Cataracts in Patients Heterozygous for Galactokinase Deficiency

Dwight Srambolian, Virginia Scarpino-Myers, Ralph C. Eagle, Jr., Barton Hodes, and Harry Harris

The role of heterozygous galactokinase deficiency in the development of presenile cataracts is presently undetermined. Erthrocyte galactokinase activity was measured from 95 normal Caucasian subjects and from 39 Caucasian patients who had developed idiopathic bilateral cataracts between ages 20 and 55. The diagnosis of heterozygous galactokinase deficiency was based on the following criteria: galactokinase activity more than 2.0 SD below the control population mean; when available, familial evidence for heterozygous galactokinase activity was used as additional evidence. Three of 39 patients (1/13) with cataracts were found to be carriers of the galactokinase deficiency allele ($P < 0.001$). Two heterozygotes had high dietary galactose intake suggesting a possible relationship between a high galactose diet and cataract formation. Dietary information was unavailable for the third heterozygote. We conclude that there is a high prevalence of heterozygous galactokinase deficiency existing in patients less than 55 yr of age with cataracts, and recommend that adults at risk restrict their consumption of dairy products.


Hereditary deficiency of galactokinase, first described in 1965, results in manifest hypergalactosemia and galactosuria. The sugar alcohol of galactose, dulcitol, accumulates in the lens from the action of aldose reductase, leading to cataracts.

Although a cause and effect relationship between galactose intake and cataract is admitted for homozygotes, the role of heterozygous galactokinase deficiency in the development of presenile cataracts is unclear. In one study, a statistically significant decrease in galactokinase activity was found in patients with cataract presenting during the first year of life, yet in the same study patients who developed cataract at a later age demonstrated no significant reduction in galactokinase activity. In contrast, a high incidence of heterozygous galactokinase deficiency was found in patients with idiopathic bilateral cataract aged 50 or less. Although heterozygotes with cataracts have been reported, other studies have found no relationship between early cataract and galactokinase deficiency.

Any study of galactokinase deficiency in the United States must take cognizance of racial variations. In American blacks, a 50% decrease in galactokinase activity is not necessarily indicative of heterozygous galactokinase deficiency. Black individuals are polymorphic for two common alleles, the GALK$^A$ allele normally found in white subjects and the GALK$^P$ allele, or Philadelphia variant, found exclusively in blacks. The red cell galactokinase activity of GALK$^P$ homozygotes is only 50% of that found in individuals homozygous for GALK$^A$. For this reason we have chosen not to include the enzyme activities from blacks in our statistical analysis.

The present study examines the prevalence of decreased red cell galactokinase activity in a Caucasian population sample who developed idiopathic bilateral cataracts before age 55. Further analysis of heterozygotes for galactokinase deficiency (GALK$^A$GALK$^G$) and normal controls (GALK$^A$GALK$^A$) was done by isoelectric focusing.

Materials and Methods

Study Populations

Thirty-nine consecutive patients between ages 20 and 55 with the clinical diagnosis of bilateral idiopathic cataract formed the study population. The patients were referred to our laboratory by ophthalmologists at the Scheie Eye Institute or in the community. No patient had clinical or laboratory evidence of diabetes mellitus, drug toxicity, or past history of eye trauma.
Table 1. RBC assays of galactokinase in Caucasians

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>n</th>
<th>Mean*</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sample (this study)</td>
<td>95</td>
<td>0.312</td>
<td>0.053</td>
</tr>
<tr>
<td>Normal sample (previous study of Tedesco et al 1975)</td>
<td>618</td>
<td>0.323</td>
<td>0.065</td>
</tr>
<tr>
<td>Total cataract sample</td>
<td>39</td>
<td>0.331</td>
<td>0.089</td>
</tr>
<tr>
<td>Normal activities in cataract sample (GALK\textsuperscript{a}GALK\textsuperscript{a})</td>
<td>36</td>
<td>0.345</td>
<td>0.078</td>
</tr>
<tr>
<td>Intermediate activity in cataract sample (GALK\textsuperscript{a}GALK\textsuperscript{b})</td>
<td>3</td>
<td>0.160</td>
<td>0.026</td>
</tr>
</tbody>
</table>

\* \mu mol substrate converted/hr per ml RBC at 37°C.
Caucasian individuals with normal galactokinase activity carry the GALK\textsuperscript{a}GALK\textsuperscript{a} genotype while individuals with intermediate activity have the GALK\textsuperscript{a}GALK\textsuperscript{b} genotype.

