Genetically Isolated populations

Implications for genetic care

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Chapter 1

General introduction and outline of the thesis
Chapter 1

GENERAL INTRODUCTION

Genetically isolated populations exist worldwide, including in the Netherlands. These populations provide unique opportunities for many different genetic investigations. The main objective of this thesis is to demonstrate these various opportunities in a young genetically isolated population in the Netherlands.

Genetically isolated populations

Genetically isolated populations are (sub)populations derived from a small number of individuals who became isolated because of a founding event and stayed ‘isolated’ for many generations. The founding events and reasons for isolation may be diverse, e.g. geographical, cultural, political, and/or religious. Often, these genetically isolated populations show a high rate of population expansion, very restricted immigration from neighboring populations, and endogamy (marriage within the community).

The common ancestral origin, bottleneck effect in the history of the population, genetic drift, and/or the founder effect cause reduction in genetic variation (for definitions of the different concepts see Box 1). This is characterized by extended chromosomal runs of homozygosity (ROH’s), increased identity-by-descent (IBD) sharing within the population, and wider intervals of linkage disequilibrium (LD).

Members of these populations also tend to show reduced non-genetic variance due to the sharing of similar environmental and cultural factors, such as climate, lifestyle, diet, and exposure to disease vectors.

Each genetically isolated population has its own evolutionary and demographic history in terms of the total number of founders, number and intensity of bottlenecks, age and duration of isolation, levels of endogamy, and other population-related factors, such as the growth and expansion during the life of an isolate. They can be divided in very old isolates (>100 generations), younger isolates (≤ 100 generations), and very young isolates (<20 generations). A very old genetically isolated population is, for example, the Saami in northern Scandinavia. Examples of younger isolated populations are Finland, Iceland, Sardinia, and the Ashkenazi Jewish population. Very young genetically isolated populations are, for example, the population of the Central Valley of Costa Rica, Hutterries, Mennonites, Old Order Amish, French Canadians of
Quebec,\textsuperscript{14} and several small genetically isolated populations in the Netherlands.\textsuperscript{15,16} In many of these genetically isolated populations genealogical records with large multigenerational pedigrees are available.

The reduced genetic variation together with endogamy may result in high prevalences of rare monogenic as well as multifactorial disorders in these populations compared to the nation-wide prevalences. However, some other genetic disorders may be relatively rare because the founders of the genetically isolated population were not carriers of these disorders.

\textbf{Box 1. Definitions of relevant concepts.}

<table>
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<th>Term</th>
<th>Definition</th>
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<tr>
<td>A genetically isolated population or founder population</td>
<td>is a population that is or was geographically, culturally or for religious reasons isolated and as a consequence has little genetic variation.</td>
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<tr>
<td>A bottleneck effect</td>
<td>occurs when there is a sharp reduction in the size of a population due to a natural disaster or similar event with, as a consequence, reduction of the genetic variation in the population.</td>
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<td>A founder effect</td>
<td>is the reduction of genetic variation that occurs when a new population is founded by a small number of individuals (founders) from a larger population and this population remains isolated from other populations.</td>
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<td>A founder mutation</td>
<td>is a gene mutation on an identical haplotype background, observed with high frequency in a genetically isolated population in which one or more of the ancestors were carriers of the gene mutation.</td>
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<tr>
<td>Genetic drift</td>
<td>is the random fluctuation in the frequency of a gene variant in a population, presumably owing to chance rather than natural selection.</td>
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<td>Identity-by-descent (IBD)</td>
<td>means that two or more people share identical genetic material from a common ancestor.</td>
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<td>Linkage disequilibrium (LD)</td>
<td>is the non-random association of alleles at different loci located on the same chromosome.</td>
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<tr>
<td>Runs of homozygosity (ROH’s)</td>
<td>are (long) regions of the genome where the copies inherited from both parents are identical.</td>
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Genetically isolated populations provide a unique opportunity for many different genetic investigations because of several benefits of these populations (Table 1):

- Identification of genes that underlie Mendelian disorders, especially autosomal recessive disorders;
- Identification of susceptibility genes predisposing to complex disorders for example asthma, psychiatric disorders, high blood pressure, and multiple sclerosis;
- Studying penetrance, phenotypic variation, and modifying factors underlying the clinical variability of Mendelian disorders;
- Implementation and evaluation of genetic carrier screening programs.

