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Chitotriosidase deficiency in the Cypriot population: Identification of a novel deletion in the *CHIT1* gene

Gavriella Mavrikiou, Petros Petrou, Theodoros Georgiou, Anthi Drousiotou *

Department of Biochemical Genetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

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ABSTRACT

Objectives: The purpose of this study was to determine the normal range of chitotriosidase activity in the Cypriot population and the frequency of the 24 bp duplication polymorphism. Furthermore, we compared the allele frequency of this polymorphism in two locations with different malaria endemicity in the past.

Design and methods: Plasma chitotriosidase activity was measured using a fluorogenic substrate. The 24 bp polymorphism was detected using PCR analysis of exon 10 of the *CHIT1* gene. Additional mutations were detected using direct sequencing.

Results: The normal range of chitotriosidase activity was found to be 9.5–44.0 nmol/ml/hr. Among 114 normal individuals genotyped for the 24 bp duplication, 7% were found to be homozygous, 36% heterozygous and 57% wild type (allele frequency 0.25). The allele frequency of this polymorphism in individuals originating from two locations with different malaria endemicity in the past was not significantly different. A novel deletion mutation in the *CHIT1* gene was identified associated with loss of chitotriosidase activity. This new deletion eliminates 29 nucleotides from exon 9 resulting in the generation of a premature stop codon, probably leading to the production of an aberrant protein molecule.

Conclusions: The normal range of chitotriosidase activity and the allele frequency of the 24 bp duplication polymorphism in the Cypriot population were found to be similar to those of other Mediterranean populations. No evidence for an association between the presence of the 24 bp duplication polymorphism and susceptibility to malaria was found. A novel deletion in exon 9 of the *CHIT1* gene was identified (allele frequency 0.01).

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1. Introduction

Human chitotriosidase (EC 3.2.1.14) is an endoglycosidase with the ability to hydrolyze chitin, a long chain N-acetyl glucosamine biopolymer which functions as a structural component of the coating of fungi, insects, nematodes and shellfish. The enzyme is synthesized by activated macrophages and is considered to be a component of the innate immune system, especially involved in defense against chitin containing pathogens [1–3]. It was first identified in patients with Gaucher disease, a lysosomal storage disorder characterized by the accumulation of lipid laden macrophages as a result of glucocerebrosidase (acid β-glucosidase) deficiency [2]. The enzyme was purified from the spleen of a Gaucher patient and characterized after cloning of its cDNA from a human macrophage cDNA library [4,5]. Because of its high values in the plasma of Gaucher patients, chitotriosidase is used as a marker of disease progression and for monitoring response to treatment [2]. Moderately elevated levels of chitotriosidase are also observed in other lysosomal storage disorders (such as GM1 gangliosidosis, Krabbe disease, Niemann–Pick disease, mucopolysaccharidoses I and IVA) [6–8] as well as in patients with atherosclerosis and coronary artery disease [9–11], β-thalassaemia [12–14], malaria [13,15] and other disorders involving activated macrophages.

Chitotriosidase is encoded by the *CHIT1* gene, located on chromosome 1 at position q31–32 [16]. The gene consists of 12 exons and spans about 20 kb of genomic DNA. A polymorphism in exon 10 of the *CHIT1* gene caused by a 24 bp duplication (c.1049_1072dup24) and leading to enzyme deficiency is frequently encountered among different populations. The presence of this polymorphism results in the activation of a cryptic 3′ splice site leading to an in-frame deletion of 87 nucleotides and the production of an abnormal mRNA. The resulting protein lacks 29 amino acid residues (Val 344-Gln 372) and is catalytically inactive. The 24 bp duplication polymorphism has been identified in high frequencies among different populations, both European and Asiatic [17–23].

Whereas the 24 bp duplication polymorphism is quite common in populations of European and Asian ancestry, it is almost absent in African populations [18,19]. This observation led to the suggestion that the presence of the mutant chitotriosidase allele might confer increased susceptibility to malaria and other infectious diseases. This

* Corresponding author at: Department of Biochemical Genetics, The Cyprus Institute of Neurology and Genetics, P.O. Box 23462, Nicosia 1683, Cyprus.
E-mail address: anthidr@cing.ac.cy (A. Drousiotou).

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notion was initially supported by a reported higher prevalence of the 24 bp duplication polymorphism among Indian patients with filariasis (27% homozygotes) compared to the healthy population screened (10% homozygotes) [20]. It was therefore postulated that the absence of the 24 bp duplication polymorphism in populations of African origin probably resulted from a selective advantage for the wild type allele. Other studies, however, did not support the aforementioned hypothesis. As shown for patients with filariasis in Papua New Guinea, there was no correlation between the presence of the mutant allele and the progression or the severity of the disease [24]. Moreover, data reported by two studies for the Sardinian population where contradictory with respect to the frequency of the mutant allele in areas of high and low malaria endemicity respectively. Piras et al. [21] reported similar frequencies for the wild type and mutant allele as those reported by Malaguarnera et al. [18] but their results regarding the association of the presence of the mutant allele with malarial infection significantly differed.

Several other mutations in the *CHIT1* gene resulting in absent or reduced enzyme activity have been described. The p.Gly102Ser missense mutation (exon 4 of the *CHIT1* gene) is frequently encountered (allele frequency 0.2–0.3) in all the populations screened (European, Asian, African). The results reported regarding the impact of this polymorphism on chitotriosidase activity are controversial since some groups' support that the polymorphism does not significantly affect the enzyme activity [25], whereas other groups suggest that the presence of the p.Gly102Ser polymorphism confers a 4–5 fold reduction in enzyme activity [19].

