

Distinct Haplotype in Non-Ashkenazi Gaucher Patients with N370S Mutation

Submitted 11/19/97; revised 11/20/97
(communicated by Ernest Beutler, M.D., 11/20/97)

Olga Amaral^{1,2}, Ana Marcão^{1,2}, Eugénia Pinto^{1,2}, Ari Zimran³, M.C. Sá Miranda^{1,2}

ABSTRACT: A new polymorphism, in intron 7 of glucocerebrosidase gene, has been identified in Gaucher Disease patients. It seems to appear only in Pv1.1⁻ alleles bearing the N370S mutation. This new sub-haplotype was only identified in Portuguese patients, of origins spanning all of the Portuguese continental territory. This finding indicates that, in the Portuguese, mutation N370S has existed in the context of two slightly different haplotypes and thus must be relatively ancient.

Keywords: haplotypes, glucocerebrosidase, Gaucher disease, Portugal

Gaucher disease (McKusick 230800) is the most frequent sphingolipidosis, usually caused by impaired activity of glucocerebrosidase (EC 3.2.1.45). Several causal mutations and 12 polymorphisms, defining only four haplotypes (the two commonest being Pv1.1⁻ and Pv1.1⁺), have been reported in the glucocerebrosidase gene (1). The most frequent Gaucher disease mutation g5841A→G (c1226A→G, N370S), which is known to be particularly frequent among the Ashkenazim has been found to be in linkage disequilibrium with the common Pv1.1⁻ haplotype (2). In Portuguese patients N370S is the most frequent mutation (3). A previously undescribed polymorphic change is reported here. It was identified in some patients, always superimposed on Pv1.1⁻ alleles bearing N370S mutation.

The new polymorphism was identified in Portuguese Gaucher type 1 patients by PCR-

RFLP analysis and subsequent sequencing of PCR products. The polymorphism was found to be a G→A transition (nucleotide g5470; GenBank accession J03059) in intron 7, resulting in the destruction of a restriction site for *Bsu* 36I.

As indicated in table 1, two groups of control individuals were studied, normal controls and N370S carriers. Gaucher Disease patients of different ethnic backgrounds were screened for the presence of the novel polymorphism encountered in Portuguese patients (Table 2). All patients had been previously genotyped, samples from non-Portuguese patients were kind gifts from researchers in Europe and Israel. The genomic DNA samples were screened by PCR-RFLP analysis.

This polymorphism was only identified in 11% of N370S alleles of unrelated Portuguese patients, whose origins span all of the continental

¹ Instituto Genética Médica Jacinto Magalhães, Unidade Enzimologia, Pr. Pedro Nunes 84, 4050 Porto, Portugal;

² Instituto Biologia Molecular Celular, Unidade Neurobiologia, R. Campo Alegre 823, 4150-Porto;

³ Gaucher Clinic, Department of Medicine, Shaare-Zedek Medical Center, Israel.

Reprint request to: M.Clara Sá Miranda, Ph.D., Head of Enzymology Department, Instituto de Genética Médica, R Campo Alegre 823, 4150 Porto Portugal, phone and fax 351-2-6092404, e-mail: samir-ue@individual.eunet.pt

1079-9796/97

Copyright (c) by The Blood Cells Foundation, La Jolla, California USA
All rights of reproduction in any form reserved

Published by Academic Press
Established by Springer-Verlag, Inc. in 1975

territory. Three generations were studied in some of such families and every carrier of N370S presented the additional polymorphism. Considering the patients' ages and the origins of the respective families it seems that both forms of N370S have coexisted in Portugal for over 150 years. The polymorphism was not identified in any of the other Portuguese individuals studied nor in the patients and/or N370S carriers of other origins.

Since this new polymorphism was not found

in samples with other geographic origins, in particular since the Israeli sample tested negatively (assuming a similar frequency at least 10 cases should have been detected), it seems plausible to infer that this new sub-haplotype appeared in Portugal relatively recently. Therefore N370S is probably quite ancient and the form detected in 11% of the Portuguese N370S chromosomes must be more recent and probably still confined to the Portuguese population.

Table 1. Samples from healthy Portuguese individuals screened for the presence of 5470 g-a polymorphism of the glucocerebrosidase gene

Portuguese control samples	Number of alleles studied	Number of N370S alleles	Number of normal alleles	Number of 5470 g-a alleles
Normal controls	100	0	100	0
Carriers screened in the population (unrelated to GD patients)	16	8	8	0

Table 2. Glucocerebrosidase alleles from Gaucher type 1 patients with various geographic origins screened for the presence of 5470 g-a polymorphism.

Countries of origin	Number of alleles studied	Number of N370S alleles	Number of 5470 g-a alleles
Portugal	78	45	5
Israel	128	108	0
Netherlands	40	21	0
United Kingdom	16	16	0
France	16	8	0
Spain	12	8	0

ACKNOWLEDGMENTS

We are grateful to Drs. Winchester (London), Caillaud (Paris), Aerts (Amsterdam), and Chabás (Barcelona) for their collaboration sending us DNA samples from their patients.

REFERENCES

1. Beutler E, Gelbart T. Glucocerebrosidase (Gaucher disease). *Hum Mutat* 8: 207-213, 1996.
2. Zimran A, Gelbart T, Beutler E. Linkage of the PvuII polymorphism with the common Jewish mutation for Gaucher disease. *Am J Hum Genet* 46: 902-905, 1990.
3. Amaral O, Pinto E, Fortuna M, Lacerda L, Sá Miranda MC. Type 1 GD: Identification of glucocerebrosidase mutations in the Portuguese. *Hum Mutat* 8: 280-281, 1996.