High calculated prevalence of glycogen storage disease among Faroese newborns

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BACKGROUND

The Faroese are a genetically isolated population descending from a small number of settlers. Several founder mutations have been identified and, in several cases, Faroese individuals with certain diseases all share the same causative mutation. Four treatable diseases with causative founder mutations form the basis of a research project that started in November 2019 and will continue until October 2022. During this period all parents are offered neonatal genetic screening for the listed disorders (Table 1).

	Glycogen storage disease IIIa	Primary carnitine deficiency (PCD)	Holocarboxylase synthetase deficiency	Cystic fibrosis*
Prevalence FO	1 : 3600	1 : 300*	1 : 1700	1 : 1775**
Prevalence WW	1 : 100.000	1 : 100.000	1 : 100.000	1 : 4800

Table 1 – Genetic disorders in the neonatal screening programme
The four disorders in the neonatal screening programme are shown along with their published prevalence in the Faroe Islands (FO) compared to prevalence worldwide (WW). *There is no cure for cystic fibrosis but children are treated to control symptoms, **the listed prevalence is the published observed prevalence, calculated prevalence from the same paper was 1:2300. Abbreviations: DK; Denmark.

Primary carnitine deficiency (PCD), holocarboxylase synthetase deficiency and cystic fibrosis are included in the Danish routine neonatal screening programme that the Faroe Islands follow. The genetic screen aims to supplement these metabolic tests, and test their sensitivity.

Glycogen storage disease type IIIa (GSD IIIa) is an inborn error of metabolism caused by mutations in the amyloglucosidase gene (AGL). Faroese patients all share a founder mutation, c.1222C>T (Santer et al., 2001). The prevalence of GSD IIIa in the Faroe Islands has been shown to be the highest worldwide (Santer et al., 2001). However, while GSDIIIa satisfies all of the criteria for neonatal screening set out by the WHO (Box 1) it is not included in the routine national screening programme as it is very rare outside of the Faroe Islands and there is no metabolic test available for detection of the disorder. Children with GSDIIIa are diagnosed when clinically symptomatic at which point permanent damage has already occurred in some (Joensen et al., 2006).

Box 1: WHO Criteria for neonatal screening

1. Disorders result in severe mental and physical morbidity and/or mortality if not diagnosed in the neonatal period
2. Disorders cannot be diagnosed by simple clinical examination
3. Disorders are easily treatable and have significantly improved prognosis with early treatment
4. Disorders are relatively common in the population (>1:10,000-15,000)
5. Disorders are detectable by a simple, rapid and reliable screening test

Wilson and Jungner, 1968

The prevalence of GSD IIIa in the Faroe Islands is the highest worldwide. Until now, screening for GSD IIIa has not been performed as no suitable test has been in place.

METHODS

Blood spots are collected from newborns. DNA is isolated and the regions of interest are copied using small molecule- molecular inversion probes (smMIPs), amplified and sequenced (Figure 1). All laboratory work is conducted at iNOVA research park. Data analysis is performed with NextSeq (JSI medical systems).

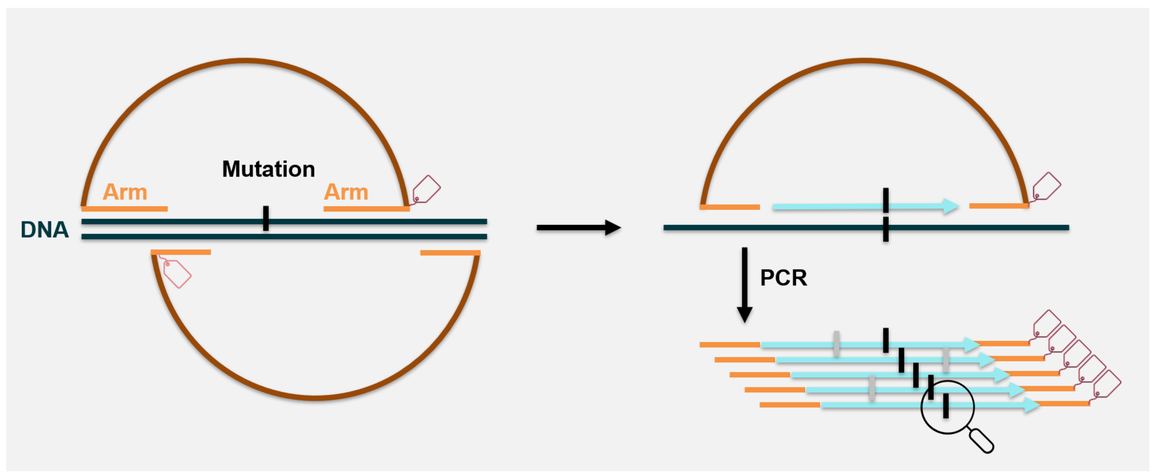

Figure 1 – small molecule- molecular inversion probes (smMIPs)
The arms are complementary to the sequences around the mutation site, i.e. the targeted region. The smMIP hybridises to DNA and gap filling through extension and ligation copies the region of interest (=capture). The single molecule tag labels each captured DNA molecule with a unique tag (pink and dark purple tags on the left diagramme). Each mutation site is covered by at least two independent smMIPs targeting both DNA strands. The copied region of interest is amplified with arms and small molecule tag by PCR and sequenced on a MiSeq. Consensus sequences are built, thereby minimising PCR and sequencing artifacts (grey vertical lines) as only variations found in all copies are considered true variations (black vertical lines).

RESULTS

Analysis has been completed for the first 278 samples. No children have been identified with any of the disorders so far, but a number of healthy carriers have been identified for all disorders. An example is shown in Figure 2. The carrier frequencies that are observed are used to calculate (calculated) prevalence (Figure 3).

