# Amylo-1,6-glucosidase deficiency (glycogenosis type III) in the Faroe Islands

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The glycogen storage disease glycogenosis type III has not yet been fully elucidated with regard to its genetic basis. Biochemical studies have revealed some unexplained heterogeneity, even within families[2 3 4 5 7 9 10]. It is not clear whether more genes, allelic or non-allelic, may be involved, and a search for biochemical abnormalities in the heterozygotes has failed to give fully consistent results which would be of considerable help in further genetic studies. Most of the published families with multiple cases support the assumption of autosomal recessive inheritance, but the relative rarity of the condition and probably also technical problems have impeded full clarification of the aetiology and pathogenesis of this condition.

During the last decades it became apparent that amylo-1, 6-glucosidase deficiency was not uncommon in the Faroe Is-

lands (population size about 38.000 in 1970). It was, therefore, decided to undertake a thorough investigation of all patients diagnosed in this region as well as of their families. It might a priori be expected that many of the cases would be related as consanguinity is known to be relatively frequent, and if so more information could be obtained about the range of variation, clinically and biochemically, seen in carriers of one and the same mutant gene in the homo- or the heterozygous condition.

The present report gives, details of a study of 7 probands with amylo 1,6-glucosidase deficiency and of their relatives. Family connections were found between 6 of the probands, and it was likely that the 7th proband was related to the same family.

## *Material*

The probands comprise all patients in Landssjúkrahúsið (the central hospital of the Faroe Islands) in Tórshavn who during the period 1955—70 were found to present amylo-1,6-glucosidase deficiency. Some of the patients had later on supplementary examinations in the University Clinic of Copenhagen. In 1972 a very extensive study of the probands and all available relatives was undertaken, giving a total of 78 examined individuals.

## *Methods*

All individuals had their medical history taken and were subjected to a clinical examination in a search for signs of glycogenosis. Blood samples for biochemical and serological analyses were obtained, and 6 of the probands had a liver biopsy. The genealogy of the probands was worked out by interviews, but not supplemented by perusal of public registers, censuses or the like.

The activity of amylo-1,6-glucosidase in the white and red cells was determined, by methods to be described, after shipment of the samples to Marselisborg Hospital, Aarhus.

*Isolation of leucocytes and erythrocytes.* For the separation of blood cells 3 ml of a 5 % Dextran T 150 (Pharmacia) solution in 0.9 % NaCl and 750 ul of a 4 % EDTA solution were placed in a piece of plastic tubing, 32 cm long, inner diameter 10 mm, which was closed at one end with a rubber stopper. Approximately 20 ml of venous blood was tapped

into the tube which was then closed at the other end with a rubber stopper, inverted 10 times and placed vertically in a rack for 75 min at room temperature[1]. After sedimentation of the erythrocytes the tube was pierced just above the layer of red cells with a cannula and the plasma containing the white cells and the platelets was tapped. The plasma was centrifuged at a speed of 150 g for 4 min and decanted. The sedimented leucocytes were washed once by resuspension in 0.9 % NaCl and centrifuged as before. They were then resuspended in 2 ml 0.1 M NaF, kept for 15 min in an icebath, centrifuged (2000 g) for 5 min, decanted and stored at —20° C. During transport the cells were kept on dry ice. For enzyme assay the cells were thawed, resuspended in 700 µl 0.1 M NaF, sonicated and centrifuged (2000 g) for 5 min at 4° C. The supernatant was used as enzyme source.

The erythrocytes were washed 5 times in cold 0.9 % NaCl using centrifugation at 1500 g. After the last centrifugation one volume of destilled water was added to the sediment, the mixture was inverted a couple of times and stored at —20° C. For enzyme assay the cells were thawed, shaken vigorously by hand and centrifuged at a speed of 2000 g for 15 min. at 4° C. The supernatant was used as enzyme source.

*Analytical procedures.* Amylo-1,6-glucosidase activity was measured by its ability to incorporate glycosyl residues into glycogen by a modification of the method described by Nelson and Larner[8]. The reaction mixture contained in a total volume of 90 µl: 9 mg rabbit liver glycogen (Sigma type III), 135 nanomoles glucose-U-C14 (specific activity 25,000 cpm/nanomole), 3 nanomoles maleate buffer pH 6.5 and 30 µl enzyme solution. After incubation for 1 h at 30° C, 75 µl of the reaction mixture was withdrawn, spotted on filter paper and immersed in icecold 66 % ethanol as described by Nelson and Larner. Each assay was accompanied by a zero time incubation. This method was applied to both leucocytes and erythrocytes. The filter paper bits from the leucocyte assay could, after washing and drying, be counted directly in 10 ml 0.5 % PPO in toluene. In the assay of enzyme from erythrocytes, the haemoglobin precipitated on the paper bits gave such heavy colour-quenching that reproducible results could not be obtained. The paper bits were therefore placed in a disposable plastic counting vial, bleached with 300 µl 30 % hydrogen peroxide and treated with 3 ml of a 1:1 mixture of Soluene (Packard) and isopropanol for 30 min at 60° C to extract the radioactive glycogen. The radioactivity was then counted in 15 ml of a 1:9 mixture of 0.1 N HCl and Instagel (Packard).