The p.Gly354Arg polymorphism (exon 10) is either encountered on its own or in combination with two more changes downstream (complex E/I 10 allele). In both cases a complete absence of chitotriosidase activity is observed [25]. The p.Ala442Gly polymorphism (exon 12) is frequently encountered among different populations but has not been associated with reduced enzyme activity, whereas the p.Ala442Val polymorphism, which is mostly encountered among individuals of African origin, has been associated with significantly reduced chitotriosidase activity [25].

The presence of polymorphisms that decrease chitotriosidase activity impedes the use of this biomarker for the diagnosis and monitoring of Gaucher disease. It is therefore important, for the correct interpretation of chitotriosidase levels, to establish the type and frequency of these polymorphisms in every population. In the present study we established the normal range of plasma chitotriosidase activity and the frequency of the 24 bp duplication polymorphism in the Cypriot population. We also showed the presence of the p.Gly102Ser polymorphism and identified a new mutation in exon 9. Furthermore, we compared the frequency of the 24 bp polymorphism in two locations of the island with low and high malaria endemicity in the past.

2. Materials and methods

2.1. Subjects and samples

For the determination of the normal range of chitotriosidase activity and the frequency of the 24 bp duplication in our population a total of 114 apparently healthy unrelated individuals were recruited. The number and geographic origin of samples were selected such that all regions of the island are correctly represented in the cohort. The study was approved by the Cyprus National Bioethics Committee and informed consent was obtained from all participating volunteers.

For the investigation of a potential correlation between the 24 bp duplication polymorphism and malaria endemicity, a different set of DNA samples (195 in total) obtained from the DNA bank of the Cyprus Institute of Neurology and Genetics were used. The samples were randomly selected. One hundred samples came from individuals originating from a region at sea level where malaria endemicity was high, and 95 samples belonged to individuals from a village at an altitude of 880 m where malaria was nearly absent.

2.2. Measurement of chitotriosidase activity

EDTA plasma was used for the determination of chitotriosidase activity using an artificial substrate as previously described [2]. Briefly, 5 μl of undiluted plasma was incubated at 37 °C for 1 h with 100 μl of a solution containing 22 μmol/L of the artificial substrate 4-methylubelliferyl-β-D-N,N′,N″triacetyl chitotrioside (Sigma M5639) in citrate–phosphate buffer, pH 5.2. The reaction was terminated by adding 1 ml of 0.25 mol/L glycine-NaOH buffer, pH 10.4. The release of 4-methyl-umbelliferone was measured using a JASCO PTL-3965 fluorometer at an excitation and emission wavelength of 365 nm and 450 nm respectively. Chitotriosidase activity was calculated as nanomoles of substrate hydrolyzed per ml plasma per hour.

2.3. DNA isolation

DNA was isolated from blood samples using the Qiagen Gentra Puregene Blood Kit (catalog number 158467) according to the manufacturer's instructions.

2.4. 24 bp duplication polymorphism detection by PCR

Genotyping for the 24 bp duplication polymorphism was performed using previously described primers (5′-GAAGAGGTAGCCAGGCTCTGG-3′) and (5′-CTGCCGTAGCGTCTGGATGAG-3′) which enabled the detection of both the normal (75 bp) and the mutant (99 bp) allele [21]. PCR reactions were carried out in 50 μl volumes containing 1 × PCR buffer (Qiagen), 0.5 mM dNTP's, 20 pmol of each primer, 0.25 units of Taq polymerase (Qiagen) and 200 ng of template DNA. DNA was initially denatured at 94 °C for 4 min and then subjected to 36 cycles of 60 s denaturation at 94 °C, 30 s annealing at 55 °C and 60 s of extension at 72 °C. Amplified fragments were separated by electrophoresis in 3% agarose gel (NewSieve) and visualized by ethidium bromide staining [Fig. 2].

2.5. Detection of additional CHIT1 mutations

DNA samples were subjected to sequencing in an attempt to identify additional mutations or polymorphisms present. Exons 4, 10 and 12 of

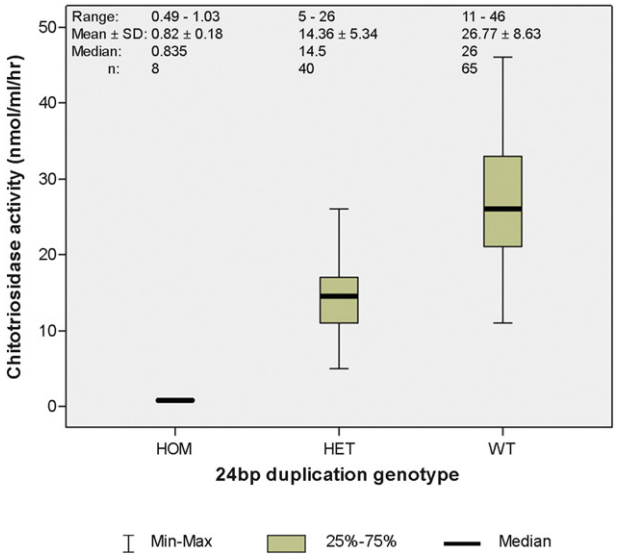

Fig. 1. Chitotriosidase activity levels for the three genotype groups of the 24 bp duplication in the Cypriot population.

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