Genetically isolated populations

Implications for genetic care

Mathijssen, I.B.
Chapter 9

General discussion and future perspectives
DISCUSSION

Genetically isolated populations provide unique opportunities for many different genetic investigations. Examples are: identification of genes that underlie Mendelian (autosomal recessive) disorders; identification of susceptibility genes predisposing to complex disorders; studying penetrance, phenotypic variation, and modifying factors underlying the clinical variability of Mendelian disorders; and implementation and evaluation of genetic carrier screening programs. The main objective of this thesis is to demonstrate these various opportunities in a young genetically isolated population in the Netherlands.

In this chapter, the main findings of this thesis will be presented and discussed in the context of the scientific literature. The chapter concludes with future perspectives for research and clinical care in genetically isolated populations.

Identification of genes that underlie autosomal recessive disorders

Genetically isolated populations are very suitable for identifying new genes because of the reduced genetic heterogeneity. A variety of strategies can be used to identify these genes. We used an approach of homozygosity mapping\(^1\) followed by Sanger sequencing of candidate genes to identify new founder mutations of two different disorders in the genetically isolated population under study (chapter 2 and 3).

Identification of new founder mutation (FADS) and reproducibility

The first disorder is fetal akinesia deformation sequence (FADS) (MIM 208150), a lethal disorder characterized by progressively reduced fetal movements, joint contractures, and severe pulmonary hypoplasia (chapter 2). We identified a homozygous missense mutation in the \(MUSK\) gene as the cause of FADS in this genetically isolated population.\(^2\) The \(MUSK\) gene is essential for the development of the neuromuscular junction. Mutations in this gene had been described before in patients with congenital myasthenic syndrome (CMS), but not in lethal FADS. However it was already shown that mutations in other genes encoding neuromuscular synaptogenesis can cause phenotypes with variable severity, ranging from CMS to FADS. With the identification of \(MUSK\) as a novel cause of lethal FADS, this gene can be included in this row of genes. To confirm our finding, we tested a cohort of 26 unsolved cases with FADS who did not come from the genetically isolated population. However, no likely pathogenic mutations in \(MUSK\) were identified.

Findings in genetically isolated populations may not be reproducible in the general population. This may especially be a problem in association studies for complex trait
gene identification, but also for identification of genes that underlie Mendelian disorders. Even if found in the general population, these mutations may only explain a small subset of the genetic causes of the disorder. This is for example the case in primary ciliary dyskinesia (PCD) (MIM 615067) and osteogenesis imperfecta type IIB/III (OI) (MIM 610682) in the genetically isolated population under study. Shortly after the publication of our article, another article was published about a family with recurrent pregnancies with lethal FADS which was caused by a homozygous frameshift mutation in MUSK. This confirms that also outside the genetically isolated population under study mutations in MUSK are a (probably) rare cause of FADS.

Identification of new founder mutation (XX-GD) and genetic heterogeneity

The second disorder is XX female gonadal dysgenesis (XX-GD), a genetically heterogeneous disorder characterized by primary amenorrhea, lack of secondary sexual characteristics, streak gonads, hypoplastic uterus, and hypergonadotropic hypogonadism (chapter 3). More than 10 patients with XX-GD are known in the genetically isolated population. About half of these patients also suffer from sensorineural deafness. The combination of XX-GD and sensorineural deafness is known as Perrault syndrome (PS) (MIM 233400). Whole exome sequencing (WES) in three patients with symptoms of PS initially showed no variants present in all three patients (personal communication), however two patients were homozygous for the c.707A>T, p.(Asn236Ile) mutation in the ERAL1 gene. Also a third female PS-patient and a male patient with sensorineural deafness turned out to be homozygous for this missense mutation. Sanger sequencing of five patients with XX-GD without sensorineural deafness in this population did not reveal the founder mutation in ERAL1, suggesting genetic heterogeneity for XX-GD in this population. We decided to perform homozygosity mapping of four of the patients with XX-GD without deafness, followed by Sanger sequencing of the candidate gene PSMC3IP. All five cases with XX-GD without deafness were homozygous for the c.74G>C, p.(Arg25Pro) missense mutation in PSMC3IP. Interestingly, also the case with presumed PS (with XX-GD and sensorineural deafness) turned out to be homozygous for the PSMC3IP founder mutation, suggesting the hearing loss in this patient has a separate cause. This case shows that also in genetically isolated populations genetic heterogeneity exists.