Protein was determined by a Lowry method and leucocyte glycogen was measured with the method of Kristmann[6]. The ratio between the extinctions at 460 nm an 390 nm was also determined in this method.

The serum proteins and enzymes listed in table II were determined by conventional methods in the laboratory of the hospital in Tórshavn.

Serological markers were determined in the Blood Group Department of the University Institute of Forensic Medicine, Copenhagen, and the HL-A types in the Tissue Typing Laboratory of the University Clinic, Copenhagen, by the routine methods worked out in these laboratories.

## *Results*

The renewed clinical and biochemical studies of the probands verified the diagnosis in all cases.

*Clinical findings.* A short summary of the case histories of the probands are given in the following.

*Individual V. 105,* male, born 1954. Since the age of 2 years pronounced abdominal distension was observable, caused by enlargement of the liver which was palpable some centimetres below the umbilical transversal and extended to the spleen. The boy was clinically without symptoms, and hypoglycaemic episodes had never been noted in spite of low levels of fasting blood glucose. At the time of the present investigation (1972) his liver was only slightly enlarged, reaching 6 cm below the right costal margin.

*Individual V. 107,* male, born 1964. This boy had shown abdominal distension since his birth. At the age of 5 prenounced hepatomegaly was observed, the liver edge being felt 25 cm below the right costal margin and reaching to the spleen. His motor development was retarded, and he became easily fatigued and dyspnoeic on exertion. Biochemical studies at that time indicated moderate liver failure. In 1972, considerable hepatomegaly was found; the liver reached the umbilical transversal and the spleen. Ten small suggillations were noted in the skin.

*Individual V. 110,* male, born 1966. A large abdomen was noted in this boy when he was born, and when he was 2 years old his liver reached the umbilical transversal. There were no clinical cymptoms, but biochemical studies performed at that time revealed liver failure and low levels of fasting plasma glucose. In 1972 he suffered from mild dyspnoea on exertion and skin bleeding following minor injuries. The liver edge was felt 2 cm above the umbilical transversal and reached the spleen. Three small suggillations were noted in the skin.

*Individual V. 111,* female, born 1970; at the age of 10 months this child passed a routine examination by the family doctor who noted that the liver filled out »the entire right side of her abdomen«. She presented no

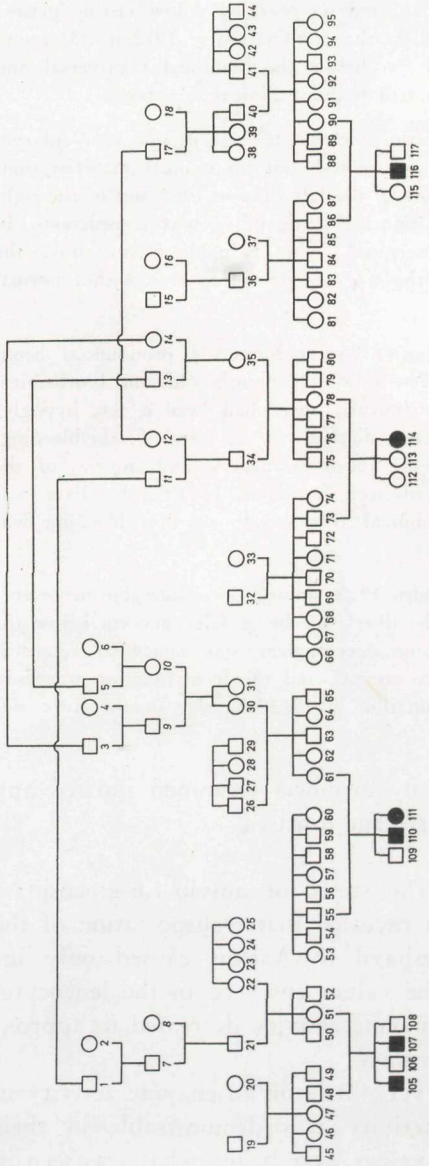


Fig. 1a: *Pedigree of the family. Black squares and circles indicate probands.*

clinical symptoms, but laboratory analyses revealed a low fasting plasma glucose and severe liver failure. At the examination in 1972 it was found that the liver was palpable 1 cm below the umbilical transversal and reached the spleen, but she was still without clinical symptoms.

