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Human β-Galactosidase Gene Mutations in Morquio B Disease

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Summary

Three different β-galactosidase gene mutations—a ^{273}Trp→Leu (mutation F) in both families, ^{482}Arg→His (mutation G) in one family, and ^{509}Trp→Cys (mutation H) in the other family—were identified in three patients with Morquio B disease who were from two unrelated families. Restriction-site analysis using *Stu*I, *Nsp*(7524)I or *Rsa*I confirmed these mutations. In human fibroblasts, mutation F expressed as much as 8% of the normal allele's enzyme activity, but the other mutations expressed no detectable enzyme activity. We conclude that the unique clinical manifestations are specifically associated with mutation F, a common two-base substitution, in this disease.

Introduction

Genetic deficiency of human acid β-galactosidase (E.C.3.2.1.23) causes G_{M1} gangliosidosis and Morquio B disease. The latter has been classified as one type of genetic mucopolysaccharidosis, with progressive and generalized skeletal dysplasia and without neurological involvement—in contrast to G_{M1}-gangliosidosis, which is a severe neurosomatic disease (O'Brien 1989). We cloned recently a full-length cDNA for human β-galactosidase (Oshima et al. 1988) and reported gene mutations in G_{M1}-gangliosidosis (Yoshida et al. 1991). Further molecular analysis revealed three different mutations in three Morquio B patients.

Material and Methods

The fibroblast strains from three Morquio B disease patients—case 1 (family 1 proband; GM01602), case 2 (family 1 affected sister; GM02455), and case 3 (family 2 proband; GM03251)—were purchased from NIGMS Human Genetic Mutant Cell Repository (Camden, NJ).

Details of the procedures of the mutation analysis and of the transient-expression experiment have been described in previous reports (Sakuraba et al. 1990; Yoshida et al. 1991). Three DNA constructs for gene expression—pCAGGS(GPF), pCAGGS(GPG), and pCAGGS(GPH)—were prepared from mutant cDNAs F, G, and H, respectively.

Results and Discussion

Northern blotting showed a single band of apparently normal size (3.0 kb) and of either normal or high intensity in all patients (data not shown). Direct sequence analysis of amplified cDNA from the patients revealed three different mutations—$^{851-852}$TG→CT (^{273}Trp→Leu; mutation F) in all three cases, ^{1479}G→A (^{482}Arg→His; mutation G) in cases 1 and 2, and ^{1561}G→T (^{509}Trp→Cys; mutation H) in case 3. It is noteworthy that mutation F contained a two-base substitution. It may have been produced either by a coincidental combination of two single-base substitutions or by a single-base substitution associated with a preexisting neutral polymorphism.

The three mutation alleles of Morquio B disease were detected also by restriction-site analysis using specific restriction enzymes (fig. 1). Mutation F did not

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Table 1

β-Galactosidase Gene Mutation in G_{M1}-Gangliosidosis and Morquio B Disease

A. Mutant Alleles

Nomenclature[a]	Mutation
A	^{51}Ile→Thr
B (J)	^{201}Arg→Cys
C	^{457}Arg→Gln
D	Duplication (nucleotides 1103–1267[b])
E	^{10}Leu→Pro
F	^{273}Trp→Leu
G	^{482}Arg→His
H	^{509}Trp→Cys
I	^{123}Gly→Arg
J	^{316}Tyr→Cys
K	^{494}Gly→Cys
L	Duplication (nucleotides 288–310[b])
P (I-1)	^{49}Arg→Cys
Q (I-2)	^{457}Arg→Ter

B. Genotypes

Phenotype and Genotype[c]	No. of Cases	Source(s)
G_{M1}-gangliosidosis:		
Infantile:		
D/D?	1	Yoshida et al. 1991 (case 4)
D/?	1	Yoshida et al. 1991 (case 2)
I/K	1	Yoshida et al. 1991 (case 3)
J/L	1	Yoshida et al. 1991 (case 1)
P/?	1	Nishimoto et al. 1991 (case 1)
Q/Q	1	Nishimoto et al. 1991 (case 2)
Late infantile/juvenile:		
B/B	1	Nishimoto et al. 1991 (case 5)
B/?	4	Nishimoto et al. 1991 (cases 3, 4, and 6); and Yoshida et al. 1991 (case 5)
Adult/chronic:		Nishimoto et al. 1991 (cases 7–12); and
A/A	11	Yoshida et al. 1991 (cases 8–11)
A/C	1	Yoshida et al. 1991 (case 7)
Morquio B disease:		
F/G	2	Oshima et al. (cases 1 and 2)
F/H	1	Oshima et al. (case 3)