The control population in the study was selected among outpatient volunteers from various divisions of the Hospital of the University of Pennsylvania. Ninety-five consecutive volunteers without a history of presenile cataracts between the ages of 18 and 55 were included in the control population.

Informed human consent was obtained prior to undertaking this study.

Assays for Erythrocyte Galactokinase Activity

Three to five milliliters of venous blood was collected in acid citrate dextrose tubes and immediately stored at 4°C. Within 24 hr of venipuncture, the red cells were washed free ofuffy coat and plasma with cold 0.85% sodium chloride. Galactokinase estimations were done in triplicate as previously described.\textsuperscript{12} Activities more than 2.0 SD below the control sample mean or less than 0.206 \mu mol/hr per ml RBC were repeated in triplicate. Additional fresh samples were obtained when activity remained low with repeat assay. Enzyme activities were expressed in \mu moles galactose-1-phosphate formed/hr per ml packed red cells at 37°C.

\textsuperscript{14}C-galactose (Amersham; Arlington Heights, IL) substrate for the assay of galactokinase was purified to remove impurities as described previously.\textsuperscript{13} Samples containing low galactokinase activity were also assayed for galactose-1-phosphate uridyl-transferase by a previously described technique.\textsuperscript{14}

Isoelectric Focusing of RBC Hemolysates

Hemolysates from all cataract and several control subjects were isoelectrofocused side by side in polyacrylamide and examined by a recently described technique specific for detection of galactokinase.\textsuperscript{12}

Statistical Analysis

A Student's t-test was used to compare mean galactokinase activity in the normal white population sample in this study to a corresponding sample of Tedesco et al.\textsuperscript{15} A chi-square analysis comparing the cataract sample carrier frequency with that reported previously in a normal population was carried out.

Results

Population Distribution of Enzyme Activities

Comparison of our results with the previously reported frequency of heterozygous galactokinase deficiency in a normal Caucasian population sample\textsuperscript{15} requires similar assay conditions. The mean galactokinase (Table 1) activity in our normal population sample (X = 0.312, n = 95) did not differ significantly (t = 1.63, P > 0.1) from the corresponding sample (X = 0.323, n = 618) of Tedesco et al.\textsuperscript{15} This suggests that the assay methods employed in both studies are comparable.

The erythrocyte galactokinase activities of the cataract population are summarized in Table 1. Three subjects in the cataract group had galactokinase activities that were more than 2.0 SD below the control population mean. Red blood cell galactose-1-phosphate uridytransferase was assayed in all three subjects and found to be within the normal range. Age, cataract type, and visual acuity for the three heterozygous patients are shown in Table 2.

Table 2. Patients with heterozygous galactokinase deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cataract type</th>
<th>Corrected visual acuity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposita II. 1, pedigree A, age 36</td>
<td>Aphakic OD, posterior subcapsular cataract OS</td>
<td>6/7.5 OD, 6/24 OS</td>
</tr>
<tr>
<td>Propositus II. 2, pedigree B, age 41</td>
<td>Dense nuclear and cortical cataract OU</td>
<td>6/60 OU</td>
</tr>
<tr>
<td>Third heterozygote, age 53</td>
<td>Posterior subcapsular cataracts OU, intermittent posterior cortical vacuoles OU</td>
<td>6/12 OD, 6/21 OS in the presence of posterior cortical vacuoles and posterior subcapsular cataracts.</td>
</tr>
</tbody>
</table>

Genetic Basis for Determination of Heterozygotes

Two of the three subjects with values more than 2.0 SD below the mean had family members available for study.

