Human Population Genetics in the Faroe Islands

A research model and a haplotype study on bipolar affective disorder

Arvaeginleikakanningar í Føroyum

Kanningarbygnaður og ein kanning innan tvípóla hýrissjúku

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Úrtak

Kring allan heimin verður roynt at koma nærri møguligum arvaeginleikum, sum kunnu hava týdning fyri eitt nú sinnisligar sjúkur.

Royndir eru gjørdar at seta í gongd verkætlanir millum fólk, sum í øldir hava búð í útjaðarøkjum. Her kann verða lættari at vinna fram við kanningum, tí møgulig avvik í arvaeginleikunum kunnu her vera í færri útgávum.

Slíkar verkætlanir eru gjørdar í Føroyum. Í fyrstu atløgu nýtt at kanna bipolera sinnisliga sjúku, eisini rópt manio-depressiv sjúka.

Fyrstu úrslitini eru við og serliga við atløgu innan kromosom 16 og 18, har serliga tvinni øki á kromosom 18 kunnu hava týdning.

Abstract

Genetic studies concerning psychiatric illnesses have for some years created hope for new insight in mental illness.

Isolated populations may offer a new approach. Here, the number of different alleles and mutations may be fewer and therefore more easy to detect.

Such models have been developed for patients in the Faroe Islands. The first illness to study has been bipolar disorder, or manic-depressive illness.

Early results concerning chromosome 16 and 18 have especially pointed to two areas on chromosome 18 as interesting.

Introduction

Interest in complex disease gene mapping in isolated populations has increased in recent years. In geographically and culturally isolated populations founded a few hundred or thousand years ago from relatively few individuals most persons with a disease may have inherited a disease gene and neighbouring markers from a single or few ancestors. A disease chromosome descending from a common ancestor not too many generations ago will share a common haplotype in the neighbourhood of the disease allele, reflecting the alleles and haplotype of the ancestral chromosome on which the disease mutation occurred. In such a population linkage disequilibrium has been found in much larger chromosome areas of up to around 10 cM as opposed to less than

1 cM often seen in large, panmixed populations. The search for linkage disequilibrium in association studies among unrelated, or very distantly related cases, with anononymous markers may be impossible for diseases in which mutations have occurred several times at several loci, none of which may need to be frequent (de la Chapelle, 1993; Houwen *et al.*, 1994; Kaplan *et al.*, 1995).

For complex diseases with locus heterogenecity it is possible that only some among several susceptibility loci are present, and this is advantageous. The smaller the group of founders the greater the probability of only some susceptibility genes may being present. Furthermore, in small populations gene frequencies may fluctuate randomly from generation to generation and alleles may be lost. Bottleneck reductions in population size due to disasters may further decrease the number of disease genes present (Holgate, 1966; Nei *et al.*, 1975; Neel and Thompson, 1978; Spiess, 1989).

Recently an approach has been applied, using a search for shared chromosomal segments among affected individuals in an isolated population who were related a few centuries ago, and in a genome wide scan used to map a gene for beningn recurrent intrahepatic cholestasis. This approach makes the localisation of recessive or dominant disease genes possible by means of a genome wide scan with a few hundred markers genotyping only a few affected individuals and their parents (Houwen *et al.*, 1994; Freimer *et al.*, 1996; Escamilla *et al.*, 1996).

As this method compares only the haplotype of affected individuals it can be applied to diseases with low penetrance.

Material and methods

Population and history of the Faroe Islands

The Faroe Islands is a small group of islands in the North Atlantic Ocean situated between Norway, Iceland and Scotland. Of the 18 main islands, 17 are inhabited. The islands cover an area of 1.399 km^2.

The origins of the population is not known, but thought to be mainly Norwegian with admixture from the British Islands. Thus, disease genes found in the Faroese population are potentially more relevant for a larger population than mutations in other genetic isolates in Europe such as Finland and Sardinia. Irish monks are believed to have visited the islands from about A.D. 725 or earlier. The islands have been raided by the Vikings from about 795 and settled from around 825 probably especially by emigration from the western part of Norway around Bergen. The islands were thus mainly populated at the same time as Iceland and it is believed that the majority of the land was already occupied around year 900. While the population in Iceland has been estimated to around 65000 in year 965 probably only a few thousand people inhabited the Faroe Islands at that time. Isolation was imposed by distance, by commercial monopoly and legislation and the principal contacts have been with Norway, Iceland and Denmark. However, the islands have had some contact with merchants, smugglers, pirates and naval forces

from Scotland, Ireland, England, the Netherlands, Germany and France. Even pirates from Algiers attacked the islands in 1629 (Young, 1979).

