Genetically Isolated populations

Implications for genetic care

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Chapter 5

Targeted carrier screening for four recessive disorders: High detection rate within a founder population

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ABSTRACT

In a genetically isolated community in the Netherlands four severe recessive genetic disorders occur at relatively high frequency (pontocerebellar hypoplasia type 2 (PCH2), fetal akinesia deformation sequence (FADS), rhizomelic chondrodysplasia punctata type 1 (RCDP1), and osteogenesis imperfecta (OI) type IIB/III. Over the past decades multiple patients with these disorders have been identified. This warranted the start of a preconception outpatient clinic, in 2012, aimed at couples planning a pregnancy.

The aim of our study was to evaluate the offer of targeted genetic carrier screening as a method to identify high-risk couples for having affected offspring in this high-risk subpopulation.

In one year, 203 individuals (92 couples and 19 individuals) were counseled. In total, 65 of 196 (33.2%) tested individuals were carriers of at least one disease, five (7.7%) of them being carriers of two diseases. Carrier frequencies of PCH2, FADS, RCDP1, and OI were 14.3%, 11.2%, 6.1%, and 4.1% respectively. In individuals with a positive family history for one of the diseases, the carrier frequency was 57.8%; for those with a negative family history this was 25.8%. Four PCH2 carrier-couples were identified.

Thus, targeted (preconception) carrier screening in this genetically isolated population in which a high prevalence of specific disorders occurs detects a high number of carriers, and is likely to be more effective compared to cascade genetic testing. Our findings and set-up can be seen as a model for carrier screening in other high-risk subpopulations and contributes to the discussion about the way carrier screening can be offered and organized in the general population.
INTRODUCTION

Autosomal recessive childhood diseases cause serious morbidity and/or mortality in at least 25 of 10,000 children.\(^1,2\) Genetic counseling and carrier testing aims to identify carrier couples with a 1-in-4 (25%) risk of affected offspring, enabling autonomous reproductive decisions, which consequently might reduce perinatal morbidity and mortality. If carrier testing is performed before pregnancy (preconception), couples are able to make the most informed reproductive choices at least time constraints, and this is therefore generally considered as the ideal timing. 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. When carrier couples are identified during pregnancy, accepting the risk of having an affected child or prenatal diagnosis are the only options. In the Netherlands, termination of pregnancy is allowed up to 24 weeks. Termination for severe fetal disorders after 24 weeks of gestation may be excepted from legal prosecution provided adherence to stringent criteria and assessment of the case by an expert committee appointed by the ministries of Health and Justice.\(^3\)

Different strategies can be used for genetic carrier testing for autosomal recessive disorders. At this moment, standard clinical care in the Netherlands, as in many European countries, is cascade genetic carrier testing. This means that testing is targeted at close relatives and partners of previously identified patients and carriers. In contrast, population carrier screening is defined as the detection of carrier status in persons who do not have an a-priori increased risk for having a child with a certain disease based on their family history.\(^4\) For example in the US, preconception and prenatal population-based carrier screening for cystic fibrosis (CF) is recommended for couples with no family history of CF since 2001.\(^5\) In some well-defined ethnic groups, the prevalence of specific genetic disorders is relatively high and the mutations in a given gene are often limited to one or a few specific, so-called founder mutations. In these subpopulations, ethnicity-(or ancestry-) based targeted carrier screening for population-specific mutations is considered an effective way to inform and identify carrier couples for disorders that are generally (very) rare. Examples are carrier screening in individuals of Eastern European Jewish (Ashkenazi) descent\(^6,7\) and carrier screening for hemoglobinopathies in ethnic populations from Africa, the Mediterranean and Southeast Asia.\(^8,9\)

