in six cases, was <2 years. Total urinary glycosaminoglycan excretion [10] was normal. Thin-layer chromatography of urinary oligosaccharides [11] identified a characteristic pattern, suggesting GM1-gangliosidosis. β-Galactosidase enzyme activity, determined using 4-methylumbelliferyl β-D-galactopyranoside [12], was 0.9–7.8 nmol/h/mg protein in white blood cells (normal range 72–390), 6–17.4 nmol/h/mg protein in fibroblasts (normal range 375–1100), and 1.3 and 5.7 nmol/h/mg protein in cultured amniotic fluid cells (normal range 350–1150 nmol/h/mg protein). β-Glucuronidase, used as a control enzyme, was normal.

Sequencing analysis of the *GLB1* gene on chromosome 3p21.33 [13,14] was performed using primers as described [15] and ABI Big Dye 3.1. chemistry. The products were run on an ABI 377 DNA Analyzer, with Sequence Navigator used for data analysis. Intragenic *GLB1* polymorphisms L10P (rs7637099), L12L (rs7614776), R521C (rs4302331), and S532G (a putative mutation/polymorphism not listed in SNP databases) [15] were analysed by sequencing exons 1 and 15. Population screening for the R59H mutation was done in a panel of 413 anonymous controls, representing different Gypsy sub-isolates in Bulgaria [16,17]. We used a PCR-based RFLP assay (*NlaIII* site created by the mutation) where exon 2 was amplified with a HEX-labeled forward primer 5′-catccttgctctgtagaatg-3′ and reverse primer 5′-acagttgtatcttctctccag-3′, and restriction fragments were length-separated on an ABI 377 DNA Analyzer.

Results and discussion

We identified a G > A transition in exon 2 of *GLB1*, c.176G > A, resulting in the substitution of histidine for a highly conserved arginine residue at position 59 (R59H). Bulgarian Gypsy disease chromosomes shared a conserved intragenic polymorphic haplotype (Table 1), supporting the assumption of a common ancestral mutation.

R59H is a known mutation reported previously in GM1-gangliosidosis patients in other parts of the world: two homozygous siblings from Italy (patients 2.1 and 2.2 in [8,9]) and eight affected subjects from Brazil (patients 1–8 in [15]). To test the possibility of a common origin, we re-examined the family history of the affected siblings diagnosed in Italy and established that the parents were Gypsy migrants from the Balkans. Analysis of *GLB1* polymorphisms showed that these patients were homozygous for the same haplotype as observed in Gypsy disease chromosomes from Bulgaria (Table 1). In Brazil, R59H accounts for over 20% of GM1-gangliosidosis alleles (in 9 out of the 40 disease chromosomes reported in [15]), with different polymorphic haplotypes suggesting a recurrent mutation. Our comparison with the haplotypes inferred from the published Brazilian data showed that the ancestral Gypsy haplotype is identical to the less common haplotype of Brazilian R59H alleles (referred to as "Brazilian 2" in the Table below; present in patients 3, 6, and 7 in Table 2 of [15]), supporting the possibility of a founder mutation spreading to South America with the Gypsy diaspora. Balkan Roma, a substantial proportion of the Gypsy community of Brazil, reached South America in the late 19th–early 20th century, as part of the large migration wave out of Romania after the abolition of Gypsy slavery [18].

Table 1
Comparison of intragenic polymorphic haplotypes of R59H alleles

	L10P	L12L (c34C > T)	R59H	R521C	S532G
Gypsy-Bulgaria	+	+	+	–	–
Gypsy-Italy	+	+	+	–	–
Brazilian 1	–	–	+	–	+
Brazilian 2	+	+	+	–	–

The screening of a panel of 413 anonymous unrelated control subjects identified eight heterozygotes, i.e., a carrier rate of 1.94% in the general Gypsy population and a predicted incidence of affected homozygotes 1 in ~10,000 newborns. The distribution was non-random: all carriers were detected among the 81 samples from a specific Gypsy group, the Rudari—an endogamous sub-isolate well represented in the Gypsy communities of both Bulgaria and Brazil [1,18], where the estimated carrier rate is 10%.

The pathogenic effects of the R59H mutation have been documented in in vitro studies [9]. All our cases displayed the classical infantile GM1-gangliosidosis phenotype. The association of R59H with cardiomyopathy, reported in the Italian patients [8,9], was also present in two of the affected children from Bulgaria, and may have gone undetected in the others. The data presented here should stimulate future phenotype studies in the genetically homogeneous group of R59H homozygotes.

Our results place GM1-gangliosidosis among the common Mendelian disorders in the Gypsy population. R59H testing should be the first choice procedure in Gypsy children with the clinical manifestations of a lysosomal storage disorder, and may be particularly useful in couples with an infant deceased from an undiagnosed condition compatible with GM1-gangliosidosis. Our findings should facilitate the diagnostic process and allow prenatal analysis, an option to which affected families have been highly receptive.

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