Addressing reproductive risk in consanguineous couples

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CHAPTER 4

First steps in exploring prospective exome sequencing of consanguineous couples

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ABSTRACT

Consanguinity is one of the most frequent risk factors for congenital disorders. In theory, prospective exome sequencing of consanguineous couples could identify couples who both are carriers of autosomal recessive diseases, and empower such couples to make informed reproductive decisions. To investigate this, we sent blood samples to our laboratory of four pairs of consanguineous parents having one or more children affected by an autosomal recessive disorder, without revealing any diagnostic information. The study was restricted to find identical, previously described, or evidently pathogenic mutations in both parents of each couple, in over 400 genes known to result in severe autosomal recessive disorders. Out of the six autosomal recessive disorders known to the four couples studied, two were correctly identified. Carrier status of one not previously known autosomal recessive disorder was discovered. As expected, given the pipeline used, large deletions, mutations in genes not present in the gene list, mutations outside the exons and consensus splice sites, and mutations that were not evidently pathogenic and previously not reported, were not identified. The restriction to detecting only couples with identical mutations diminishes the risk of revealing unsolicited findings and shortens the time needed for analysis, but also results in missing couples with different mutations in the same gene. In addition to the proposed pipeline, couples should be offered testing for carrier status of frequent disorders that can present themselves by large deletions, non-exonic mutations or compound heterozygous mutations (e.g. thalassemia, spinal muscular atrophy, cystic fibrosis). Even though sensitivity is reduced, offering exome sequencing prospectively will increase reproductive options for consanguineous couples.

INTRODUCTION

Consanguinity is a frequent phenomenon: it is estimated that about 10% of people worldwide are in a consanguineous relationship or have consanguineous parents (Bittles and Black, 2010a). Compared with children of non-consanguineous parents, children of consanguineous parents have an extra risk (2-2.5% in first cousins) of being affected by a hereditary disorder, mostly involving autosomal recessive (AR) conditions (Hamamy et al., 2011). This higher risk is additional to the risk resulting from a possible family history of an AR disease. The extra 2-2.5% risk at population level is not equally shared by all first-cousin couples. From these percentages it can be estimated that fewer than 8-10% of first-cousin parents are at high risk (25% or more) for disorders not already known in the family (Teeuw et al., 2010). Consequently, some 90-92% of them have no increased risk at all.

Exome sequencing, in theory, promises to become a useful tool for prospectively identifying those consanguineous couples in which both partners carry a deleterious mutation in the same gene (Makrythanasis et al., 2014; Sheridan et al., 2014; Alkuraya, 2013). Exome sequencing is already frequently used in affected children – with or without consanguineous parents – to determine the causative gene (Bamshad et al., 2011; Dixon-Salazar et al., 2012; Gilissen et al., 2012). For the application of this technique in health care to prospectively identify consanguineous couples at high risk, it should (1) have a high sensitivity; (2) produce few ambiguous results and preferably a low number of unsolicited findings; and (3) give results within an acceptable course of time.

The expected yield of this approach can be investigated *in silico*, leaving the question unanswered regarding whether expectations would be realized in practice. In order to explore the potential of exome sequencing in prospective consanguineous parents in practice in our own laboratory, DNA was sampled from four consanguineous couples that already had a child with an autosomal recessive disorder. Subsequently, these samples were offered to the laboratory that had not been involved in the diagnosis in the child earlier on, with the request to diagnose the carrier status of both parents by means of exome sequencing. No details about the existing recessive disorders were revealed. The analysis was restricted to finding identical, previously described or evidently pathogenic mutations in a large, but limited number (#437) of well-known genes known to cause severe recessive childhood disorders, in both parents (Bell et al., 2011). The aim was to establish the extent to which the laboratory would indeed identify, in both parents, the causative mutations, and whether other outcomes of the analysis of the exome would appear.

METHODS

For this proof-of-principle project we obtained approval from the Medical Ethical Committee of the VU University Medical Center Amsterdam, The Netherlands.

Subjects

With the aim of including three to five consanguineous couples with an affected child in this study, two of the authors (MT & PZ) approached eight couples known from a previous study (Teeuw et al., 2010). They were known to be carriers of identical mutations of at least one AR disorder from a set gene list (Bell et al., 2011). Four couples gave informed consent for participation after having been counselled with regard to the objectives and possible additional outcomes of the study, such as unsolicited findings. The couples were asked whether they wanted to receive information about possible carrier status of AR disorders not yet known to them, and about unsolicited findings of medical relevance.