The size of the population remained around 4000 inhabitants from the late 13th century until around 1800. A count in 1769 shows that it was then 4773. As severe epidemics occurred periodically the size of the population has occasionally been less. It is believed that up to around one third of the population died from the plague around 1349. A severe smallpox epidemic occurred in Torshavn in 1709. Periods of starvation have occurred as the islands were never self-sufficient in grain. From 1801-1860 the population doubled to around 9000. Since then the population has increased to around 45000 today. Immigration has been sparse and the expansion has mainly been by increasing the number of children per amily. For centuries the population was scattered in about 85 small villages each containing an average og 10-12 families. Internal subisolates have been present up till today. Today one third of the population lives in Torshavn,and two thirds of the population lives in Streymoy and Eysturoy. As one of the remotest communities of Europe the Faroese have maintained their own national and cultural identity, including their own language and the medieval ballad tradition of ring dance (Wang, 1996).

The Faroese have had a Home Rule Government within the Kingdom of Denmark since 1948.

Psychiatric morbidity on the Faroe Islands

In the 19th century it was believed that there was a greater prevalence of mental disorder on the Faroe Islands than in other parts of Scandinavia. Today the incidence and pattern of psychiatric disorders as valued from hospital admissions are similar compared to Denmark (Joensen and Wang, 1983; Wang, 1981, 1984; Palmquist and Wang, 1997).

Concerning affective disorders the occurrence of attempted suicide and suicde is low on the Faroes (Wang,1996).

Collection of bipolar cases and diagnostic assessment

Well documented cases with bipolar disorder were sought among patients treated at the Department of Psychiatry. National Hospital, Torshavn, which is the only psychiatric department in the Faroe Islands. Furthermore patients who themselves and whose ancestors predominantly originated from a relatively isolated regions of the Faroe Islands were selected. The interviews were performed by an experienced psychiatrist (A.G.W.) using a brief version of Present State Examination. On the basis of hospital notes and the interview a clinical narrative was made for each patient. Final diagnosis was made as a best estimate by an experienced psychiatrist who independantly had reviewed the clinical narrative and other relevant material. The diagnosis were made in accordance with ICD-10, Diagnostic Criteria for Research and DSM-IV (WHO, 1996). The eight patients included were 3 males and 5 females. Age of onset

Table 1. *Probability p(m) that a random chromosomal segment of a ceratin size is shared among eight descendants related g generations ago.*

Talva 1. Yvirlit yvir hvussu sannlíkt tað er (p(m)), at eitt petti av kromosomi av ávísari stødd kann finnast felags hjá 8 sjúkum, sum eru í familju g ættarlið aftur í tíðina.

Size of segment 10 cM		p(m)
g=5	0 out of 8	0.86157
	1 out 0f 8	0.12958
	2 out of 8	0.00853
	3 out of 8	0.00032
	4 out 0f 8	<0.00001
g=6	0 out of 8	0.93547
	1 out of 8	0.06266
	2 out of 8	0.00184
	3 out of 8	0.00003
	4 out of 8	<0.00001
15 cM		
g=6	0 out 0f 8	0.95382
	1 out of 8	0.04523
	2 out of 8	0.00094
	3 out of 8	0.00001
	4 out of 8	<0.00001

was between 17 and 33 years. All eight held a bipolar diagnosis by ICD-10 and a bipolar type I doagnosis by DSM-IV.

Genealogic assessment

Cases related six to ten generations ago were sought in order to obtain an reasonable size of shared chromosomal segments around the putative disease gene(s). A genealogical search of church and civic records of birth, marriages and deaths were made for each patient. Lineages were traced back as long as possible and the shortest possible distance between any pair of the patients parents were recorded. Exellent church records exist from around 1750 while incomplete church and civic records exist from even earlier. All eight patients could be connected in one or several ways. The father in generation 1 was born around 1620, but the birth year of his wife is unknown. The shortest possible distance between the 28 possible pairs made by connecting each person from the parent generation with each other were calculated. The average number of generations relating two patients were 6.2. Only two pairs were related as short as three or four generations ago. In order to reconstruct the haplotypes preferably both parents of the patients should be collected and genotyped. However, due to the strict inclusion criteria based on severity of phenotype, geography and treatment response two parents could only be collected for two of the eight bipolar patients, while one parent could be collected for the remaining five patients. Parents still lack from the eighth patient. This was not considered a major obstacle as haplotypes can be inferred except for markers in which the parent and child are identical heterozygotes, in which case additional markers may be genotyped if necessary.