Genetic heterogeneity was experienced before in this genetically isolated population. Several patients with autosomal recessive retinitis pigmentosa (RP) are known in this population, and initially it was assumed that it was caused by a single type of RP. However, clinical heterogeneity was observed, with the majority of the patients having RP with preserved para-arteriolar retinal pigment epithelium (PPRPE), while this
phenotype was not found in the remaining RP-patients.\textsuperscript{11,12} In the patients with PPRPE, a homozygous mutation in \textit{CRB1} was identified, causing RP12 (OMIM 600105).\textsuperscript{13} In the remaining patients three other causes of RP were identified (Table 1).\textsuperscript{14} Also in other genetically isolated populations both non-allelic\textsuperscript{15} as well as allelic heterogeneity\textsuperscript{16} have been demonstrated, which can complicate the genetic mapping of some disorders. However, despite this caution, the chance of experiencing allelic and non-allelic heterogeneity in genetically isolated populations is reduced and these populations are very useful for identifying new genes.

**Phenotypic variation**

In outbred populations it may be difficult to study phenotypic variation in patients with the same homozygous mutation because extended pedigrees and larger series of patients with the same mutation are rare and non-genetic variance (environmental and cultural factors) influencing the phenotype may be more evident. Genetically isolated populations, however, provide a unique opportunity for studying phenotypic variation, penetrance, and modifying factors underlying the clinical variability of autosomal recessive disorders. The majority of the patients are homozygous for the same founder mutation, tend to show reduced non-genetic variance, and frequently it is easy to re-contact patients for follow-up studies as many of the inhabitants stay within the community.

In the genetically isolated population under study we studied the long-term clinical course and variability of 30 patients with retinitis pigmentosa type 12 (RP12) due to a homozygous founder mutation in the \textit{CRB1} gene (chapter 4). The age of diagnosis and the clinical course (in terms of visual acuity, visual fields, and ophthalmic findings) showed marked interindividual variability. This supports the hypothesis that the phenotype of patients with \textit{CRB1} mutations is modulated by other factors.\textsuperscript{14}

In this genetically isolated population, phenotypic variation was also studied in several patients with pseudoxanthoma elasticum (PXE) caused by a homozygous founder mutation in \textit{ABCC6}.\textsuperscript{17} Also, considerable interindividual phenotypic variability was demonstrated for this disorder.

As in both genetic disorders, RP12 and PXE, the interindividual difference between the individuals with the same homozygous mutation is very high, studying genotype-phenotype correlations for these disorders are expected to be fruitless. Both genetic disorders can be used for unraveling genetic and environmental factors modifying the phenotypes.
Knowledge of founder mutation enhances better care

Each genetically isolated population shows a unique profile of rare disease alleles, with some genetic disorders (much) more prevalent compared to the general population, while others are less prevalent.