Genetically isolated populations are very suitable for identifying new genes because of the reduced genetic heterogeneity. Many studies in these populations have been focused on the identification of genes for Mendelian disorders. As the reduced genetic variation and high degree of inbreeding mainly causes an increased incidence of autosomal recessive disorders, it is not surprising that many of the identified founder mutations show an autosomal recessive mode of inheritance. However, also large pedigrees with (mainly late-onset) autosomal dominant disorders may be present in genetically isolated populations and proved to be useful for the identification of genetic causes these rare dominant disorders.¹⁷

**Table 1.** Advantages and limitations in using genetically isolated populations in genetic research (partly derived from Peltonen et al¹⁷ and Kristiansson et al¹⁸).

<table>
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<th>Advantages</th>
<th>Limitations</th>
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<td>Limited/ reduced genetic heterogeneity</td>
<td>Unable to generalize the results in other populations</td>
</tr>
<tr>
<td>Limited/ reduced non-genetic heterogeneity</td>
<td>Chance of stigmatization</td>
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<tr>
<td>Limited immigration and migration</td>
<td></td>
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<tr>
<td>Elevated levels of endogamy and/or consanguineous unions</td>
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<tr>
<td>Increased prevalence of genetic disorders</td>
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<tr>
<td>Reduced phenotypic heterogeneity</td>
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<td>Reduced chance of non-allelic heterogeneity</td>
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<tr>
<td>Wider intervals of LD</td>
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<td>Fewer regions of very low LD ('holes')</td>
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<tr>
<td>Good availability of genealogical records</td>
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<td>High participation rates in studies</td>
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LD = linkage disequilibrium
A variety of strategies can be used for gene mapping. Homozygosity mapping is an efficient strategy for identifying rare autosomal recessive disorders. This approach takes advantage of the high degree of inbreeding in these populations and the assumption that the disease locus would be inherited identical-by-descent in affected individuals. Not only the disease gene will be homozygous but also the polymorphic markers located nearby the disease locus will show homozygosity-by-descent. This makes it possible to map autosomal recessive disorder by searching for regions of homozygosity that are shared by different affected individuals, similar to the method used in consanguineous families. Homozygosity mapping can be followed by Sanger sequencing of candidate genes in the IBD-region or by whole exome sequencing (WES) or whole genome sequencing (WGS) with special attention to (candidate genes in) this homozygous region. With the increasing availability and the decreasing costs of WES and WGS it will become more common to sequence all patients’ exomes/genomes and find shared homozygous variants after filtering out non-significant variants.

Genetically isolated populations in the Netherlands
The general Dutch population is relatively outbred. However, more than ten genetically isolated populations exist. The main reasons for genetic isolation in these Dutch populations are (former) geographical isolation (islands) and religion. Several founder mutations have been identified in these genetically isolated populations, ranging from zero to more than 10 in each population. Many of these founder mutations show an autosomal recessive mode of inheritance, but also large pedigrees with autosomal dominant disorders are present in these populations, such as hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) (MIM 605714). In a young genetically isolated population in the South West of the Netherlands, compromising 20,000 inhabitants, extensive research has been conducted on unraveling genetic risk factors involved in complex disorders. This study is called the ERF (Erasmus Rucphen Family) cohort study and is part of the Genetic Research in Isolated Population (GRIP) program. The study mainly focuses on unraveling neuropsychiatric diseases, cardiovascular diseases, and metabolic diseases, such as depression, function and structure of the arterial wall, and type 2 diabetes. Another genetically isolated population in the Netherlands is the Ashkenazi Jewish (AJ) population. Approximately 37,000 to 53,000 Jews live in the Netherlands, 90-95% of whom are of AJ descent. This population is at increased risk of several severe autosomal recessive disorders, such as Tay-Sachs Disease, Familial dysautonomia, Canavan disease, and Bloom syndrome. The American College of Medical Genetics (ACMG),
recommends screening for eight disorders in this population.\textsuperscript{29} Between 1 in 4 and 1 in 5 Ashkenazi Jews are carriers of one or more of these disorders.\textsuperscript{29}

**The genetically isolated population under study**

The research in this thesis is mainly performed in one specific genetic isolated population in the North West of the Netherlands. Originally, this village was the location of a harbor of a nearby village. In 1357 the inhabitants of this nearby village dug a new exit to its sea harbor and dammed up the original harbor. Local fisherman and farmers settled at the old harbor and established a new settlement. The village was founded an estimated 22 generations ago by seven to 20 families. It is a typical Roman Catholic village, in contrast to its Protestant environment. Due to religious and social factors, it is still a close-knit village. The village has about 21,500 inhabitants, about 7100 persons of reproductive age (20-45 years), and a birth rate of about 250 births/year. Detailed genealogical records of this population are available.