Details about the couples are presented in Table 1 (columns 2, 3 and 4). Each of the first two couples had a child with lamellar ichthyosis. There was a difference in severity of the disorder: couple 1 had a child with severe lamellar ichthyosis (MIM#242300) and couple 2 a child with lamellar ichthyosis in the neonatal phase, which was asymptomatic later in infancy. Couple 3 had a child that was diagnosed with beta-thalassemia (MIM#613985) caused by homozygous mutations. This couple had another child with retinitis pigmentosa (RP; MIM#268000) in whom the causative gene was discovered simultaneously through exome sequencing (in another centre) in the course of our project. Couple 4 lost their first child through spinal muscular atrophy (SMA) type 1 (MIM#253300) and their second child to non-ketotic hyperglycinemia (MIM#605899), both in infancy.

For each participating couple, blood was drawn, coded and sent pairwise to the laboratory without any additional information about the identity of the parents or the relevant disorders.

Analysis

Exome sequencing was performed as described previously (Wolf et al., 2014) and according to standard protocols. The procedure consisted of three steps: (1) specific capture of the exome using SeqCap EZ Human Exome Library v3.0 kit (Nimblegen); (2) sequencing performed with 100 bp paired-end reads on a Hiseq2000 (Illumina, San Diego, CA); and (3) data processing using an in-house pipeline.

For data interpretation, variants were filtered for identical heterozygous variants, present in both partners of each couple, in the set gene list known to cause severe childhood recessive disorders (Bell et al., 2011). Identified variants were cross-checked

with the medical literature and, in compliance with standard procedures, a prediction was made regarding the possible pathogenicity of the mutation. As a result of this approach, gene or exon deletions and mutations in genes other than those mentioned in the list of Bell et al. (2011) were not identified during the analysis. Finally, after de-blinding, results were compared to the pre-existing information. In cases of unidentified mutations, unfiltered data of the couple were consulted.

RESULTS

The results of the exome sequencing are presented in Table 1 (columns 5 and 6). The results in couple 1 were straightforward since both parents were found to be carriers of the pathogenic mutation in *TGM1*, and no other pathogenic mutations were found. In couple 2, the mutation in *TGM1* was initially identified, but the mutation was interpreted as not certainly pathogenic since this mutation had not been described in literature and was not present in the online Human Gene Mutation Database Pro (HGMD pro). In couple 3, although the mutations in the *HBB* and *MERTK* gene were present in the unfiltered data, they were both removed by the applied filter steps. The *HBB* mutation because it was an intronic mutation located outside the consensus splice site, and the *MERTK* mutation because genes associated with retinitis pigmentosa were not present in the applied gene list. In couple 4, the carrier status of both partners of the pathogenic mutation in *GLDC* was correctly identified, while the carrier status for a two exon deletion in *SMN1* was not identified as the applied data analysis pipeline did not contain an algorithm to detect larger deletions. In addition, the carrier status of both parents for pathogenic mutations in the *NPHS1* gene causing congenital nephrotic syndrome was identified. This disease was not present in the family and therefore not known to the couple.

DISCUSSION

In this first exploration, exome sequencing was performed in four consanguineous couples that were, in retrospect, both carriers of seven autosomal recessive disorders. The interpretation of the exome data revealed identical heterozygosity in two parent couples for three autosomal recessive diseases (two of the previously known diseases and one not previously known to the couple). Moreover, as was expected based on the used filtering strategy, there were no unsolicited findings. If this technique had been offered preconceptionally, information on these mutations would have been provided to the parents.

Because of the strategy chosen at the start of the project, not all known disorders were identified**.** This highlights the importance of awareness of both potency and

limitations of using next-generation sequencing (NGS) for this purpose. Communication between clinicians and lab specialists is essential in order to reach attunement concerning the possible outcomes and mutual expectations when implementing a new technique such as NGS (Rigter et al., 2013).

In couple 3, the first disorder was filtered out because the *HBB* gene contained an intronic mutation outside the splice consensus site. In couple 4, the SMA type 1 exon deletion was filtered out, since the analysis pipeline only identifies single nucleotide variants and small indels. Both disorders are examples of more frequent AR disorders: hemoglobinopathies especially in the population originating from Africa, the Middle East, the Indian subcontinent, South-East Asia, and the Mediterranean area, while SMA type 1 is prevalent throughout many different ethnic groups (Modell and Darlison, 2008; Muralidharan et al., 2011). In addition to NGS with the present pipeline, it is worth considering testing for hemoglobinopathies separately using traditional means (e.g. HPLC), as well as testing for the exon 7, and 8 deletions in the *SMN1* gene, or implementing tools such as XHMM (Fromer et al., 2012) for the detection of large deletions and duplications in exome sequencing data.