In the genetically isolated population under study, founder mutations for 17 Mendelian disorders are currently known (Table 1). The carrier frequencies of the autosomal recessive disorders are generally (very) high, ranging from about 2 to 14%.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM</th>
<th>Genetics</th>
<th>Gene</th>
<th>Mutation</th>
<th>Carrier frequency genetic isolate</th>
<th>References concerning genetic isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)</td>
<td>125310</td>
<td>AD</td>
<td>NOTCH3</td>
<td>c.1732C&gt;T; p.(Arg578Cys)</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Chondrodysplasia punctata rhizomelic type 1 (RCDP1)</td>
<td>215100</td>
<td>AR</td>
<td>PEX7</td>
<td>c.875T&gt;A; p.(Leu292X)</td>
<td>6.1%</td>
<td>18</td>
</tr>
<tr>
<td>Fetal akinesia deformation sequence (FADS)</td>
<td>208150</td>
<td>AR</td>
<td>MUSK</td>
<td>c.1724T&gt;C; p.(Ile575Thr)</td>
<td>8.1-11.2%</td>
<td>2,18</td>
</tr>
<tr>
<td>MUTYH-associated polyposis coli (MAP)</td>
<td>608456</td>
<td>AR</td>
<td>MUTYH</td>
<td>c.527A&gt;G; p.(Tyr176Cys)</td>
<td>9.8%</td>
<td></td>
</tr>
<tr>
<td>Osteogenesis imperfecta type IIb/III (OI)</td>
<td>610682</td>
<td>AR</td>
<td>CRTAP</td>
<td>c.21_22dupGG; p.( Ala8fs)</td>
<td>4.1%</td>
<td>6,18</td>
</tr>
<tr>
<td>Perrault syndrome (PS)</td>
<td>233400</td>
<td>AR</td>
<td>ERAL1</td>
<td>c.707A&gt;T; p.(Asn236Ile)</td>
<td>9.2%</td>
<td>8</td>
</tr>
<tr>
<td>Phenylketonuria (PKU)</td>
<td>261600</td>
<td>AR</td>
<td>PAH</td>
<td>c.1315+1G&gt;A</td>
<td>9.2%</td>
<td>19</td>
</tr>
<tr>
<td>Polymerase gamma (POLG)-related disorders</td>
<td>203700</td>
<td>AR</td>
<td>POLG</td>
<td>c.2243G&gt;A; p.(Trp748Ser)</td>
<td>7.9%</td>
<td></td>
</tr>
<tr>
<td>Pontocerebellar hypoplasia type 2 (PCH2)</td>
<td>277470</td>
<td>AR</td>
<td>TSEN54</td>
<td>c.919G&gt;T; p.(Ala307Ser)</td>
<td>1.5-14.3%</td>
<td>18,20-23</td>
</tr>
<tr>
<td>Primary ciliary dyskinesia (PCD)</td>
<td>615067</td>
<td>AR</td>
<td>CCDC114</td>
<td>c.742G&gt;A; p.(Ala248Thrfs)</td>
<td>10%</td>
<td>5</td>
</tr>
<tr>
<td>Pseudoxanthoma elasticum (PXE)</td>
<td>264800</td>
<td>AR</td>
<td>ABCC6</td>
<td>c.3775delT; p.(Trp1259Glyfs)</td>
<td>9.3%</td>
<td>17,24,25</td>
</tr>
<tr>
<td>Retinitis Punctata Albescens (RPA)/Juvenile Retinitis Pigmentosa (JRP)</td>
<td>604537</td>
<td>AR</td>
<td>LRAT</td>
<td>c.12delC; p.(Met5CysfsX53)</td>
<td>6.0%</td>
<td>12,14</td>
</tr>
<tr>
<td>Retinitis pigmentosa type 12 (RP12)</td>
<td>600105</td>
<td>AR</td>
<td>CRB1</td>
<td>c.3122T&gt;C; p.(Met1041Thr)</td>
<td>4.1%</td>
<td>9-14</td>
</tr>
<tr>
<td>Usher syndrome type 2A (USH2A)</td>
<td>276901</td>
<td>AR</td>
<td>USH2A</td>
<td>c.13274C&gt;T; p.(Thr4425Met)</td>
<td>2.7%</td>
<td></td>
</tr>
<tr>
<td>Usher syndrome type 2C (USH2C)</td>
<td>605472</td>
<td>AR</td>
<td>ADGRV1</td>
<td>c.4108T&gt;G; p.(Phe1370Val)</td>
<td>7.0%</td>
<td></td>
</tr>
<tr>
<td>(Warburg) micro syndrome (WARBM)</td>
<td>614225</td>
<td>AR</td>
<td>RAB3GAP2</td>
<td>c.3085G&gt;T; p.(Glu1029*)</td>
<td>2.2%</td>
<td>26</td>
</tr>
<tr>
<td>XX female gonadal dysgenesis (XX-GD)</td>
<td>233300</td>
<td>AR</td>
<td>PSMC3IP</td>
<td>c.74G&gt;C; p.(Arg25Pro)</td>
<td>4.4%</td>
<td>Submitted</td>
</tr>
</tbody>
</table>

Table 1. Founder mutations identified in the genetically isolated population under study.
In most of the genetically isolated populations in the Netherlands just zero to a handful of Mendelian disorders have been identified,\textsuperscript{27,28} being much less than in the genetically isolated population under study. There are several possible reasons for this difference; the founder individuals in the other populations were carrier of none or only a few genetic disorders, the founder population is less isolated and/or younger and/or larger, insufficient knowledge about the disorders is present in the population, the founder population is assumedly not as cooperative in genetic research (e.g. because of religious barriers), and insufficient recognition of patterns of disorders in the population. Recognition of these (patterns of) disorders is greatly enhanced by drawing pedigrees during genetic counseling in members of the genetically isolated population. The genetically isolated population under study is very cooperative in genetic research.

Knowing which disorders are more prevalent in a genetically isolated population, including their specific genes and mutations, is important for making a rapid and correct (differential) diagnosis by clinicians, for genetic counseling to help individuals, couples and families in the population to understand and adapt to the medical, psychological, familial and reproductive implications of the genetic disorder(s),\textsuperscript{29} and for research in these populations. For some genetically isolated populations (online) databases with an overview of genetic disorders (much) more prevalent in these populations are available. Examples are the Amish, Mennonite, and Hutterite Genetic Disorder Database (www.biochemgenetics.ca/plainpeople),\textsuperscript{30} the Finnish Disease Database (www.findis.org),\textsuperscript{31} and the Israeli National Genetic Database (www.goldenhelix.org/israeli).\textsuperscript{32} Currently, such a database is not available in the Netherlands and it is debated whether we should develop it. Besides the above mentioned advantages, there may be disadvantages, such as stigmatization. This may be a reason not to develop an open access database but instead a database only accessible to health care providers.