In the past two decades, founder mutations for several disorders have been identified in this genetically isolated population. Many of the genetic causes of these disorders are originally identified in patients in the genetically isolated population first, and then in patients in the general population of the Netherlands and worldwide. Examples are retinitis pigmentosa type 12 (RP12) (MIM 268000),\textsuperscript{30} pseudoxanthoma elasticum (PXE) (MIM 264800),\textsuperscript{31} pontocerebellar hypoplasia (PCH2) (MIM 277470),\textsuperscript{32} osteogenesis imperfecta type IIB/III (OI) (MIM 610682),\textsuperscript{33} and primary ciliary dyskinesia (PCD) (MIM 615067).\textsuperscript{33}

**Preconception carrier testing and carrier screening**

Because of the high carrier frequencies of founder mutations in (some) genetically isolated populations, these populations may be very suitable for implementation and evaluation of genetic carrier screening programs.

Carrier testing and carrier screening aims to identify couples with a 1-in-4 (25%) risk of having offspring with an autosomal recessive disorder in order to enable autonomous reproductive decision making, which consequently might reduce perinatal morbidity and mortality. Carrier testing is targeted at close relatives and partners of previously identified patients and carriers, whereas carrier screening is targeted at individuals without an a priori increased risk for having a child with a certain disorder based on their partners' personal or family history.\textsuperscript{34}

Ideally, the carrier test is performed before conception (preconception) because this enables carrier couples to make the most informed reproductive options at least time
constraints. These reproductive options include refraining from having children, pre-implantation genetic diagnosis (PGD), prenatal diagnosis (chorionic villus sampling or amniocentesis), sperm/egg donation, adoption or accepting the genetic risk.

**Different strategies for carrier testing**

Carrier testing can be offered to different groups; couples with a positive family history of one or more genetic disorders (cascade genetic carrier testing), high risk populations based on ancestral background (ancestry-based carrier screening), and the general population irrespective of risk status (universal or pan-ethnic carrier screening). The standard practice in the Netherlands, as in many European countries, is cascade genetic carrier testing, in which carrier testing is targeted to close relatives and partners of previously identified patients and carriers. However, this way of carrier testing identifies only a minority of carrier couples since the majority of affected children are born in parents without a known positive family history. Beside this, many family members do not come forward for testing and many people of non-reproductive age have to be tested.

Targeted carrier screening is however offered in some well-defined ethnic groups. In these groups the prevalences of specific genetic disorders are relatively high. This facilitates ethnicity- (or ancestry-) based carrier screening for population-specific disorders, and is considered an effective way to inform and identify carrier couples for disorders that are generally (very) rare. An example is carrier screening for hemoglobinopathies in ethnic populations from Africa, the Mediterranean and South East Asia. Next to this screening offer, carrier screening programs have been introduced in some genetically isolated populations, because carrier frequencies of some disorders may also be very high in these groups and the mutations in a given gene are often limited to one or a few (founder) mutations. Examples of screening programs in genetically isolated populations are the programs offered to people of Eastern European Jewish (Ashkenazi) descent, in different isolated (mainly Arab and Druze) communities in Israel, and in the Saguenay-Lac-Saint-Jean region of Quebec, Canada.

Technological advances have enabled the development and offer of preconception expanded carrier screening (ECS) in which couples without an a priori increased risk of having a child with a genetic disorder can be screened for several (hundreds of) disorders simultaneously. This also enables the implementation of universal or pan-ethnic carrier screening. An increasing number of mainly commercial laboratories offer these expanded universal carrier screening (EUCS) panels.
Carrier testing in the genetically isolated population under study