Also in genetically isolated populations, rare genetic disorders can be relatively prevalent. In these genetic isolates, the vast majority of the couples are both members of the same population. As a result of the common ancestral origin and genetic bottleneck effect, many disease-causing founder mutations can occur at relatively high frequencies.
In a small community in the Netherlands, with common ancestral origin, several genetic disorders are more frequent than in other parts of the Netherlands. In the past decades, multiple patients with four rare severe disorders have been identified in this village: pontocerebellar hypoplasia type 2 (PCH2) (MIM 277470), fetal akinesia deformation sequence (FADS) (MIM 208150), rhizomelic chondrodysplasia punctata type 1 (RCDP1) (MIM 215100), and osteogenesis imperfecta (OI) type IIB/III (MIM 610682). Children with these disorders suffer significant morbidity and have a severely reduced lifespan. In this community, prospective parents are at relatively high risk of having a child with one of these four severe disorders. In 2012, this warranted the start of a preconception outpatient clinic in collaboration with the local midwifery practice, aimed at couples planning a pregnancy.

In this article the results of the first year of the outpatient clinic are presented. It is shown that targeted (preconception) carrier screening of high frequent genetic disorders can be a well-suited way of carrier testing in genetically isolated populations.

**PATIENTS AND METHODS**

**Setting**
Targeted carrier screening was offered in a fishing village along the former Zuiderzee in the Netherlands, which was founded in the 14th century by seven to 20 families. It is a typical Roman Catholic village, in great contrast to its Protestant environment. Due to religious and social factors, it is still a close-knit village. The village has about 21,000 inhabitants, about 7,100 persons of reproductive age (20 to 45 years), and a birth rate of about 250 births/year.

**Genetic disorders**
In the past two decades, founder mutations have been identified for PCH2, FADS, RCDP1, and OI IIB/III. An estimated 2-4 of the 250 children born in the genetically isolated village are annually affected with one of these four severe autosomal recessive diseases. The clinical characteristics of the four diseases, the genes, and mutations are described in Box 1.

In the community described, also other (non-lethal) disorders are highly frequent. Founder mutations have been found for phenylketonuria (PKU) (MIM 261600), primary ciliary dyskinesia (PCD) (MIM 615067), retinitis pigmentosa (RP) (MIM 268000), and pseudoxanthoma elasticum (PXE) (MIM 264800).
Box 1. The four severe autosomal recessive disorders occurring in high frequency in the genetically isolated community in the Netherlands.

**PCH2** is a progressive neurodegenerative disorder characterized by hypoplasia/atrophy of the cerebellum and pons, progressive microcephaly, extrapyramidal dyskinesias, dystonia, seizures, and severe cognitive and motor handicaps. Most infants die during infancy or childhood. In 2008 the *TSEN54*-gene was identified in patients with PCH2 from the genetic isolate. All affected individuals from the above-mentioned genetic isolate carry the homozygous c.919G>T (p.Ala307Ser)-mutation in the *TSEN54*-gene.

**FADS** generally refers to a heterogeneous group of disorders characterized by decreased or absent fetal movements, multiple joint contractures, pulmonary hypoplasia, poor muscle bulk, and craniofacial anomalies (ocular hypertelorism, low-set ears, retromicrognathia). The pregnancy is usually complicated by intrauterine growth retardation, polyhydramnios, and premature birth. Most infants die within two hours of birth due to severe pulmonary hypoplasia. The specific founder mutation of FADS was recently identified in the genetic isolate through homozygosity mapping of two FADS patients. All patients in the village are homozygous for the c.1724T>C (p.Ile575Thr)-mutation in the *MUSK*-gene.

**RCDP1** is a peroxisomal disorder characterized by rhizomelia, widespread epiphyseal calcifications, profound growth retardation, severe intellectual disability, cataracts, and ichthyosis. The majority of patients do not survive the first decade of life and some die in the neonatal period. Individuals with RCDP1 from the genetic isolate carry the homozygous c.875T>A (p.Leu292X)-mutation in the *PEX7*-gene.