**Carrier screening in genetically isolated populations**

In the genetically isolated population under study four severe recessive genetic disorders occur at relatively high frequency; pontocerebellar hypoplasia type 2 (PCH2) (MIM 277470), fetal akinesia deformation sequence (FADS), rhizomelic chondrodysplasia punctata type 1 (RCDP1) (MIM 215100), and osteogenesis imperfecta (OI) type IIB/III. Children with these disorders suffer significant morbidity and have a severely reduced lifespan. An estimated 2 to 4 of the 250 children born annually are affected with one of these disorders. In September 2012 we started an outpatient clinic in the genetically isolated population offering carrier screening for these four disorders, aimed
at couples planning a pregnancy (chapter 5).

Genetic carrier screening showed a very high carrier frequency of 33% of the four tested disorders. Not only in individuals with a positive family history for one of the disorders, but also in individuals with a negative family history, the carrier frequency was high (57.8% versus 25.8%). In the first year, four PCH2 carrier couples were identified. Using cascade genetic carrier testing, in which carrier testing is targeted to close relatives and partners of previously identified patients and carriers, the carriers and carrier couples without a positive family history would not have been identified.

Targeted carrier screening programs for multiple disorders have been introduced in a few other genetically isolated populations worldwide, for example in people of Eastern European Jewish (Ashkenazi) (AJ) descent, in different isolated (mainly Arab and Druze) communities in Israel and in the Saguenay-Lac-Saint-Jean region of Quebec, Canada. Also in these populations, carrier frequencies are high and these programs are quite successful in terms of providing couples with meaningful options for autonomous reproductive choice (the primary aim of carrier screening). Targeted carrier screening in the genetically isolated population under study, but also in other genetically isolated populations with a high prevalence of specific disorders, is thus an effective method to identify carriers and is likely to be more effective compared to cascade genetic testing.

In the genetically isolated population under study, also other (non-lethal) disorders are highly frequent, for example phenylketonuria (PKU) (MIM 261600), primary ciliary dyskinesia (PCD) (MIM 615067), and retinitis pigmentosa (RP) (see Table 1). If there is a positive family history for (one of) these other disorders, we offer further genetic counseling for the specific disorder(s). Because of the less severe nature of these disorders, therapeutic options in some of them, and the (presumably) different reproductive consequences, it is preferred not to include these less severe disorders in the test panel with the severe disorders, but instead to offer one or more separate test panels for which the couples can give separate informed consent.

It is important to carefully consider if and how to offer carrier screening for milder disorders in genetically isolated populations and in the general population. In the AJ population carrier screening for Gaucher disease (GD) was included in the test panel because it is one of the most prevalent recessive disorders in this population, for which testing is simple and the test sensitivity is high. However, the disorder is frequently mild or asymptomatic and therapeutic options (enzyme replacement therapy) exists. It is therefore ethically questionable to systematically carry out carrier screening for this disorder.
We recently identified a relative frequent founder mutation for \textit{MUTYH}-associated polyposis coli (MAP) (OMIM 608456) in the genetically isolated population under study. Individuals who inherit \textit{MUTYH} mutations from both parents (biallelic) have a colorectal cancer (CRC) risk of about 43\% at age 60 and the life-time risk is assumed to be close to 100\% in the absence of timely surveillance.\textsuperscript{43} The lifetime risk for duodenal cancer is about 4\%.\textsuperscript{43} Surveillance by colonoscopy and gastro-duodenoscopy is advised in MAP patients. In the genetically isolated population under study, the carrier frequency of MAP is 9.8\% (compared to 1-2\% in the general population\textsuperscript{44,45}). The chance of being homozygous for this mutation in this population is about 1 in 400, being much more frequent than in the general population. The first steps have been taken in informing at-risk families and offering genetic testing for MAP in the genetically isolated population under study (see Future perspectives).

In the AJ population there is already experience with carrier screening for late-onset oncogenetic disorders. In this population, three breast-cancer founder mutations (two \textit{BRCA1}-mutations and one \textit{BRCA2}-mutation) are more prevalent than in the general population. It is estimated that over two percent of the AJ population is a carrier of one of these mutations.\textsuperscript{46} Genetic testing of these three founder mutations is offered on a research basis, in for example Israel, the UK, and Canada, to individuals of AJ descent, regardless of personal or family history of cancer.\textsuperscript{47-49} Most women with mutations would not have been qualified for genetic testing based on personal and/or family history of cancer.\textsuperscript{50} It has been argued that genetic testing of the general population of Jewish women is justified.\textsuperscript{50} In these studies, carrier screening for founder mutations in \textit{BRCA1} and \textit{BRCA2} is offered separately from the carrier screening for the recessive founder mutations.

\textbf{Expanded carrier screening and genetically isolated populations}

Meanwhile, 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.\textsuperscript{51,52} An increasing number of mainly commercial laboratories offer these screening panels.\textsuperscript{39,53} The existing (commercial) ECS panels may be less suitable for inhabitants of genetically isolated populations, as the carrier frequencies of several autosomal recessive disorders in these populations can be very skewed from nationwide or worldwide carrier frequencies.