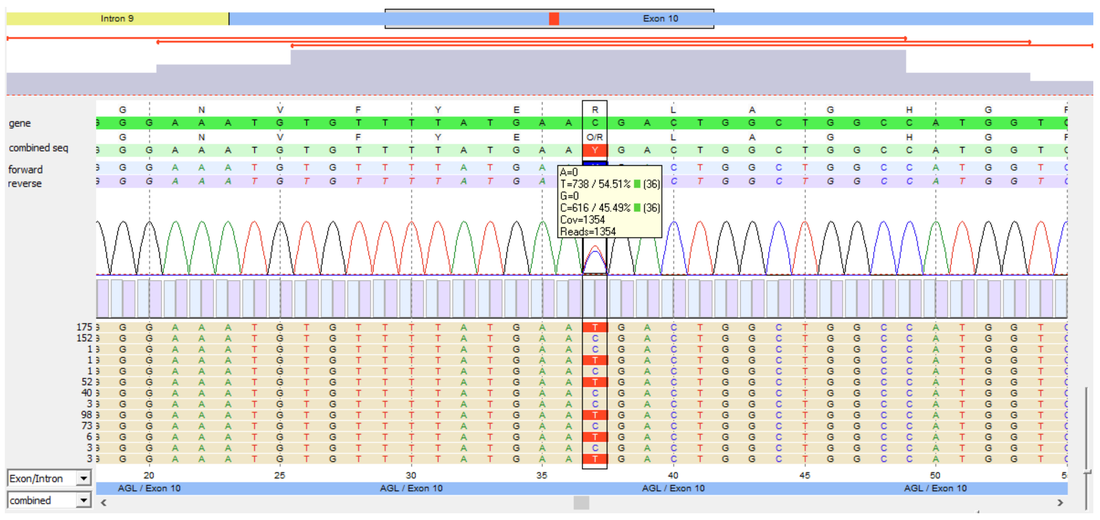

Figure 2 – Example of a healthy GSD IIIa carrier.
Note the three red lines at the top of the figure, below the exon number, showing the three smMIPs that individually capture the region of interest. Of the 1354 reads, 738 individual DNA molecules had a T and 616 had a C at the c.1222 position of interest. The number of unique DNA molecules captured with each smMIP are shown on the far left, bottom panel, for the three smMIPs.

Carrier frequency of GSD IIIa among Faroese newborns indicates a calculated prevalence of GSD IIIa at 1: 1745; twice as high as previously published (Figure 3). The numbers are still low and the calculated frequencies differ from published numbers, especially for GSD IIIa and HLCS. By the end of the project we aim to have analysed 1500 samples. Observed prevalence of GSD IIIa in the Faroe Islands is 1: 3330 (16 cases). No new case has been identified since 2008 – approximately 8000 children have been born since then. Statistically, we would expect between 2 – 4 cases of GSD IIIa since 2008.

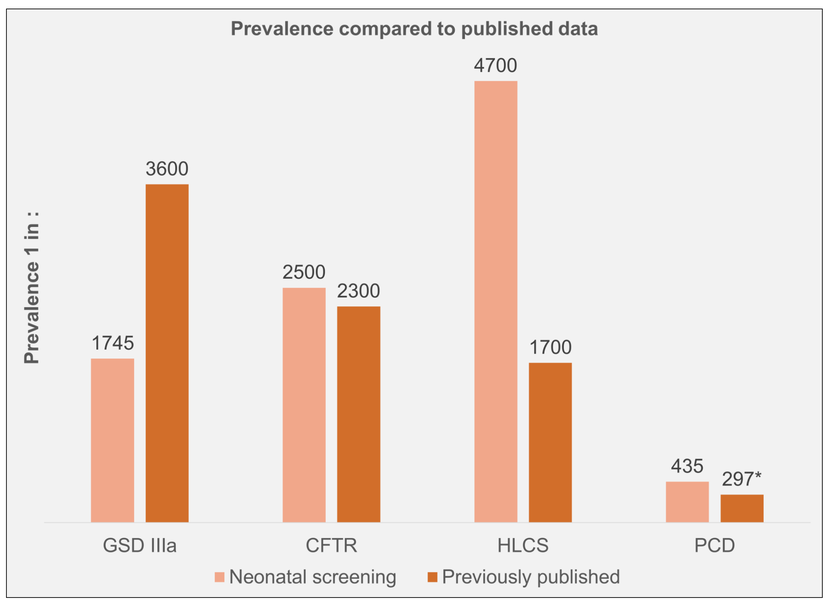

Figure 3 – Calculated prevalence for the four disorders compared to published data.
Carrier frequency was used to calculate prevalence in the population, shown as 1 in X. In the neonatal screening project 278 samples were analysed for all conditions. In the previously published studies **the number of samples included** in the analysis were; GSD IIIa: 272, CFTR: 881, HLCS: 217, and PCD 26462. *PCD prevalence is based on mutation status following population screening for low blood carnitine. Prevalence for the other conditions is based on mutation screening of DNA from random Faroese PKU cards.

The frequencies of all four disorders are high in the Faroe Islands. We find the second highest prevalence for GSD IIIa, a disorder that has never been part of a screening programme in the Faroe Islands.

CONCLUSION

- For the first time, all Faroese newborns are offered genetic screening for four disorders with high prevalence in the Faroe Islands
- The prevalence of GSD IIIa is high in the Faroe Islands
- The smMIP method can be used as a simple, rapid and reliable screening test to screen for GSD IIIa in Faroese newborns
- Screening for GSD IIIa enables affected children to start treatment before they become symptomatic

ACKNOWLEDGEMENTS

The project is funded by Innovationsfonden, Sjúkrakassagrunninum and CTD felagnum.

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