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## Annotation:

# Fabry's Disease: The Search for a Regulator Gene Mutation in Man

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Fabry's disease (angiokeratoma corporis diffusum) joins a growing number of inherited disorders for which a specific metabolic defect has been identified. The disease, first reported in 1898, is characterized by the accumulation of a glycolipid, ceramide trihexoside, in various tissues, giving rise to characteristic skin and eye lesions, renal dysfunction, and cardio- and cerebrovascular disease [1, 2]. Pedigree analyses have established typical X-linked transmission of the disorder [3], and there is evidence for linkage to the $Xg^a$ locus [4].

One characteristic of large molecular weight substances for which there is no parallel with simpler compounds of intermediary metabolism is their assembly from and degradation to large stable building blocks. The existence of storage disorders has provided a tool for identifying the enzymatic steps in the complicated metabolic pathways of complex lipids [5], just as such classical diseases as phenylketonuria and alkaptonuria served to confirm metabolic relationships among simpler molecules. Because of the difficulty in working with large molecular weight substrates, enzyme assays usually depend upon use of artificial substrates.

The ceramide trihexoside stored in Fabry's disease is a sphingosine derivative containing an acyl group attached to the β-amino group and an oligosaccharide (glu-glu-gal) on the α′-hydroxyl (glu = glucose; gal = galactose; acyl = fatty acid, $C_{22}$-$C_{24}$):

$$C_{15}H_{29}\text{-}CH(OH)\text{-}CH(NH\text{-}acyl)\text{-}CH\text{-}O\overset{\beta}{\text{—}}glu\overset{\beta}{\text{—}}glu\overset{\alpha}{\text{—}}gal.$$

This material is derived from glycolipids of red blood cell membranes and other nonneural cells [1]. Sugar moieties are removed stepwise, and the inability in Fabry's disease to hydrolyze the terminal galactose residue results in accumulation of ceramide trihexoside.

Brady et al. [6] demonstrated a deficiency of a nonspecific galactosidase, which was shown to be an α-galactosidase [7]. However, affected males still have 10%–

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20% of normal α-galactosidase activity using conventional assays with artificial substrates, suggesting the possibility of multiple enzymes with α-galactosidase activity, only one of which would be affected in Fabry's disease.

Three papers in the current issue report investigations of the nature of the primary gene defect, with major attempts to distinguish whether the decreased α-galactosidase activity represents a structural gene mutation or the effect of a putative regulator gene. Despite the detailed understanding of both negative and positive regulatory systems in *Escherichia coli* [8], it should be remembered that there is still no disorder in man proved to be due to a mutation in a regulatory gene. Thus, the mechanisms of thalassemias, von Willebrand's disease, and the porphyrias remain to be elucidated. The report [9] that infusion of normal plasma containing α-galactosidase activity (ceramide trihexosidase activity) "induced" circulating levels in a Fabry's disease patient higher than in the infused plasma seems to have stimulated consideration of the X-linked locus for Fabry's disease as a possible regulator gene.

Beutler and Kuhl, Wood and Nadler, and Ho et al. have all demonstrated the existence of two forms of α-galactosidase in normal tissues (liver, leukocytes, or skin fibroblasts) on the basis of thermal inactivation and electrophoresis. The thermolabile, anodally migrating form (α-galactosidase A) is absent in patients with Fabry's disease. The thermostable, less anodal form (α-galactosidase B) is present in both normal persons and patients with Fabry's disease and constitutes about 15% of the normal activity. This selective deficiency of a particular component of enzyme activity is analogous to the findings in Tay-Sachs disease [10]. Ho et al. and Beutler and Kuhl showed that treatment of α-galactosidase A with neuraminidase shifts its mobility to that of the B enzyme.

As pointed out by the authors, absence of α-galactosidase A activity could have several mechanisms: (1) alteration of the structural gene for the polypeptide chain, giving rise to an enzymatically inactive product; (2) alteration of a regulator gene, causing decreased synthesis (or conceivably faster degradation) of the structural gene product; (3) lack of an enzyme involved in synthesis or attachment of carbohydrate side chains to the enzyme's polypeptide backbone, so that functional enzyme is not formed.

The third possibility can be dismissed. As in other glycoproteins, treatment of normal α-galactosidase A with neuraminidase to remove sialic acid groups has no influence on activity in the assay procedure (Ho et al.). Whatever the role of carbohydrate side chains, their presence or absence seems to make little difference to the catalytic function of enzymes, at least in vitro.