We made an inventory of six Dutch genetically isolated populations and their autosomal recessive founder mutations, and investigated whether the founder mutations
were covered in the (preconception) expanded carrier screening tests of carrier screening providers (chapter 8). The great majority of these founder mutations are not covered in the ECS panels of the five selected providers. This also applies to most of the founder mutations present in genetically isolated populations in other countries. Offering a (commercial) routine ECS panel to inhabitants (of the majority) of the genetically isolated populations is not appropriate because it may give false reassurance to couples with an increased risk of having a child with a founder mutation related disorder they are not being tested for. It is important that descendants from genetically isolated populations are aware that a (commercial) routine ECS panel may not be appropriate and may give false reassurance. Currently, this information is not mentioned by expanded carrier test providers.

Ideally, a customized ECS test will be developed for each country/region in which both country/region-specific mutations as well as genetic isolate-specific founder mutations are present. This approach may also reduce potential stigmatization of genetically isolated populations, while specific mutations still are included. However, preventing high healthcare costs may be an important reason against a country/region wide screening test instead of a genetic-isolate wide screening test. Also, the preferences of the target population are important in this context.

In May 2016 the Academic Medical Center (AMC) Amsterdam started a non-profit offer of expanded carrier screening for 50 severe recessive disorders (www.dragerschaps-test.nl). Most of the severe disorders currently known in Dutch genetically isolated populations are included.

It is expected that in the near future WES or even whole genome sequencing (WGS) will be used for carrier screening. The first steps have already been taken. A great advantage is that all known disease genes can be screened, including very rare disease genes prevalent in genetically isolated populations and in consanguineous couples. However, correct interpretation of test-results when using WES or WGS is complex and it is very important that only variants known to affect function (and not variants of unknown clinical significance) are reported. Also, the identification of carrier couples for mild disorders which are unlikely to alter reproductive plans is an important point to take into consideration.

Recent developments and results of carrier screening in the genetically isolated population under study:

- In June 2014 the carrier testing panel offered in the outpatient clinic was expanded from four to five recessive disorders by adding a founder mutation in the POLG
gene to the panel after the discovery of a couple of patients with this disorder in the population. Mutations in the \textit{POLG} gene cause a continuum of phenotypes with variable severity and age of onset. We expect that homozygosity for the founder mutation c.2243G>A, p.(Trp748Ser) is lethal in the early embryonic period, based on the expected deleterious effect of the mutation and the absence of individuals with homozygosity for this mutation. However, compound homozygosity of the above mentioned founder mutation combined with another mutation may cause a phenotype with intellectual disability, seizures, ataxia, liver dysfunction, neuropathy and/or progressive external ophthalmoplegia.

- After five years of carrier screening, in total 12 carrier couples for one of the four diseases have been identified; five for PCH2, three for FADS, two for OI, and two for RCDP1. The chance of being a carrier couple for one of the four disorders is about 4%. Beside this, eight carrier couples for \textit{POLG} have been identified (in five of them both partners are carrier of the above mentioned \textit{POLG} founder mutation, in three of them one of the partners is carrier of the founder mutation and the other of another mutation).

- In the first year of the outpatient clinic the majority (89%) of the couples decided to test simultaneously. However, five years later the opposite is the case as most couples prefer sequential testing, in which one of the partners is tested first and DNA testing is only performed on the other partner if the first one turns out to be a carrier. The main reason for this is not to pay the deductible excess (obligatory deductible excess was 385 euros in 2017) of both partners.

- Since May 2016, some couples (n=6 until November 2016) from the genetically isolated population under study asked for the AMC ECS test instead of the targeted screening test. Only carriers for one of the five disorders also present in the targeted screening test were identified. This is not surprising, as in the genetically isolated population no patients with the other disorders included in the screening panel are known.

- As mentioned above, the first steps have been taken in implementing genetic testing for MAP in the genetically isolated population under study.

\textbf{Evaluation of the preconception carrier screening offer}

When introducing carrier screening, it is important to investigate the (potential) beneficial and harmful implications of the screening as well as preferences regarding genetic counseling. Many studies have been published about the implementation of carrier screening for a single disorder. 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. However, studies about carrier screening for multiple disorders simultaneously are scarce. Evaluating the screening offer in the genetically isolated population under study provides lessons for optimizing the screening in this population as well as for ECS in the general population, although not all results can be generalized because of social and cultural differences between the genetically isolated population and the general population.