In the genetically isolated population under study it was already observed decades ago that many children died at a young age. At that time it was not possible to offer carrier testing as the underlying genetic causes were not identified yet. Genetic counseling only consisted of crude risk estimations and giving advice about reducing risks for future pregnancies, for example by promoting exogamous marriages (Figure 1).
With new molecular techniques the genetic causes of several disorders in the population have been identified, making (preconception) counseling and carrier testing possible. In the genetically isolated population under study about 1 to 2 children were born annually with the genetic disorder pontocerebellar hypoplasia type 2 (PCH2) (MIM 277470). In 2007 the founder mutation for this disorder was identified (published in 2008). All parents of children with PCH2 who cooperated in the study were informed about the identification of the founder mutation and were offered genetic counseling.
counseling. Also the opportunity to perform carrier testing in family members was discussed. However, it was only after the birth of two children in one month in 2010 in the genetically isolated population that many family members of these two patients asked for genetic counseling and carrier testing. We started with cascade genetic testing in an outpatient clinic in the village. When drawing the pedigrees it was noted that three other severe autosomal recessive disorders were relatively frequent in this population; rhizomelic chondrodysplasia punctata type 1 (RCDP1) (OMIM 215100), osteogenesis imperfecta type IIb/III (OI) (MIM 610682),46 and fetal akinesia deformation sequence (FADS) (OMIM 208150).47 Children with these disorders suffer significant morbidity and have a severely reduced lifespan. In September 2012, we therefore started a preconception outpatient clinic offering carrier screening for four autosomal recessive disorders in this genetically isolated population under study. The start of the outpatient clinic was accompanied by attention of local and national media (See Appendices).

Participants’ experiences with carrier screening
When introducing carrier screening, it is important to investigate the (potential) beneficial and harmful implications of the screening. Many studies have been published about the uptake of testing, knowledge, psychological impact, health perception, stigmatization, discrimination, satisfaction, and reproductive decisions of screening, for example for cystic fibrosis (CF),48,49 haemoglobinopathies,49 and fragile-X syndrome.50 In general, these studies demonstrated that screening is well received by the participants without major adverse psychological effects, and showed that the participants intended to base their reproductive decisions on the test results.34 However, these studies mainly focused on screening for one disorder with a relative small proportion of detected carriers, and mostly no carrier couples. Few studies have been published about the evaluation of carrier screening for multiple disorders,42,51 including studies aimed at individuals of Ashkenazi Jewish descent.52,53
With the offer of ECS, it is very important to investigate its (potential) beneficial and harmful aspects, determinants involved in (successful) implementation, and the preferences regarding genetic counseling, both in genetically isolated populations as well as in the general population.
OBJECTIVES AND OUTLINE OF THE THESIS

The main objective of this research was to demonstrate the various opportunities for genetic research and clinical care in a young genetically isolated population in the Netherlands.

Part I of this thesis focuses on the discovery of two new autosomal recessive disorders and the phenotypic variation in another disorder in the genetically isolated population under study. The following research questions were addressed:
1. What is the genetic cause of fetal akinesia deformation sequence (FADS) in the genetically isolated population? (Chapter 2)
2. What is the genetic cause of XX-gonadal dysgenesis (XX-GD) in the genetically isolated population? (Chapter 3)
3. What is the age of onset, long-term clinical course, and clinical variability in patients with retinitis pigmentosa type 12 (RP12) in a large cohort of patients with the same homozygous mutation in the CRB1 gene in the genetically isolated population? (Chapter 4)

In Part II of this thesis the implementation and evaluation of carrier screening in (a) genetically isolated population(s) in the Netherlands is addressed and the following research questions were formulated:
4. What are the first year experiences with target carrier screening for four severe autosomal recessive disorders in the genetically isolated population? Is targeted genetic carrier screening an effective method to identify high-risk couples for having affected offspring in this high-risk population? (Chapter 5)
5. How do individuals from the genetically isolated population experience the carrier screening for four recessive disorders? What are the psychological consequences, how is the understanding and satisfaction, what are the preferences regarding genetic counseling, and what are the reproductive intentions and decisions? (Chapter 6)
6. What are critical factors involved in successful and responsible implementation of population-based expanded carrier screening? What can be learned from two already implemented initiatives (carrier screening in two high-risk communities; the genetically isolated population under study and the Ashkenazi Jewish population)? (Chapter 7)
7. Which Dutch genetically isolated populations and autosomal recessive founder mutations are known? Are these founder mutations covered in the (preconception) expanded carrier screening tests of carrier screening providers? Are these screening tests appropriate for use in founder populations? (Chapter 8)
REFERENCES


Introduction