The galactokinase activity of the proposita (II.1) in pedigree A was 3.4 SD less than the normal population mean (0.312) while that of the propositus (II.2) in pedigree B was 2.5 SD below the mean (Fig. 1). In pedigree
A, the mother of the proband (II.1) had a decreased enzyme activity greater than 2.0 SD below the population mean; the father was deceased. In pedigree B, the proband (II.2) had one parent (I.1) with similar low galactokinase activity, whereas the other parent (I.2) had a normal value. His wife (II.1) and two children (III.1, III.2) all had normal values. Because the carrier frequency for the GALK<sup>G</sup> allele in the normal Caucasian population is approximately 1/300, it is highly unlikely that intermediate galactokinase activity would occur in the parents of both probands by chance variation. This finding supports presumptive heterozygosity for both individuals.

The third presumptive heterozygote had a galactokinase activity 2.3 SD below the normal population mean. Familial evidence for heterozygosity was not available. Both parents were deceased, and he had neither children nor siblings.

**Dietary History**

In two heterozygotes the dietary history suggested a possible relationship between galactose intake and cataract formation.

Propositus (II.2) in pedigree B had consumed one quart of milk daily and one-half gallon of ice cream weekly since childhood. In addition, he frequently ate cheese. In contrast, his 70-year-old father (pedigree B, individual I.1) who had a similarly reduced galactokinase activity, seldom consumed dairy products and did not develop senile cataracts. This suggests that the combined effect of galactokinase deficiency and high dietary galactose intake contributed to cataract formation in the proband.

The third presumptive heterozygote consumed large quantities of milk and dairy products. He complained

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**Fig. 1.** Partial pedigrees and RBC galactokinase activities in families of two probands with activity below 0.200. Arrow indicates proband.

**Fig. 2.** On the left is a slit lamp photomicrograph of the lens of the proband, WM, during the period of decreased vision to 20/100. On the right is a slit-lamp photomicrograph 1 wk later after vision returned to 20/25.
of recurrent attacks of blurred vision, lasting approximately 1 wk. When first examined, minor axial posterior subcapsular lens opacities reduced his visual acuity to 20/25 bilaterally. During an attack which reduced the vision in his right eye to 20/100, slit-lamp biomicroscopy revealed a radiating pattern of multiple vacuoles in the posterior lens cortex (Fig. 2). Approximately 75% of the vacuoles had resorbed 1 wk later, and his visual acuity eventually returned to baseline level. During a 16-mo-period, the patient experienced four additional attacks that involved both eyes. Despite the unavailability of confirmatory family data, we believe that the occurrence of these unusual transient lens opacities in a patient with low galactokinase activity and high dietary galactose intake strongly favors heterozygosity for the GALK\textsuperscript{G} allele.

Proposita II.1 of pedigree A was unavailable for questioning concerning diet.

Red Blood Cell Isoelectric Focusing

Hemolysates from all cataract patients and several individuals with normal red cell galactokinase activity were isoelectrofocused side by side in polyacrylamide to detect galactokinase variants. Isoelectric focusing revealed no difference in the isoelectric points of galactokinase from cataract and control subjects. Importantly, no difference of GALK\textsuperscript{G} carriers from GALK\textsuperscript{A} homozygotes was detected.

Incidence of Heterozygotes in Caucasian Cataract Sample

On the basis of the observed genotype, supported by appropriate family data and dietary history, three of 39 (1/13) white individuals with presenile cataract are carriers for a GALK deficiency allele and have the genotype GALK\textsuperscript{G}GALK\textsuperscript{A}. To determine if heterozygosity for galactokinase deficiency is unusually frequent in a population with presenile cataracts, the carrier frequency (1/13) in our Caucasian cataract sample was compared with the normal population frequency (1/309) of Tedesco et al\textsuperscript{15} by chi-square analysis. The highly significant difference ($\chi^2 = 16.6, P < 0.001$) between samples strongly suggests that there is an association between carriers of the GALK\textsuperscript{G} allele and the development of bilateral presenile cataract.