The carrier screening for four disorders offered in the outpatient clinic was evaluated by administering questionnaires (both attendees and non-attendees) and conducting semi-structured interviews with carrier couples (chapter 6). The attendees were highly satisfied and would recommend screening to others. All identified carrier couples made reproductive decisions based on their results, such as refraining from having children, prenatal diagnosis (PND), and preimplantation genetic diagnosis (PGD). No major adverse psychological effects and no feelings of stigmatization were reported (Mathijssen et al, in press).

We have also identified challenges when offering carrier screening. Although the majority of the people were highly familiar with the genetic disorders and carrier testing and knowledge was high, some people wrongly mentioned an increased risk of having an affected child if both partners are carriers of different recessive disorders. It is important to emphasize this topic in the counseling of carrier screening for multiple disorders. Most participants recalled their test results correctly, but two couples reported being carrier of another disorder than reported to them. It can be expected that, when expanding the number of disorders screened for and more carriers being identified, recall of test results will become more difficult, and thus requires explicit attention in information provision and counseling.

The majority of the participants stated that couples should always have pre-test consultation, and did not prefer a GP over a genetic counselor. In a focus group study with forty US genetics professionals, there was consensus that ECS should be accompanied by pre- and post-test genetic counseling, preferably by a clinician with expertise in communicating genetic information. In a Dutch online survey, most potential users of ECS also preferred face-to-face consultation but the majority preferred the test to be offered via their GP, probably due to the strong primary care structure in the Netherlands. In the genetically isolated population under study, counseling of about 200 individuals a year is achievable, but when considering a nation-wide ECS, face-to-face counseling with a genetic counselor is impossible because there are not enough genetic
professionals. Counseling by non-genetic professionals (e.g. GPs), more personalized information, using for instance interactive computerized information/apps, and easily accessible telephone contact with a genetic professional, could be a solution. Most individuals in the genetically isolated population were informed about the existence of the carrier screening and the outpatient clinic by close influencers (family/friends). When introducing ECS in other populations, it might be worthwhile to use these influencers to raise awareness about the screening-test offer. In the general population, however, community support is less evident as there is no specific community with which people can identify themselves.

Although not all results of the evaluation of carrier screening in this genetically isolated population may be generalizable to the ECS in the general population, some of the recommendations may be useful. Important in this context is that we have found no evidence that screening for multiple disorders will cause major adverse psychological effects. Moreover, the recall and interpretation of test results may become more challenging for participants in multi-disorder carrier screening, which requires explicit attention in information provision and counseling.

**Factors contributing to successful implementation of carrier screening**

The implementation of the carrier screening in the genetically isolated population under study was proved to be very successful. We can learn from this and other implemented initiatives by identifying critical factors involved in this successes (chapter 7).

Our study identified several critical factors, from a user perspective, in the genetically isolated population under study that contribute to successful implementation:

- **Familiarity.** The familiarity with the genetic disorders and carrier screening was high compared to the general population. Of the respondents, 62% knew someone with a severe genetic disorder and 82% had heard about carrier screening.

- **Perceived benefits & perceived social barriers.** The respondents were highly positive about carrier screening in their community and perceive high personal benefits and low social barriers (e.g. stigmatization) to carrier screening.

- **Acceptance of reproductive options.** The respondents showed a relative high acceptance of reproductive options, such as PND and PGD, and also termination of pregnancy in case of an affected fetus was considered acceptable.

- **Community support.** Although the initial demand for carrier screening did not entirely come from the community itself, the high social adhesion of the community facilitated the implementation process after its introduction by health care professionals.
These above mentioned important factors were also present in the AJ population being another high-risk population.\textsuperscript{38}

For population-based ECS, the above mentioned factors for successful implementation may be less evident as the general population is expected to be less familiar with genetic disorders and carrier screening, the perceived risk of being a carrier is low, and people are expected to be unaware of the possible benefits of the carrier screening. To ensure successful implementation of population-based ECS, efforts should be made to increase knowledge about genetic disorders, create awareness, and address personal benefits of carrier screening in a non-directive way.

\textbf{(Ethical) challenges}

Although genetically isolated populations provide unique opportunities for many different genetic investigations, several potential (ethical) challenges exist. Some of these issues may especially be important in genetically isolated populations compared to the general population.

\begin{itemize}
\item \textbf{Unequal access to genetic care}
The unique opportunities for many different genetic investigations in genetically isolated populations may increase genetic research and the development of carrier screening programs in these populations. However, this may limit attention to other populations or the general population. This is for example the case in Tay-Sachs disease, in which research is almost exclusively focused on the AJ population, leaving other groups including French Canadians who have a high prevalence of the disease, less well served.\textsuperscript{64} Research attention to BRCA1/2 in the AJ population may well generate similar disparities.\textsuperscript{64}

Furthermore, in many countries, including the Netherlands, carrier screening is only offered to subpopulations with a known increased risk of being a carrier of a selected group of disorders, causing unequal access to these services. Offering ECS to all individuals may result in equitable application of genomic technology.\textsuperscript{65} However, several interviewed stakeholders in a Dutch study by Van der Hout et al.\textsuperscript{66} mention that ECS might hinder rather than promote equity of access to carrier screening as the specific needs of people belonging to a specific risk group might be lost from sight when offering ECS.