**OI** is a congenital bone disorder characterized by susceptibility to bone fractures, severe bone deformities, and short stature. It can be subdivided into several types with a wide range of severity. Patients from the genetic isolate have OI type IIB (perinatally lethal OI) or OI type III (progressively deforming OI). Most cases of OI type IIB die within the first year of life due to respiratory failure. OI type III-patients already have multiple fractures, starting intrauterine or in the newborn period with progressive deformities, resulting in short stature and severe ambulatory restriction. The genetic cause of both types of OI in the genetic isolate is the c.21_22dupGG (p.Ala8fs)-mutation in the *CRTAP*-gene.
Outpatient clinic

In collaboration with the local midwifery practice, we started an outpatient clinic in September 2012 providing a preconception care consultation and offering genetic screening for the four founder mutations of PCH2, FADS, RCDP1, and OI IIB/III in the genetically isolated village, aimed at couples planning a pregnancy. Local policymakers, family physicians, midwives, and gynecologists were informed about the outpatient clinic and the offer of carrier screening. The start of the outpatient clinic was accompanied by attention of local and national media.

Individuals were not actively recruited but came to the outpatient clinic on their own initiative, or were referred by their general practitioner, midwife or gynecologist. If people preferred, counseling could also take place at the outpatient clinic in the Academic Medical Center (AMC) in Amsterdam, which is located about 30 km from the village. Prior to the appointment, all counselees received an information leaflet containing information about the diseases and genetics.

A genetic counselor from the Department of Clinical Genetics of the AMC counseled individuals or couples attending the clinic. Genetic counseling included personal and family medical history, provision of information about the four genetic diseases, autosomal recessive inheritance, carrier testing, and the reproductive options for carrier couples. If other genetic diseases occurred in the family, including other than those stated above, further genetic counseling for these diseases was offered. Individuals visiting the outpatient clinic in the village were also offered the opportunity to be given a standard preconception care consultation by one of the local midwives in addition to the appointment with the genetic counselor. The goal of this preconception care is to reduce the risk of adverse health effects for the baby and mother by optimizing the woman’s health and knowledge before planning and conceiving a pregnancy. This preconception consultation included information on general risk factors before pregnancy, health promotion, and preventive measures (if needed).

Couples were able to choose between simultaneous DNA testing in both partners or sequential DNA testing, in which one of the partners was tested first and DNA testing was only performed on the other partner if the first one turned out to be a carrier. Individuals originating from the genetically isolated village were tested for the four founder mutations. Individuals not originating from the village were only tested if the partner was carrier of one of the diseases (sequencing of the whole specific gene was performed). Results of the carrier tests were available within four weeks. Results were reported by the
genetic counselor to the individuals by phone. Subsequently, all individuals received a letter containing a summary of the genetic counseling and test results. Carrier couples were offered one or more additional genetic counseling sessions with the genetic counselor, in which they received more information about the specific disease and their reproductive options. An opportunity was offered to speak with a specialized social worker.

The costs of the genetic counseling and DNA analysis were reimbursed by the health insurances of the individuals (except for their own compulsory risk excess if not paid before because of other medical expenses).

RESULTS

Demographics

Between September 2012 and September 2013, a total of 203 individuals (92 couples and 19 individuals) were counseled. Of these individuals, 107 (52.7%) were female and 96 (47.3%) were male. The mean age was 29.8 years (SD 4.8; range 17-44). Of all women visiting the outpatient clinic, 43/107 (40.2%) were pregnant at the time of counseling. The mean pregnancy duration was 12 weeks (range 5-33 weeks). One woman was more than 24 weeks pregnant (35 weeks) when receiving the results. In this case, the couple was informed prior to testing that if they both turned out to be carrier, they could choose for prenatal diagnosis. In case of an affected fetus and a wish of the parents to terminate pregnancy, this case should be assessed by an expert committee.