Statistical evaluation

The affected persons may share a haplotype identical by descent around a disease gene or identical by descent by chance. If only one or a few less informative markers are genotyped in a chromosomal area the affected persons may share marker alleles identical by state.

The probability that the affected persons

Table 2. *The probability of segment sharing by chance with two neighbouring markers not linked to the disease in proportions out of 16 chromosomes. P(com) is the probability when the most common allele at each locus makes the pair, p(IBS) are the probabilities summed for all possible pairs for the different haplotypes.*

Talva 2. Víst verður hvussu sannlíkt tað er, at eitt felags petti við tveimum grannamørkum verður arvað, mett sum tøl í mun til 16 kromosom. P(com) er hvussu sannlíkt tað er, tá títtasti allelur gongur sum par, p(IBS) er hvussu sannlíkt tað er, tá allir møguleikar eru við fyri ymsar haplotypir.

	p(com)	p(IBS)
any 3 out of 16	0.23778	0.70104
any 4 out of 16	0.14720	0.24294
any 5 out of 16	0.06729	0.08386
any 6 out of 16	0.02350	0.02584
any 7 out of 16	0.00639	0.00666
any 8 out of 16	0.00137	0.00139

share a haplotype which is by chance identical by descent can be calculated from formulas derived by Houwen *et al.* (1994) (Table 2). A s shown even as little as 2 out of 8 individuals sharing a haplotype identical by descent is a rare event which even a genome wide scan only occurs a few times for persons related six or more generations ago.

When considering alleles at two neighbouring markers and which alleles are in linkeage equilibrium the probability of segmentsharing just by chance is dependent on the marker allele frequencies. For two neighbouring markers with 6 alleles each with frequencies of 0.4, 0.2, 0.15, 0.1 and 0.05 these probabilities are shown in Table 2.

It is crucial to choose which amount of sharing that is needed before a chromosomal area is considered iteresting. If the treshhold is set too high the disease gene region may be missing and if the treshhold is set too low to many false positive regions will have be tested with additional markers.

Genetic analyses

DNA was prepared from whole blood, using a standard triton lysis, nuclear lysis protocol with sodium chloride/isopropanol precipitation. DNA amplification was employed using a Perkin-Elmer thermocycler with optimised reaction conditions for each primer set and analysed on the ABI Prism 310 Genetic Analyzer.

As part of an ongoing genome wide scan twelve markers were tested on chromosomes 16 and 18 (Table 3).

Results

Preliminary results are shown in Table 3. On chromosome 16 at least 10 alleles were shared for the most distal marker tested D16S2622. Evidence of segment sharing did not recieve support from the marker proximal for D16S2622.

On chromosome 18 for marker D18S877 11 out of 16 alleles were shared while for marker D18S541 13 out of 16 alleles were shared. This did receive some support from neighbouring markers.

Discussion

As the Faroese population descended from Scandinavian and British ancestors, and probably are founded from much fewer people than Iceland, disease genes found in this population are of great potential relevance.

Etiological heterogenity which makes mapping of disease genes difficult is very likely in bipolar affective disorder. In the present study several measures were taken in order to identify an apparently homogenous subform of bipolar disorder. Cases related around six generations ago whose ancestors originated from a certain regions of the Faroe Islands were selected. Furtermore only cases responding to lithium treatment as defined by Grof *et al.* (1994) were collected.

Though both parents were alive for only two out of the eight patients only in a few instances were the haplotype not inferable due to the patient and parent being of identical genotype. Thus, in reality, the search for a common haplotype may not be greatly impeded even when only one of the parents are available.

We will collect a representative sample of around 100 persons from the relevant regions of the Faroe Islands in order to evaluate the allele frequency in the background population. This may also allow significant allele sharing between a fraction of the affected persons to be detected.