\item \textbf{Discrimination and stigmatization}
Most studies have revealed no predominant feelings of stigmatization among carriers,\textsuperscript{52} although in some studies concerns exist about social stigmatization of
carriers (e.g. in the AJ population) and self-stigmatization of carriers expressed by poorer health perception (e.g. in CF carrier screening). The genetic cause of PCH2 has been discovered in the genetically isolated population under study. Several individuals, including inhabitants of the genetically isolated population and health care providers, call the disorder after the name of the village. This should be avoided as this suggests that the disorder is exclusive to the genetically isolated population under study, which may increase stigmatization. The choice of words laboratories and clinicians use to describe carrier screening test panels in de AJ population (such as “Ashkenazi Jewish Genetic Disease Panel”) raises similar concerns. However, it is difficult to think of another name that covers the content of the test.

Beside this, media attention about the carrier screening offer(s) in genetically isolated population(s) may trigger stigmatization. The reasons for health care providers to participate in media interviews may be to inform the genetically isolated population as well as the general population about the chance of having a child with an autosomal recessive disorder including the opportunity of carrier screening. However, journalists often filter out the comments about the general population and focus on the more intriguing genetically isolated population itself (see Appendix).

Due to carrier screening, the number of children born with the screened autosomal recessive disorders may reduce and these disorders may be increasingly seen as preventable disorders. This may cause disability-based stigmatization by making the society less tolerant of affected individuals and their parents. A test targeted to a particular subpopulation may cause population/ethnicity-based stigmatization. Offering population-based ECS might decrease this possible stigmatization by offering a ‘universal’ test rather than targeted to a particular subpopulation.

- Undue pressure on individual choice
  Individuals may feel an obligation to participate in genetic research and/or may feel a pressure to participate in carrier screening. This may especially be a concern in socially tight communities.

**FUTURE PERSPECTIVES**

**Research perspectives**

Research in the genetically isolated population under study has proved to be successful and this population offers several opportunities for further research. For each research
opportunity it is however very important to carefully consider the potential beneficial and harmful aspects for the population. Some of these potential harmful aspects are discussed in the Ethical considerations-section. Other aspects are incidental findings and concerns in the participants about sharing genetic research data.

Suggestions for further research are:

- Although founder mutations for 17 Mendelian disorders are currently known in the genetically isolated population under study, founder mutations for several other disorders still have to be identified. We are currently searching for Mendelian causes of intellectual disability and hereditary cardiac disorders, among others.
- For several disorders in the genetically isolated population, considerable phenotypic variation in individuals with the same founder mutation was shown. This population offers a unique opportunity for unraveling genetic and environmental factors modifying the phenotypes.
- As genetically isolated populations are also very valuable for identification of susceptibility genes predisposing to complex disorders (see introduction of this thesis), also the genetically isolated population under study gives an opportunity for complex trait gene identification.
- Genetically isolated populations may be more suitable for designing induced pluripotent stem cell (iPS) models compared to the general population because of the higher chance of finding multiple individuals with (the same) long ROHs that include disease risk loci. After reprogramming adult cells into pluripotent stem cells, these cells can be converted to differentiated cells (such as neurons), giving the opportunity to study the underlying functional mechanisms contributing to disease risk and for drug development. The first steps for designing iPS cell models for brain related-disorders (e.g. autism) in the genetically isolated population under study have already been taken.
- With the identification of a relative frequent founder mutation for MUTYH-associated polyposis coli (MAP) in the genetically isolated population under study, questions arise about carrier testing for this disorder in the population. (How) should we implement carrier screening for this late-onset oncogenic disorder? What are the beneficial and harmful aspects of carrier testing for late-onset disorders from a user perspective? Should we combine this testing with the offer of preconception carrier screening for severe recessive disorders? These questions are not only important for the genetically isolated population itself but will also provide lessons for the implementation of carrier screening for late-onset and (possibly) treatable disorders in the general population.
With the increased mobility of humans, genetically isolated populations become less isolated and eventually will disappear. Performing genetic research in these populations should therefore not be postponed.

- New genetic testing technologies and decreasing costs will inevitably cause expansion of the use of ECS from selected high-risk populations to several other patient groups or even the general population. The question is whether we should offer ECS in high-value pregnancies, for example in couples undergoing IVF/ICSI/PGD, and to gamete donors or to the general population. Also, the question is which disorders are appropriate to be included in the screening panel based on clear and transparent criteria (e.g. severity of the disorder, age of onset, availability of treatment, carrier frequency). Further studies are needed to investigate the preferences of the couples/donors/recipient and the implications for the health care system, including the availability of trained (genetic) counselors, and (down-stream) costs.