A positive family history of carriers/affected persons of one of the four severe genetic diseases was reported in 46 (22.7%) individuals. In 157 (77.3%) individuals, no positive family history of the diseases within the close family (up to the third degree) was known, but in most families one or more children within the second or third degree had died in infancy of unknown cause. In all couples, at least one of the partners partially originated from the genetically isolated village. Only two (1%) individuals had no ancestors originating from the village. Of 197 individuals, 28 (14.2%) reported to be related to their partner (data were missing for six individuals); none were first cousins, eight (28.6%) were second cousins, seven (25.0%) were third cousins, and 13 (46.4%) were more distantly related.

In total, 100 (49.3%) individuals (of whom 21% were pregnant), visited the outpatient clinic in the village and 103 individuals (50.7%) visited the clinical genetics department in the AMC in Amsterdam (of whom 63.1% was pregnant).
Carrier frequencies

In total, 196 of 203 individuals (97%) decided to have the DNA test after counseling. Ninety-two couples were counseled. In 82 couples (89.1%) both partners were tested simultaneously. In seven couples only one partner was tested. Initially, three women visited the outpatient clinic without their partner. After they turned out to be a carrier of one of the diseases, their partner was also tested. All other 19 individuals who visited the counseling individually without their partner chose to participate in testing. Since they were not a carrier, there was no reason for the partner to visit the outpatient clinic. Table 1 shows the results of the carrier tests. Overall, 33.2% (65/196) of tested individuals were identified as being a carrier of at least one disease: 14.3% were carriers of PCH2, 11.2% of FADS, 6.1% of RCDP1, and 4.1% of OI. Five (7.7%) of the carriers were carriers of two diseases. Of the 45 tested individuals with a family history of the four diseases, 57.8% were carriers of one of the diseases and, of these, one (2.2%) was carrier of two diseases. Of the 151 tested individuals without a family history of the four diseases, 25.8% were carriers of one of the diseases and, of these, four (2.6%) of two diseases.

## Carrier frequencies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Carrier frequency (all individuals)</th>
<th>Carrier frequency (reported positive family history)</th>
<th>Carrier frequency (reported negative family history)</th>
<th>Carrier couples identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCH2</td>
<td>28/196 (14.3%)</td>
<td>17/45 (37.8%)</td>
<td>11/151 (7.3%)</td>
<td>4</td>
</tr>
<tr>
<td>FADS</td>
<td>22/196 (11.2%)</td>
<td>6/45 (13.3%)</td>
<td>16/151 (10.6%)</td>
<td>0</td>
</tr>
<tr>
<td>RCDP1</td>
<td>12/196 (6.1%)</td>
<td>1/45 (2.2%)</td>
<td>11/151 (7.3%)</td>
<td>0</td>
</tr>
<tr>
<td>OI IIB/III</td>
<td>8/196 (4.1%)</td>
<td>3/45 (6.7%)</td>
<td>5/151 (3.3%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65/196</strong> (33.2%)</td>
<td><strong>26/45</strong> (57.8%)</td>
<td><strong>39/151</strong> (25.8%)</td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

PCH2, pontocerebellar hypoplasia type 2; FADS, fetal akinesia deformation sequence; RCDP1, rhizomelic chondrodysplasia punctata type 1; OI IIB/III, osteogenesis imperfecta (OI) type IIB/III.

* Some individuals are carrier of two genetic disorders.

### Carrier couples

Of all 92 tested couples, four couples, all none pregnant, were identified as carrier couple of PCH2 facing a 1-in-4 risk of having an affected child with PCH2 in each pregnancy. Subsequently, two carrier couples conceived a pregnancy and both chose for prenatal diagnosis (chorionic villus sampling). Both unborn children turned out to be unaffected with PCH2. One of these carrier couples already had two children and refrained from
having more children, the other couple had not made a decision about their future reproductive choice at the time of post-test counseling. Figure 1 shows the pedigrees of two of the four carrier couples. In the first pedigree (Figure 1a), family history was negative for all four diseases. In the second pedigree (Figure 1b), the mother of the woman was identified as a carrier of PCH2, but only the father of the woman (who is not a carrier of PCH2) has a positive family history of PCH2. Using cascade testing, neither couple would have been identified.