*Individual V. 114*, female, born 1959. At the age of one year enlargement of the liver and spleen was noted, but no clinical manifestations were reported apart from fracture of the left tibia in 1962 and of the right femur in 1965; furthermore, abnormal fatigability was conspicuous. In 1972 the liver was slightly increased, being palpable 5 cm above the umbilical transversal, whereas the size of the spleen was within normal limits.

*Individual V. 116*, male, born 1961, presented a pronounced hepatomegaly at an examination when he was 10 months old, and biochemical studies revealed liver failure. Clinically there had been a few hypoglycaemic episodes, mild haemorrhagic diathesis in the form of skin bleedings and prolonged bleeding following tooth extractions and injuries of the skin. In 1968 his left tibia had been fractured. In 1972 his liver was palpable 4 cm above the umbilical transversal; no skin bleeding was noted.

*Individual V. 118*, female, born 1953, showed a considerable hepatomegaly at the age of one year, the liver edge being felt some cm below the umbilical transversal. Her motor development was somewhat retarded, but otherwise she was clinically normal, and the liver function was only slightly reduced. At the examination in 1972 the size of the liver was normal.

None of the other family members examined showed any clinical signs of glycogen storage disease.

*Biochemical findings.* The study of amylo-1,6-glucosidase activity in the blood cells revealed that transportation of the blood samples from Tórshavn to Aarhus caused only insignificant reduction of the values observed in the leucocytes whereas the erythrocyte enzyme activity decreased to approximately 20 % of normal values.

The patients presented very little or no enzyme activity in their leucocytes. Some activity was demonstrable in their erythrocytes although all values were below what was seen in controls (cf. table I).

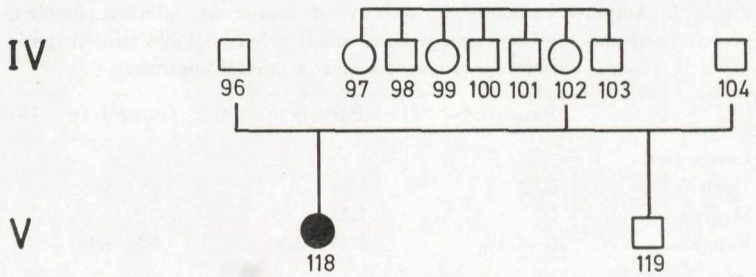


Fig. 1b: *Pedigree of the family. Black circle indicates the proband.*

In the parents, the leucocyte activity was on the average lower than in normal individuals, but the 2 distributions overlapped considerably, but were outside the range of values seen in the patients. The red celles of the heterozygous parents showed an activity which with one exception coincided with the distribution observed in the patients.

Liver function was studied in all probands; all of them except no. V. 105 and V. 118, who were the oldest, gave evidence of impaired function. Surgical biopsy of the liver was done in all but one (no. 111) of the patients. The histological picture indicated glycogen storage disease, but biochemical analyses were only performed in two cases (no. 107 and 116); here no amylo-1,6-glucosidase activity could be demonstrated in the liver cells.

The glycogen contents of the leucocytes of the probands and their parents showed values within the normal range. The ratio between extinction at 460 and 390 nm of the iodine-glycogen complex was low in leucocyte glycogen from the patients, indicating the highly branched glycogen structure. The parents showed no abnormalities in this respect.

The genealogical examination revealed that the mothers of 6 of the probands were related (see pedigree 1a, b), and at least one of the fathers was connected with this large family; the 7th proband was, according to the information given by the

*Table 1.* Amylo-1,6-glucosidase activity of leucocytes (glucose, nmole/g protein/min) and erythrocytes (glucose, nmole/g haemoglobin/min) in patients, definite heterozygotes and in normal controls.

| | Patients (n = 7) | Parents (n = 9) | Controls (n =18) |
|---|---|---|---|
| *Leucocytes* | | | |
| Mean | 0.12 | 1.51 | 3.04 |
| Median | 0 | 1.33 | ... |
| Range | 0—0.48 | 0.49—2.56 | 1.62—4.48 |
| *Erythrocytes* | | | |
| Mean | 0.122 | 0.126 | 0.225 |
| Median | 0.120 | 0.113 | ... |
| Range | 0.109—0.136 | 0.105—0.229 | 0.153—0.363 |

family, also connected with the other probands, but it was not possible to determine the exact relationship.