Discussion

Decreased galactokinase activity in patients 10–50 yr of age with cataracts had not been found in some previous studies.\textsuperscript{3,9} However, another study did show a statistically significant increase of partial galactokinase deficiency in patients with presenile cataracts.\textsuperscript{4} In our study, three of 39 patients whose cataracts developed between the ages of 20 to 55 yr had reduced galactokinase activity.

The failure of some previous studies to establish a statistically significant relationship between decreased galactokinase activity and the development of presenile cataract may be explained by the analytical methods employed and the characteristics of the patient population studied. Beutler et al studied all patients with cataract irrespective of etiology.\textsuperscript{3} No mention was made of a previous history of drug toxicity, trauma, diabetes mellitus or other known causes of cataracts in his study population. In effect the patient population was heterogeneous with respect to the etiology of their cataracts. Although Magnani et al excluded patients with traumatic or diabetic cataract, his negative conclusion based on a comparison of the mean value of galactokinase activities in cataract and control population is misleading.\textsuperscript{9} One would not expect to find a significant difference between population means, when the incidence of heterozygous galactokinase deficiency is so low.

A previous report of decreased galactokinase activity in patients with presenile cataract employed single determinations of enzyme activity and did not evaluate genetic transmission or dietary history.\textsuperscript{4} It is noteworthy that our study has given familial evidence for transmission of the GALK\textsuperscript{G} allele in two of the three heterozygotes. Furthermore, documentation of a high galactose diet was possible in two presumptive heterozygotes.

The importance of inquiring about diet in heterozygotes for galactokinase deficiency is emphasized by a comparison of propositus II.2 in pedigree B to his father (pedigree B, individual I.1). Both had similar galactokinase activities. In contrast, his 75-yr-old father drank little milk, ate dairy products sparingly, and did not develop presenile cataracts. The son who developed cataracts by age 34 consumed large quantities of ice cream and milk.

The determination of a cause and effect relationship between decreased galactokinase activity and cataract formation rests with the proper investigation of diet. The activity of galactokinase is highest during the early years of life when milk is the major component of the diet.\textsuperscript{17,18} Although galactokinase activity is lower in adulthood, the usual concomitant decrease in milk intake, and hence, galactose intake prevents significant formation of dulcitol by aldose reductase. However, high galactose intake in the presence of partial galactokinase deficiency, as in heterozygotes, could lead to the production of excess dulcitol.\textsuperscript{19}

It is noteworthy that there was no difference in isoelectric points between the GALK\textsuperscript{G} carriers and the GALK\textsuperscript{A} homozygotes; only one enzyme activity band was found for each sample. Several possibilities exist
for the occurrence of this pattern. Although human erythrocyte galactokinase has been reported to be a dimer, studies of mouse x human hybrid cell lines suggest the enzyme is a monomer. If galactokinase is indeed a monomer, the polypeptide produced by GALK would not be detectable by our method of enzyme activity staining. Several additional mechanisms could explain the failure to observe hybrids of known polymeric enzymes is heterozygotes. If a hybrid enzyme is rapidly dissociated into its subunits, the hybrid forms could be lost during the course of the electrofocusing separation. Furthermore, there may be some structural feature of the alternative polypeptide chains which prevents their association in the same polymeric molecule. It also is well recognized that structural variants will not differ in mobility if the involved amino acid substitutions do not produce a net change in electric charge. Finally, galactokinase deficiency may be the consequence of a gene deletion or of a mutation resulting in no enzyme protein expression.

Although only three heterozygotes were identified in a population of 39 adult Caucasians who developed bilateral cataracts before age 55, this finding is significant. The prevalence of galactokinase deficiency in the normal Caucasian population is one in 310. The probability that the one in 13 rate found in this small sample occurred by chance is less than 1:1000. For this reason it seems sensible that moderate restrictions in the consumption of dairy products during adulthood should be recommended for those at risk.

Key words: cataracts, galactokinase, heterozygote, galactose, erythrocytes

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References