Cholestasis Familiaris Groenlandica/ Byler-like disease in Greenland – A population study

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ABSTRACT

Objectives. Cholestasis Familiaris Groenlandica (CFG, or progressive familiar intrahepatic cholestasis type 1 (PFIC1)) is a very common lethal recessive inherited disease in Greenland. A missense mutation, 1660G>A (asp554asn) in the gene ATP8B1 causes the disease (Klomp et al. 2000). Study design. A family study examining medical files from the period 1951-2003 from East Greenland resulted in 46 cases of PFIC1 and more than 220 relatives showing carrier status. Further, random blood sample testing 953 anonymous persons from 11 major cities or districts all over Greenland have been analysed for carrier status of the mutation. Methods. A sensitive PCR method is developed to distinguish between normal and mutant alleles for ATP8B1 in the Greenland population. Results. The mutation 1660G>A is found in all areas of Greenland, and the frequency of the mutant allele vary all over the country. A shockingly high frequency for the mutant allele is found in East Greenland in Ittoqqortoormiit (0.16) and in Tasilaq (0.077), whereas in Northwest Greenland lower frequencies are found in Uummannaq and Ilulissat (0.032), and Maniitsoq (0.005). Conclusions. The high frequency of the mutation in East and Northwest Greenland strongly indicates that routine screening of the population for carrier status should be done.

Key words: Cholestasis Familiaris Groenlandica; intrahepatic cholestasis; Byler disease; population study in Greenland; most common recessive lethal disease.

INTRODUCTION

In Greenland, progressive familial intrahepatic cholestasis type 1 (PFIC1) occurs. The disease called Cholestasis Familiaris Groenlandica was first described by Nielsen I-M (1), and its chromosomal location was later mapped to chr18 by linkage analysis (2,3). A missense mutation, 1660G-A (asp554asn) in the gene ATP8B1, is documented to cause the disease (4). To calculate the frequency of this recessive disease, medical files for the period 1951 to 2003 were investigated, and a total 34 cases of PFIC1 were recorded for East Greenland. The birth rate in the same period was 50-100 person per year in East Greenland, resulting in an estimated frequency of the disease allele \( q = 0.1 \) and a carrier frequency \( 2pq = 0.2 \) (2). A more precise estimation of the frequency for the mutated allele in the ATP8B1 gene was done by random blood sample testing. 953 anonymous blood samples from 12 areas in West and East Greenland were analysed for the purpose of deciding whether routine screening for the disease allele in specific areas in Greenland should be recommended.

MATERIAL AND METHODS

Data from families with known affected children in Greenland and data found by studying medical files from the period 1951-2003 for affected dead children in Tasiilaq were included in this study. Blood samples were collected for DNA isolation and PCR analysis. Additionally, 953 random selected blood samples obtained from Statens Serum Institute’s biobank were analysed by PCR. The DNA were isolated from 1-to-3-year-old blood
samples collected on filter paper originally for screening for phenylketonuria (PKU). A mutation specific PCR method is designed to discriminate between the tree genotypes: normal (N,N), carrier (N,S) and affected (S,S). The three different primers used in the 1660G-A mutation sensitive PCR are:

* BYL-S-F (mutant allele)
  AGCCAGGCAGCCTCTCTCA;
* BYL-N-F (normal allele)
  CCAGGCAGCCTCTCTCG;
* BYL-R (reverse primer)
  GCAAGACATTGTAAATCC-3’.

BYL-R is radioactively labelled by phosphorylation with P\(^{32}\)γ-ATP, the primer ratio in the PCR is: 1(BYL-S-F)/2,5(BYL-N-F)/3,5(BYL-R). The PCR-fragment for the normal allele (128bp) and the mutant allele (130bp) is separated on a 7% denaturing polyacrylamide gel and bands visualized by autoradiography (figure 1). The PCR is carried out at standard conditions (5). This study has been approved by the Scientific Committees for Copenhagen and Fredriksberg (journal nr. 01-094/01).