As heterozygosity could not be diagnosed with certainty by the methods used in the present study it was impossible to perform ordinary linkage studies. It was, however attempted to look for possible associations within sibships between a factor influencing the enzymatic level and each of the marker systems taken separately. If sib pairs alike with respect to a given marker show significantly lower activity differences than those sib pairs which present dissimilarity with respect to the marker, this may be a reflection of genetic linkage with the gene determining the level of enzyme activity. Lack of significant associations does not in any way exclude linkage as it may i.a. be caused by the fact that the methods employed reflect the genetic variation very poorly. When sibships showing variation with respect to a given marker were used in the analyses, all results were non-significant except for the comparison between ADA phenotypes and enzyme levels. The mean intrapair enzyme activity difference was found to be significantly lower (P between 0.05 and 0.01) in ADA identical than in ADA different sib pairs.

The serological markers were also used to check the alleged biological relationships. It appeared most likely that individuals IV, 53 and 54, according to the results obtained in the MNSs and the HL-A systems and considering the rarity of crossing-over in these systems, had only their mother in common with the remaining members of the sibship (individuals 55—60). In the sibship comprising individuals V. 112, 113 and 114 one of the haplotypes observed in no. 112 was not seen in any of the parents, nor in the two sibs; the results obtained in the Rh system

*Table 2.* Results of laboratory examinations of the probands; serumprotein and some enzymes

| Individual No. | Sex | Age year | Hb | Pl. | PP | Chol. | Alan. | Asp. | LDH | Alk. ph. | Tot. Pr. | Alb. | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 105 | M | 17 | 14.9 | 168 | 82 | 175 | 11 | 32 | 200 | 74 | 76.0 | 48.3 | 3.0 | 6.2 | 9.4 | 9.1 |
| 107 | M | 8 | 13.2 | 137 | 82 | 278 | 54 | 141 | 305 | 102 | 81.0 | 51.2 | 2.7 | 7.2 | 10.5 | 9.4 |
| 110 | M | 6 | 13.0 | 250 | 95 | 191 | 50 | 155 | 310 | 200 | 76.0 | 47.2 | 3.2 | 7.6 | 9.3 | 8.7 |
| 111 | F | 2 | 11.3 | 267 | 300 | 485 | 50 | 200 | 440 | 188 | 79.0 | 45.6 | 4.0 | 10.9 | 9.9 | 8.6 |
| 114 | F | 12 | 13.9 | 145 | 86 | - | 25 | 72 | 265 | 117 | 77.0 | 48.3 | 2.9 | 7.0 | 6.9 | 11.9 |
| 116 | M | 10 | 14.4 | 175 | 129 | 147 | 45 | 95 | - | 78 | 69.0 | 47.6 | 2.8 | 5.7 | 6.3 | 6.6 |
| 118 | F | 18 | 11.5 | 261 | 100 | 108 | 4 | 20 | 160 | 25 | 71.0 | 47.4 | 2.8 | 6.7 | 6.7 | 7.4 |
| Normal range (adults) | | | 12.1-17.6 | 150-500 | 70-130 | 180-250 | 5-35 | 8-40 | <241 | 20-150 | 66.0-82.0 | 44.4-58.6 | 2.3-4.3 | 3.1-5.9 | 4.7-7.9 | 6.2-11.0 |

Abbreviations: Hb = haemoglobin (g/100 ml); Pl = platelets (mia/l); PP = protrombin-proconvertin (%); Chol. = cholesterol (mg/100 ml); Alan. = alanine aminotransferase (units); Asp. = aspartate aminotransferase (units); LDH = lactate dehydrogenase (units); Alk. ph. = alkaline phosphatase (units); Tot. Pr. = total protein (g/l); Alb. = albumin; $\alpha_1$ = $\alpha_1$-globulin (g/l); $\alpha_2$ = $\alpha_2$-globulin (g/l); $\beta$ = $\beta$-globulin (g/l); $\gamma$ = $\gamma$-globulin (g/l).

also failed to support the information that these three individuals were full sibs; it was more likely that only V. 113 and 114 were full sibs. Incidentally, the rare Rh-gene D- -, which has formerly been described in the population of the Faroe Islands, must be present in V. 114, his father and the sibs of the father in order to explain the Rh-types in this part of the kindred.

*Discussion*

The relationships established between the 6 probands and the possibility that the 7th proband was also related to the other as well as the distribution of the patients in the larger family give strong support to the assumption of autosomal recessive inheritance.