The differentiation of structural and regulator mutations has been approached in two quite different ways. The first is to seek direct evidence of structural alteration of the enzyme. The simplest method employs antibody against the enzyme, either precipitating or inactivating the enzyme, to determine whether an inactive but cross-reacting product is present. Cross-reaction could be demonstrated by reacting Fabry's disease extracts with antibody and then testing for residual antibody capacity for reaction with a standard amount of normal enzyme. Unfortu-

nately, none of the authors had antiserum against α-galactosidase. With efforts to prepare the enzyme from normal plasma [2], it should be possible to obtain such antisera in the future. In the absence of low levels of α-galactosidase A activity, it is impossible to compare kinetic, electrophoretic, or thermal properties of the possibly altered enzyme in Fabry's disease patients with the normal enzyme. Patients with partial deficiencies might be informative, if these were due to allelic mutations.

Ho et al. interpret the thermal, isoelectric, and electrophoretic properties of neuraminidase-treated α-galactosidase of liver from controls and one patient with Fabry's disease to indicate that the various forms separated by isoelectric focusing share a common polypeptide backbone and differ in the number or position of sialic acid (NANA) residues. Rapidly migrating forms (α-galactosidase A) revert to the mobility of the B enzyme in controls, while the residual activity in the patient does not change in electrophoretic mobility after neuraminidase. After neuraminidase treatment, however, the single isoelectric-focused peak of α-galactosidase is thermolabile in control preparations but thermostable in the material from the Fabry's patient. Thus, these authors conclude that the primary gene defect could be a structural gene mutation causing alteration of the enzyme's polypeptide backbone so as to become a poor acceptor of sialic acid residues. Since the sialic acid residues are not essential for activity, the alteration of the backbone also must interfere with catalytic function either by involving the active site or by altering the enzyme conformation. Little is known about the control of carbohydrate attachment to proteins, so that further studies will be necessary to clarify the biochemical abnormality.

The second approach to differentiating structural and regulator gene mechanisms is indirect and depends upon demonstrating genetically that the structural gene product segregates in autosomal fashion, while the disease is clearly X-linked. Beutler and Kuhl have screened leukocytes from 225 normal persons for an electrophoretic variant of α-galactosidase A, so that its segregation in families could be determined. Unfortunately, no variant was found, but additional search seems to be indicated, since a single rare variant could be highly instructive. Meanwhile, Beutler and Kuhl have taken advantage of electrophoretic differences between the presumably homologous enzyme in interspecies hybrids. Horse-donkey male hybrids (hinnies and mules) have electrophoretic forms of α-galactosidase corresponding to mobility of both horse and donkey, suggesting that the electrophoretic pattern may be autosomally determined. Similar studies could not be carried out with human-mouse somatic cell hybrids because the α-galactosidases of the two species were not electrophoretically distinguishable. Chinese hamster-human somatic cell hybrids [11] might be suitable for such studies. As noted by Beutler and Kuhl, however, the autosomal component of the α-galactosidase patterns could involve either the amino acid sequence of the polypeptide chain or the attachment of the carbohydrate moieties. If, in fact, the various forms of α-galactosidase demonstrable by electrophoresis or isoelectric focusing are interconvertible by alteration of sialic acid content, the interpretation of interspecies bands on electro-

phoresis must be tentative. Furthermore, the findings in equine hybrids depend upon the assumption of similar gene content for human and equine X chromosomes, for which data are quite limited.

Thus, the issue of the primary gene defect in Fabry's disease remains unresolved, though much detailed new information is provided by these three papers. Purification of the enzyme, preparation of specific antibody, and characterization of the process of carbohydrate attachment are needed. The possibility is always open that different mechanisms may be at play in different families with Fabry's disease. Clinically, greater interfamilial than intrafamilial variation has been noted [2]. The present papers differ somewhat in the levels of activity found in hemizygotes and in heterozygotes. Perhaps not all the differences can be attributed to differences in laboratory methods, although some of the cases were studied by more than one of the groups.

The studies reporting that infusion of a certain amount of plasma containing normal enzyme led to an increasing level of activity in the recipient with Fabry's disease [9] need confirmation and explanation, because mixing of control and Fabry's tissues or plasma seems not to give more than an additive result. Also, such "induction" by a regulator that could cross cell boundaries should lead to normal levels of activity in carrier females in vivo and in cultures of cells in vitro.

Finally, caution must be exercised in assuming that an enzyme present in abnormal amount (either deficient or elevated levels) without any *detectable* structural protein abnormality reflects a regulator gene effect. For example, G6PD Hektoen is associated with 400% activity and four times the normal rate of synthesis in fibroblasts, yet was found upon careful investigation to have an amino acid substitution [12]. Similarly, pseudocholinesterase Cynthiana is a structural gene variant present at three times the normal concentration of enzyme molecules [13]. Thus, structural gene mutations which initially may be difficult to demonstrate may greatly influence the rate of synthesis or degradation of the enzyme, at any level from DNA to messenger RNA to protein product. As attempted here by Beutler and Kuhl and in the mouse by Ohno [14], a search for combinations of X-linked and autosomal mechanisms in the production of specific enzymes seems a promising general approach to regulatory mechanisms in mammals.

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