**Future clinical perspectives**

It is important to continue to provide good genetic care for the inhabitants of the genetically isolated population under study and to continue to adjust the care to the demands of the population.

Currently, several aspects and developments are important to take into consideration.

- Until recently, the majority of inhabitants of the genetically isolated population under study knew someone with one of the four (five) severe recessive disorders. However, after the setup of the outpatient clinic, presumably the number of children born with one of these disorders has been reduced drastically (personal communication). Long term follow up is needed to assess the actual impact of screening. Parallel to this reduction, the awareness of the severity and impact of these disorders will presumably diminish. It will be a challenge to keep the inhabitants fully informed of the disorders.

- The question is whether ancestry-based carrier screening is the preferred way of carrier screening in this and other genetically isolated populations, or whether we should offer population-based ECS, in which a wide array of diseases is screened not aimed solely at specific high-risk groups. The five severe recessive disorders frequently found in the genetically isolated population are included in the population-based ECS panel in the Netherlands and this panel is thus suitable for use in this population. We suspect that the mutations in other genes included in this ECS panel are not present in the isolated population because no patients with these disorders are known in the
population. However, as population-based ECS might decrease possible stigmatization by offering a ‘universal’ test rather than targeted to a particular subpopulation, this way of carrier screening should be considered, taking into account the costs of the test(s) and the demands of the population.

- We experience that genetic counseling in the genetically isolated population is becoming increasingly complex, as illustrated by the pedigree in Figure 1. Besides the four (five) severe recessive disorders included in the carrier screening panel, other (frequently) less-severe recessive disorders are also highly frequent. We offer additional genetic counseling for these disorders if (one of these) disorders are present in the family or if the counselees ask for further information about these disorders. The question is whether carrier screening for these disorders has to be introduced in a more systematic way by offering one or more separate test panels (see under Carrier screening in genetically isolated populations).

- During pregnancy, cell-free fetal (placental) DNA (cfDNA) is circulating freely in maternal blood. This cfDNA can be used for non-invasive prenatal testing (NIPT) to screening for fetal aneuploidy. In the Netherlands, NIPT was first offered only to women at increased risk of fetal aneuploidy, but is since April 2017 available to all pregnant women within the TRIDENT-2 study. The discovery of cfDNA also enables non-invasive prenatal diagnosis for monogenic disorders (MG-NIPD).

---

**Figure 1.** Example of a pedigree of a carrier couple for OI and PCD identified by carrier screening in the genetically isolated population under study. The woman is affected with PKU.
Squares indicate males; circles, females; diamonds, gender unspecified; arrows, probands; shaded symbols, affected; spot, carrier; PKU, phenylketonuria; OI, osteogenesis imperfecta type IIb/III; FADS, fetal akinesia deformation sequence; PCD, primary ciliary dyskinesia; POLG, Polymerase gamma (POLG)-related disorders; IUFD, intrauterine fetal death.
NIPD for de novo\textsuperscript{80} and paternally inherited autosomal dominant conditions\textsuperscript{81} is relatively straightforward and is already offered for some conditions. However, NIPD for autosomal recessive and de novo maternal autosomal dominant conditions remains challenging because of the predominance of the maternal mutant allele in the cfDNA sample.\textsuperscript{82} The first proof-of-concept studies have been performed.\textsuperscript{83} NIPD for the five severe autosomal recessive disorders in the genetically isolated population under study may even be more complicated because the quantity of informative SNPs may be reduced as a consequence of the common ancestry of the couples. The demands of the carrier couples for developing a NIPD test for these disorders are high and we are currently trying to set up NIPD for these disorders.

- Knowing which disorders are more prevalent in genetically isolated populations, including their specific genes and mutations, is important for making a rapid (differential) diagnosis by clinicians, genetic counseling, and research in these populations. Currently, such a database is not available in the Netherlands but we are considering setting up a database. It is still debated whether this database should be an open access database or, to prevent stigmatization, a database only accessible for health care providers.

**CONCLUSION**

Genetically isolated populations provide unique opportunities for many different genetic investigations. This is illustrated in this thesis by different investigations in a young genetically isolated population in the Netherlands. In this population we identified two new founder mutations, we studied genetic heterogeneity, phenotypic variation, and we implemented and evaluated carrier screening for multiple recessive disorders to enable reproductive choices. Although genetically isolated populations may differ in various aspects from other populations and not all research findings may be representative for the general population, investigations in these populations may be helpful in understanding causes of genetic disorders and may provide lessons for further successful and responsible implementation of expanded carrier screening.
REFERENCES