Parallel during this first years’ experience of the outpatient clinic, two children whose parents did not visit the preconception consultation of the outpatient clinic were diagnosed in the AMC with one of the four diseases. One child was diagnosed postnatally with PCH2. The other was prenatally identified with OI, based on routine ultrasound finding at 19+2 weeks gestation and confirmed with DNA analysis on amniotic fluid. This pregnancy was terminated. Neither of the couples were aware of the opportunity of targeted carrier screening in the outpatient clinic.

**DISCUSSION**

In this article we describe our first-year experiences of targeted genetic screening of four severe autosomal recessive disorders in a genetically isolated community. Genetic testing showed a very high carrier frequency (33.2%) among the 196 people we tested. In this way, relatively few people have to be tested per detected carrier.

In the literature, we found other studies about carrier screening programs in genetic isolates, mainly in Israel. For example, a targeted carrier screening program for severe and frequent diseases was implemented in different isolated (mainly Arab and Druze) communities in Israel. As was the case in the current genetically isolated community, the purpose of these carrier screening programs was to give individuals informed options regarding family planning. However, in these genetic isolates, prenatal testing and termination of pregnancy was not well accepted, but early identification of carriers also gives the opportunity to choose a partner by carrier status or performing PGD. In the genetic isolate as presented here, however, termination of pregnancy is generally well accepted and two carrier couples chose for prenatal diagnosis.

In many genetically isolated villages, the reported rate of consanguinity is high, while in the current genetic isolate the percentage of individuals reporting consanguinity is relatively low. 14.0% Of individuals reported to be related (second cousins or more distantly related). This is most probably an underestimation as genealogy in this endogamous community shows that most couples are related several generations ago. Because of the
high carrier frequencies in the village, the chance of meeting a partner who is a carrier of the same autosomal recessive condition may be as high as observed in cousin marriages.\textsuperscript{19} Even in individuals without a positive family history of affected persons or carriers, the carrier frequency was high (25.8\%). In most of these families however, one or more children within the second or third degree had died in infancy of unknown cause. It is not unreasonable to suggest that some of these children had one of the four disorders.

Because of the high prevalence of several genetic disorders, this village was well known by counselors of the Department of Clinical Genetics of the AMC for genetic counseling and scientific research. A great advantage of this knowledge was that the genetic services could be developed based on community needs, and could thus be delivered more effectively. The genetic counseling and testing was embedded in the regular preconception care setting, offered in the home village of the participants, and aligned with local policymakers.

In a one-year period 203 individuals were counseled, a relatively high uptake compared to the birth rate of 250 births/year. However, the uptake is not complete while parents may also decide to decline screening or are not informed about the offer. During the study period two children were diagnosed with one of the four diseases among couples who did not attend the clinic.

Although reproductive genetic counseling preferably takes place before conception, a large percentage of women visiting the outpatient clinic (40.2\%) were pregnant at the time of the genetic counseling. This high pregnancy rate was observed previously in other screening programs recommending preconception screening such as the population-based CF screening program in the US.\textsuperscript{20,21} It is possible that the pregnancy rate in our study was so high because the genetic test was offered only recently. In that case the pregnancy rate in counseled couples is expected to drop in the coming years as more couples become aware of this preconception care initiative. To increase the number of persons aware of the genetic test offer, and to lower the number of women who are already pregnant, we will continue to inform the population (for example through local media) and educate the local medical staff referring to the clinic. Another possibility is to raise awareness through education at school. This has been done successfully for many years in Jewish high schools in several countries for genetic conditions common in the Ashkenazi Jewish population.\textsuperscript{22}