The present study reports preliminary results from chromosomes 16 and 18. Concerning chromosome 16 the most distal marker on chromosome 16p showed evidence of increased marker allele sharing, which is interesting as we earlier have found possible evidence of linkeage to chromosome 16p13.3 with a maximum LOD score of 2.76 for D16S510 which is located around 3 cM proximal to D16S2622. However, as the distance to the marker proximal to D16S2622 is 14 cM additional testing of markers in this region is necessary.

Concerning interesting markers on chromosome 18, 13 out of 16 alleles were shared for marker D18S541. This marker is located distal on chromosome 18q, proximal in the area in which Freimer *et al.* (1996) found evidence of haplotype sharing in Costa Rica. More markers will be tested in that area in the near future. D18S877 is located on chromosome 18q12, i.e. in the pericentrometric region. Earlier reports have been about positive LOD scores at this marker (1.56 for a narrow phenotypic model) in a large family (Ewald *et al.* 1995). Some evidence of increased allele sharing were also found at marker D18S976 located close to D18S62. As the neighbouring markers were located 18 and 16 cM away additional markers will be tested in that area. The marker D18S542 which is the marker closest to the perhaps most interesting marker in the study by Berrettini *et al.* (1994) and Stine *et al.* (1995). D18S37 also showed some evidence of increased sharing and additional markers will be tested in the near future in that area.

The present study is part of a larger project (FAROGENE) investigating several different psychiatric diseases in the Faroe Islands, including subtypes of affective disorder.

Acknowledgements

The project has been supported with grants from: Danish hospital foundation for medical research, region of Copenhagen, the Faroe Islands and Greenland; The Danish research counsils; Research fund of Føroya Sparikassi; Ministry of Education, culture and research, the Faroese Government.

Table 3. *Markers on chromosome 16 and 18 are shown with analysis results.*
Talva 3. Mørk nýtt á kromosom 16 og 18 eru víst saman við rannsóknarúrslitum.

Table 3.	A	B	C	D	E	F	G	H	
D16S2622 (14 cM)	83 83	83 75	83 83	79 75	75 87	83 83	83 89	83 83	10/16
D16S748 (6 cM)	205 205	196 205	208 193	193 208	208 193	205 193	202 193	208 193	6/16
D16S2619 (17 cM)	152 156	152 148	152 148	148 152	152 156			148 148	5/12
D16S403 (8 cM)	137 135	137 135	135 143	139 137	135 145	135 135	135 138	135 135	9/16
D16S769 (7 cM)	253 265	261 261	253 257	257x261	257x261	265 261	253 257	265 257	5/16
D16S753 (15 cM)	249 261	257 245	261 261	253 257	245x253	257 245	249 257	233 249	4/16
D16S771 (3 cM)									
GATA22F09 (15 cM)									
D16S2624 (7 cM)	140 136	136 136	136 140	144 136	140x144	136 136	148 136	144 136	9/16
D16S518 (19 cM)									
D16S422 (13 cM)	188 188	200 208	204 198	200 188	206 200		180 200	204x206	4/14
D16S539	166 154	166 170	166 178	154x1709	170 170	154x170	166x170	166 166	6/16
D18S59 (18 cM)	152 152	162 162	162 162	170 166	166x170	168 168	158 164	154 164	3/16
D18S976 (16 cM)	186 186	186 186	182 186		186 190	190 186	186 190	178 186	9/14
D18S843 (12 cM)	186 189	189 192	186x189		192 192	195 189	183 196	189 192	5/14
D18S542 (17 cM)	195 189	195 181	189 203	189 191	195 189	189 189	189x195	189 195	8/16
D18S877 (11 cM)	129 129	129 133	121 129	129 126	129 129	129 126	125 129	129 129	11/16
DS18S536 (10 cM)	142 142	146 146	146 168	150 150	142 142	146x150	154 148	146 146	6/16
D18S851 (6 cM)	264 264	264?268	264 260	264x272	260x272		272 272	264x268	5/14
D18S858 (4 cM)	191 194	200 194	194 200	194 194			200 203	194x200	6/10
D18S64 (17 cM)	194 196	198 196	194x206	196 212	198 198		194 194	194 196	5/14
GATA26C0 (13 cM)	272 276	284x292	272 292	284 284	276 272	272x288	288 280	288 292	4/16
D18S541 (14 cM)	272 272	272 272	272 272	280 272	280 272	272 272	272x280	272 272	13/16
D18S844	201 198	198x95	195x201	192x198	195 195	198 204	198x192	198 201	6/16

x symbolizes genotypes for which the haplotype could not be determined.

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