**RESULTS**

**Family study approach**
A total 46 persons with PFIC1 were found in medical files from the period 1951-2003. Screening relatives to these 46 persons for the 1660G-A mutation revealed 203 persons showing carrier status. The geographic distribution of persons with carrier status found is limited to 5 out of 16 different districts (Table I).

| City/Area     | Family project | Random blood samples on PKU cards |             |             | Allele frequency (q) | Carrier frequency in (2pq) |
|---------------|----------------|----------------------------------|-------------|-------------|----------------------|---------------------------|
|               | CFG patients   | CFG patients alive               | CFG patients dead | Carriers detected | Number of mutant alleles | Number of persons |              |
| Tasiilaq      | 33 *           | 2                                | 31            | 203         | 15                   | 97                      | 0,077              | 14,2% **     |
| Ittoqqortoormiit | 1              | 1                                | 0             |             | 14                   | 44                      | 0,061              | 27,0% **     |
| Qaanaaq       | 0              |                                  |               |             |                      |                         |                   |              |
| Upernavik     | 3              | 0                                | 3             | 12          | 4                    | 87                      | 0,023              | 4,5%         |
| Uummannaq     | 4              | 1                                | 3             |             | 5                    | 79                      | 0,032              | 6,2% ***     |
| Ilulissat     | 0              |                                  |               |             | 7                    | 109                     | 0,032              | 6,2% ***     |
| Qasigiannguit | 0              |                                  |               |             |                      |                         |                   |              |
| Aasiaat       | 0              |                                  |               |             | 4                    | 103                     | 0,020              | 3,8%         |
| Qeqertarsuaq  | 0              |                                  |               |             |                      |                         |                   |              |
| Sisimiit      | 0              |                                  |               |             | 3                    | 98                      | 0,015              | 3,0%         |
| Manitssoq     | 0              |                                  |               |             | 1                    | 99                      | 0,005              | 1,0%         |
| Nuuk          | 2              | 1                                | 1             |             | 4                    | 104                     | 0,019              | 3,7%         |
| Paamiut       | 3              | 1                                | 2             | 5           | 0                    | 47                      | 0,000              | 0,0%         |
| Narsaq        | 0              |                                  |               |             |                      |                         |                   |              |
| Qaqortoq      | 0              |                                  |               |             | 3                    | 86                      | 0,017              | 3,3%         |
| Nanortalik    | 0              |                                  |               |             |                      |                         |                   |              |
| Total         | 46             | 6                                | 40            | 220         | 60                   | 953                     |                   |              |

* Journals for dead persons are investigated only in Tasiilaq
** Screening of all persons highly recommended
*** Screening of pregnant women highly recommended
Random blood sample testing
Using the random blood sample testing method, 953 anonymous persons were screened for the mutated allele by the sensitive PCR method using DNA from PKU cards. Persons with carrier status were found in 11 out of 12 different cities or districts, compared to 5 out of 16 district using the family study method (Table I). In addition, using the random analysis approach, 15 persons with carrier status born in 1999 were found in Tasiilaq area, compared to only 7 persons with carrier status detected by the family study approach in the same year in Tasiilaq.

This strongly demonstrates that the random analysis approach is superior and much more sensitive than the family study approach when screening for carrier status for the 1660G-A recessive disease mutation.

DISCUSSION
The disease allele frequency in Greenland varies between the cities or districts studied. A mutant allele frequency higher than 0.03 results in a risk of one person being affected with PFIC1 out of 1,000 births. The highest allele frequencies were found on the East coast in Ittoqqortoormiit (0.16) and Tasiilaq (0.077), resulting in the risk of 1 out of 39 respectively 169. We therefore strongly recommend routine genetics screening on the East Coast and in the Uummanaq-Illulissat areas. This disease allele is one of the most common lethal alleles in the world.

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REFERENCES
1. Nielsen I-M, Ørnvold K, Jacobsen BB, Ranek L. Fatal Familial Cholestasic syndrome in Greenland Eskimo Children. Acta Paediatr Scand; 1986; 75: 1010-1016.
2. Eiberg H, Nielsen IM. Linkage studies of Cholestasis Familiaris Groenlandica/ Byler-like disease, with polymorphic protein and blood group markers. Human Heredity 1993; 43: 250-256.
3. Eiberg H, Nielsen IM. Linkage of Cholestasis Familiaris Groenlandica/Byler disease to chromosome 18. Int J Circumpolar Health 2000; 59: 57-62.
4. Klomp LWJ, Bull LN, Knisely AS, Doelen MAM, Juin JA, Berger R, Forget S, Nielsen IM, Eiberg H, Houwen RHJ. A missense mutation in FIC1 is associated with Greenland Familial Cholestasis. Hepatology 2000; 32: 1337-41.
5. Eiberg H, Møller J, Berendt I, Mohr J. Assignment of granular corneal dystrophy Groenouw type / (CDGG1) to chromosome 5q. Close linkage to IL9, DSS210 and DSS119. Eur J Human Genet 1994; 2: 132-138

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