In the last few years, studies have been reported on possibilities for preconception carrier screening for several hundreds of severe recessive childhood diseases by next-generation sequencing (NGS).\textsuperscript{23-25} In this way couples can comprehensively be screened for carrier status of these disorders, especially if there is no positive family history of autosomal recessive diseases. Several commercial laboratories are now offering expanded panels.\textsuperscript{26} Expanded carrier screening using NGS could be cost-effective and the costs are decreasing each year.\textsuperscript{24}
In the genetic isolate described in the current article, cascade preconception genetic testing after a positive family history and/or carrier screening for several hundreds of severe recessive childhood diseases by an existing (commercial) routine expanded screening panel may be less suitable ways of carrier identification. Cascade genetic testing is considered highly inefficient as most affected children are born into families without a family history of disease, many family members do not come forward for testing, and many people of non-reproductive age have to be tested. With cascade genetic testing, the carrier couples shown in Figures 1a and 1b would not have been identified.

**Figure 1.** Examples of pedigrees of carrier couples identified by targeted carrier testing. (a) PCH2 carrier couple without positive family history of the four diseases identified. (b) PCH2 carrier couple. Note that the mother of the woman is carrier of PCH2, while the father has a positive family history of PCH2 but is no carrier himself.

Squares indicate males; circles, females; diamonds, gender unspecified; double lines, consanguinity; arrows, probands; shaded symbols, affected; spot, carrier; P, pregnancy; PCH2, pontocerebellar hypoplasia type 2; FADS, fetal akinesia deformation sequence.
Carrier screening for several hundreds of severe recessive childhood diseases has advantages such as preventing the stigmatization of a population and causing fewer carriers to be missed. However, this form of carrier screening also has several disadvantages.\textsuperscript{28-31} Besides the current costs of expanded carrier screening by NGS, it has been argued that the interpretation of results can be difficult for now (e.g. variants of unknown clinical significance)\textsuperscript{23} and the range of disorders is very diverse.\textsuperscript{25,26} Moreover, in the current commercial screening panels some population-specific genes or mutations are not covered whereas other covered genes and mutations are not present in the specific subpopulation. For example, mutations in the specific genes for PCH2, FADS and OI are not covered in the screening test for 108 disorders,\textsuperscript{25} and in the proposed screening test for 448 severe autosomal recessive diseases the specific gene for FADS is not present.\textsuperscript{23,24} This is also the case for several mutations in the targeted carrier screening program in different isolated (mainly non-Jewish) communities in Israel.\textsuperscript{16-18} This emphasizes that screening by an existing (commercial) routine expanded screening panel is not appropriate for each population. We therefore propose to develop a customized NGS screening test for each country/region in which both country/region specific mutations are present as well as genetic isolate specific founder mutations, which can be updated regularly. This approach may also reduce potential stigmatization of genetic isolates, while specific mutations still are included.

Further research is needed to investigate participants’ informed choice to screening, psychological impact, also in the long term, and satisfaction among individuals who have decided to be tested or who refrained from testing. Moreover, as members of a subpopulation may feel stigmatized or discriminated as a result of carrier status information,\textsuperscript{32} this aspect needs to be addressed.

In the community described, testing was offered for four severe genetic disorders. As described earlier, other non-lethal disorders are also highly frequent in the described community. If (one of) these other founder mutations was present in the family, we offered further genetic counseling for the specific disease(s). Because of the less severe nature of these diseases, therapeutic options in some of them, and the (presumably) different reproductive consequences, these less severe disorders should preferably be offered in one or more separate test panels for which the couples can give separate informed consent.\textsuperscript{26,30}

Our results stress the importance of offering targeted carrier screening, instead of cascade genetic testing, within genetically isolated populations with a high prevalence of specific genetic disorders. Carrier screening using an isolate specific panel is worthwhile, as we have shown here. As NGS becomes more routine and costs are falling, it is expected that
isolate specific panels will be replaced. Our findings and set-up can be seen as a model for carrier screening in other high-risk subpopulations and contributes to the discussion about the way carrier screening can be offered and organized in the general population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Targeted carrier screening for four disorders