[a] Nomenclature of Nishimoto et al. (1991) is given in parentheses.
[b] Source: Oshima et al. (1988).
[c] The common mutation for each phenotype is underlined.

create any new restriction site, but the introduction of an additional substitution ^{847}T→A induced by an appropriate upstream primer created an *Stu*I site; the 147-bp PCR product was digested to produce a 121-bp fragment. Mutation G created an *Nsp*(7524)I site, and the 101-bp PCR product was digested to 61-bp and 40-bp fragments. Mutation H created an *Rsa*I site, and the 103-bp PCR product was digested to 57-bp and 46-bp fragments. A partial digestion was observed for cases 1 and 2 in *Stu*I- and *Nsp*(7524)I-site analyses and for case 3 in *Stu*I- and *Rsa*I-site analyses. The PCR products from the cDNA without these mutations were not digested by any of the enzymes used in the present study. We concluded that all cases were compound heterozygotes—either genotype F/G (cases 1 and 2) or genotype F/H (case 3).

We have not found any polymorphism in the β-galactosidase cDNA in the Japanese population, but the base sequence designated as polymorphism E [^{63}T→C (^{10}Leu→Pro)] was found in two cases from family 1.

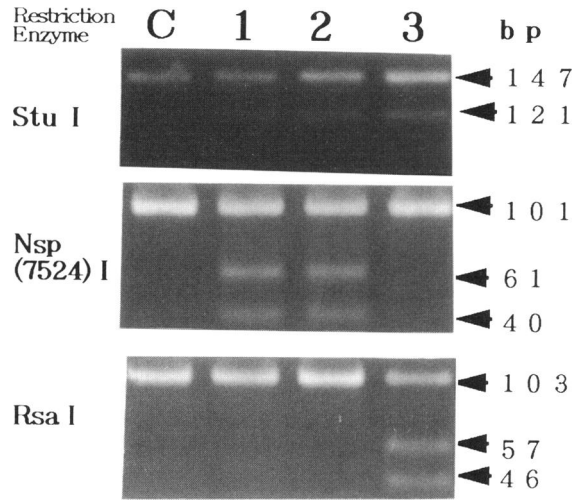

Figure 1 Restriction-site analysis of PCR-amplified β-galactosidase cDNA. Three amplified cDNA fragments—nucleotides 824–960, 1421–1521, and 1505–1607—were amplified and subjected to restriction-site analysis using *StuI* (top gel), *Nsp*(7524)I (middle gel) and *RsaI* (bottom gel), respectively. The first fragment contained an additional base substitution, ^{847}T→A, which created an *StuI* site in the presence of mutation F (nucleotides 851–852) (see text). The fragments with mutation G (nucleotide 1479) or mutation H (nucleotide 1561) were digested by *Nsp*(7524)I or *RsaI*, respectively; normal cDNA fragments without these mutations were not digested by any of the three enzymes. Electrophoresis was performed in 3% agarose gel. Lane C, Control fibroblasts. Lanes 1–3, Cases 1–3.

In retrospect it was seen that this substitution was in fact observed also in the λgt11 cDNA library from human placenta of non-Japanese origin (Oshima et al. 1988) and is considered to be a neutral polymorphism.

The pathogenesis of different phenotypic expressions is not clear in human β-galactosidase deficiency. Including those in the present report (table 1), 14 different β-galactosidase gene mutations have been observed, and some correlations with clinical phenotypes are suggested. Our previous gene-expression study detected no enzyme activity toward a fluorogenic substrate (4-methylumbelliferyl β-galactoside) in mutant β-galactosidase genes in G_{M1}-gangliosidosis, except for two mutant alleles—mutations A (^{51}Ile→Thr) and B (^{201}Arg→Cys), for adult and juvenile forms, respectively (Yoshida et al. 1991).

In the present study, the common mutant F allele in Morquio B disease was found to express a low but measurable enzyme activity (8% of control value), whereas the other two mutations expressed no detectable enzyme activity. We tentatively conclude that the three mutant β-galactosidase genes expressing detectable enzyme activity may be closely related to the pathogenesis of late-onset G_{M1}-gangliosidosis (mutations A and B) or Morquio B disease (mutation F). Further characterization of mutant gene products—e.g., in terms substrate specificity, enzyme kinetic studies, and intracellular turnover of the enzyme protein—is expected to reveal further the phenotype-genotype relationship in human β-galactosidase deficiency.

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