The clinical picture observed in the probands brought no new features, but the 6 probands known to belong to one and the same family reflected the variation which may be observed in patients carrying the same gene (in the homozygous condition).

A rough estimate of the incidence of the condition may be obtained as it is known that about 14,000 children were born alive in the Faroe Islands during the period 1953—70, and 7 of these were later diagnosed as cases of amylo-1,6-glucosidase deficiency. This leads to a higher estimate of the incidence than found in any population studied previously. The presumed high rate of consanguinity is of course an important factor in the explanation of this observation, but additional possibilities should also be considered. As the prognosis of the disease seems to be relatively good, it may be expected that a mutant gene of this type, when introduced into a population with high frequency of intermarriage, may give rise to a high number of cases.

The determination of amylo-1,6-glucosidase activity in the leucocytes seems to have provided a reliable diagnosis in all the present probands. The definite heterozygotes show a median value which lies between that of the patients and the normal controls, but as the distribution of values seen in heterozygotes overlaps the distribution in normals to a considerable extent

it is obvious that a diagnosis of heterozygosity cannot be established in single individuals by this method. Healthy sibs of patients (with a chance of being heterozygous of 67 %) and sibs of parents of patients (with a chance of heterozygosity around 50 %) have as expected medial values between those seen in definite heterozygotes and in normals.

The determination of enzyme activity in the erythrocytes does not permit any distinction between patients and definite heterozygotes. It is possible that the presence of measurable activity in the patients is an artifact due to unspecific binding of radioactive glucose to glycogen and protein in the assay; this may also take place in the assay of the enzyme in leucocytes, but the results here show zero activity in the patients, corresponding to the much lower protein concentration in this assay. The median value of erythrocyte activity in normals is much higher than that of the patients and the heterozygotes, but the distributions overlap considerably. Thus, the examination of erythrocytes by the methods adopted here is less informative than the study of leucocyte activity.

It seems clear that more reliable methods of defining heterozygotes are needed for practical as well as theoretical purposes. Genetic counselling cannot be sufficiently exact as long as such methods are lacking, and they will be needed if more effective preventive measures such as screening of newborns in this and other high risk populations are to be introduced. Linkage analyses would also have to await the appearance of methods for reliable heterozygote detection. The present suggestion of a possible association between the gene determining ADA variants and enzyme activity may be due to chance alone as a considerable number of tests for association was performed. It might, however, be worthwile to attempt studies of the genetic relation between the genetic ADA variation and amylo-1,6-glucosidase deficiency by somatic cell hybridization; positive results obtained in this way could be of high value in the continued studies which aim at a more complete elucidation of type III glycogenosis.

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## ABSTRACT

Seven cases of glycogenosis type III (amylo-1,6-glucosidase deficiency) in two probably related families from the Faroe Islands are presented. The group of patients comprised two pairs of sibs. In a total of 78 members of the 2 families case histories were obtained and clinical examinations, analyses of amylo-1,6-glucosidase activity in erythrocytes and leucocytes, determinations of red cell, serum and enzyme groups as well as HL-A types were performed. In addition, all patients were subjected to studies of liver function. The distribution of patients in these families supports the assumption of autosomal recessive inheritance.

Heterozygotes could not be diagnosed with certainty by the methods of enzyme activity analysis employed.

The incidence of glycogenosis type III with amylo-1,6-glucosidase deficiency was found to be high in the Faroe Islands.

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## ÚRTAK

Sagt verður frá sjey tilburðum av glycogenosis flokki III (amylo-1,6-glucosidasu troti) í tveimum, kann henda seg, skyldum ættum í Føroyum. Í sjúklingabólkinum vóru tvey systkinapør. Frá til samans 78 fólkum úr báðum ættum vórðu fingnar sjúkrasøgur, og vórðu fólkini læknakannað, eins og kannað varð amylo-1,6-glucosidasu virksemi í reyðum og hvítum blóðkroppum, flokking av reyðum blóðkroppum, sveitavatni og kveikabólkum eins og HL-A flokkum. Umframt varð starvsemi hjá livrini kannað hjá øllum. Fyrikomingin av sjúklingum í hesum báðum ættum bendir á autosomalt dvínandi ættarbregði.

Við teimum rannsóknarháttum, ið nýttir vórðu viðvíkjandi kveikavirksemi, bar ikki til við vissu at gera av heterozygotar.

Títtleikin av glycogenosis flokki III við amylo-1,6-glucosidasu troti varð funnin at vera høgur í Føroyum.