MOLECULAR CHARACTERIZATION OF MUTATIONS CAUSING FUCOSIDOSIS IN ITALY

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INTRODUCTION

Fucosidosis is an autosomal recessive disorder characterized by accumulation of fucoglycoconjugates within lysosomes due to deficiency of alfa-L-fucosidase. The disorder was originally described in Italy (Durand P. et al., 1966) where at least 14 patients have been observed, 7 of whom have been reported (Borrone C. et al., 1974; Filocamo M. et al., 1982; Giovannini M. et al., 1978; Porfiri B. et al., 1981). More than 80 patients have been described in the world literature. Two clinical phenotypes have been discriminated based primarily on the age of onset and severity of the disease (Koussef G. et al., 1976). They are: fucosidosis type 1, with onset at about 6-8 months of age and death within the first 6 years of life; fucosidosis type 2 with onset at one or two years of age and survival into adulthood.

We have followed at the Gaslini Institute 12 patients with fucosidosis and performed 5 prenatal diagnoses which resulted in 2 affected foetuses (Durand P. et al., 1979). Other subjects from these families, who died within the first ten years of life, probably were also affected with fucosidosis. We are currently studying the molecular basis of the mutations which cause this disorder in 7 Italian patients, with the aim of correlating clinical and molecular heterogeneity. A detailed knowledge of the basic defect is also essential for the planning of future gene or replacement therapy.

METHODS

Fibroblasts derived from skin biopsies or lymphoblastoid cell lines from controls and patients, from a G2/6 HGPRT hepatoma cell line and from an Hela cell line were cultured in RPMI 1640 supplemented with 15% fetal calf serum and used as source of genomic DNA or total RNA. DNA was
extracted following a published procedure (Kunkel L. et al., 1977),
digested with restriction endonucleases and blotted as previously
described (Roncuzzi L. et al., 1985). Total RNA was isolated by the
guanidinium/cesium chloride method (Chirgwing J.M. et al., 1979). Poly A+
RNA was purified using Hybond mAP (Amersham) according to the
manufacturer's instructions. RNAs were electrophoresed either through
agarose gels after denaturation by glioaxal and dimethylsulfoxide
(McMaster G.K. and Carmichael G.G., 1977) or through agarose gels
containing formaldehyde, after denaturation by formamid and formaldehyde,
(Maniatis T. et al., 1982).

Probes AF3 (Fukushima H. et al., 1985) and AF11B (Willems P.J. et
al., unpublished data) representing partial cDNA of the alfa-fucosidase
gene, were radioactively labelled by either nick translation or
oligolabelling and used for hybridization. Probe AF3 covers 1054 bp of
the coding sequence of the alfa-L-fucosidase gene, while probe AF11B
covers 646 additional bp of the same coding sequence on the 3' side of
the gene. These two contiguous partial cDNAs are separated by an EcoRI
site.

RESULTS

Genomic DNA from six patients and four controls was digested with
the following restriction enzymes: EcoRI, HindIII, BglI, MspI, TaqI, XmnI
and hybridized with probes AF3 and AF11B (see Methods). Among these
enzymes only EcoRI revealed, with both probes, a variant pattern in
patient 3. Using AF3 as probe, five fucosidosis patients and all the
controls showed a pattern of 6.5, 4.5, 3.0 and 2.0 Kb fragments. In
addition to these bands patient 3 showed a 6.0 Kb band (fig.1). Using
AF11B as probe, five patients and all the controls showed a 1.5 Kb band,
but patient 3 showed only a 6.0 Kb band (fig.2).

The variant EcoRI pattern observed in patient 3, can be interpreted
as due to a mutation causing the loss of an EcoRI site situated in the
coding sequence of the alfa-L-fucosidase gene (see Methods). The loss of
this site produces the 6.0 Kb fragment instead of the 4.5 and 1.5 Kb
fragments. Patient 3, as already observed by J.S. O'Brien's group, is
homozygous for this mutation, as shown by the absence of any 1.5 Kb
fragment with AF11B probe. A second 4.5 Kb fragment located at the 5' end
of the gene is still present when Southern blots are hybridized with the
AF3 probe. Its presence is documented in figure 2 by a band of 4.5 Kb of
decreased intensity in patient 3 with respects to the other individuals.
Genomic DNA from both parents of patients 3 showed both 6.0 and 1.5 Kb
EcoRI fragments after hybridization with the AF11B probe, which indicates
their heterozygosity for this mutation (data not shown).

Genomic DNA from six patients was also digested with PvuII,
electrophoresed and hybridized with AF3 in order to define the
distribution in the Italian population of a previously described
polymorphisms (Darby J.K. et al., 1986). Only one out of six fucosidosis
patients was heterozygous for this polymorphism (6.0 and 7.0 Kb

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