It is possible that both partners of a consanguineous couple are carriers of the same AR disorder, but have two different mutations. If the filter strategy were to be expanded to predict the risk of compound heterozygosity in children, this would make the analysis much more complicated and laborious. When just focusing on identical mutations, the *extra* risk caused by mutations identical-by-descent is obviated as well as the risk from mutations that are identical-by-state. This results in a substantially lower *a posteriori* risk of AR disorders for tested consanguineous couples. However, in some diseases compound heterozygosity may be relatively frequent, even in children of consanguineous parents (for example when there are many different mutations, and one of which is very frequent, like the CFTRΔF508 mutation in cystic fibrosis) (Ten Kate et al., 1991; Ten Kate et al., 2010). Adding frequent mutations to the exome filter, followed by analysis of the gene sequence to identify any mutation in the partner, may be considered.

Couple 2 would not have been informed prospectively about the mutation in *TGM1*, because the mutation had not been described in the literature, nor was it present in the online Human Gene Mutation Database Pro (HGMD pro) and, therefore, it was interpreted as possibly – but not clearly – pathogenic. Given the mild phenotype of this child, it is at least questionable whether the parents would have desired sharing of this information preconceptionally. Furthermore, even in cases of previously described mutations, parents should be aware that for some mutations it is difficult to predict the severity of the phenotype in the child.

Table 1. Parents with offspring's inbreeding coefficient, phenotypes, genotype and parental genotypes and the interpretation resulting from this study

	Pre-existing knowledge			Results of exome sequencing	
Couple #	Inbreeding Coefficient (F)	Phenotype	Known genotype in child (homozygous state)	Exome data in parents (heterozygous state)	Outcome
1	1/16	Lamellar ichthyosis	TGM1 gene c.160C>T p.(Arg54*) (NM_000359)	TGM1 gene c.160C>T p.(Arg54*) (NM_000359)	Pathogenic, leading to lamellar ichthyosis in homozygous state
2	1/32	Lamellar ichthyosis	TGM1 gene c.1115T>C p.(Val372Ala) (NM_000359)	TGM1 gene c.1115T>C p.(Val372Ala) (NM_000359)	Identified and filtered out: Interpreted as not pathogenic
3	5/64	Beta-thalassemia	HBB gene c.93-21 G>A (IVS1-110 (g>a)[1]. (NM_000518)	HBB gene c.93-21 G>A (IVS1-110 (g>a)) (NM_000518)	Removed by filter (intronic)
		Retinitis pigmentosa	MERTK gene c.2179C>T p.(Arg727*) (NM_006343)	MERTK gene c.2179C>T p.(Arg727*) (NM_006343)	Removed by filter (gene list)
4	1/64	Non-ketotic hyperglycinemia	GLDC gene c.1270C>T p.(Arg424*) (NM_000170)	GLDC gene c.1270C>T p.(Arg424*) (NM_000170)	Pathogenic, leading to non-ketotic hyperglycinemia in homozygous state
		Spinal Muscular Atrophy type 1	SMN1 gene Exon 7/8 deletion (NM_000344)	Not identified	Not identified: Exon deletion
		Not applicable	Not applicable	NPHS1 gene c.2398C>T p.(Arg800Cys) (NM_004646)	Pathogenic, leading to congenital nephrotic syndrome in homozygous state

[1] *mutations not revealed before sampling*

Naturally, the applied gene list needs continuous updating with newly discovered genes causing severe early-onset AR disorders. Moreover, genes associated with severe AR intellectual disability are not yet included in this list and can be added. At the same time, an ethical debate should focus on extending the offer of preconception screening as couples may prefer to have various options. In addition to testing for severe AR disorders, discussion is needed on a screening offer for disorders perceived as less severe: for example for RP (as in our couple 3) or genes causing hereditary forms of deafness.

If informed correctly about possible implications and available reproductive options, the parents might opt for what is most suitable for their situation. Above all, in particular for disorders without immediate consequences to the child's health, the child's anticipatory right to an open future should be safeguarded, requiring prudent disclosure policies (Bredenoord et al., 2013).

It was found earlier that information regarding the reproductive risk and the possibility of genetic carrier testing is appreciated by the target population, preferably at an early stage -even before marriage has taken place (Teeuw et al., 2014). Exome sequencing and analysis will nowadays take approximately two months (without confirmation by Sanger sequencing). This makes it particularly suitable for use in the preconceptional phase.

Our straightforward and feasible approach, even though sensitivity is reduced by the use of filters, increases reproductive options for consanguineous couples compared to the current situation. If (future) consanguineous parents and their referring care givers are carefully informed about potential pitfalls like missed mutations, missed diseases and findings of unknown significance, we suggest that, as long as financial conditions are in place, this technique can already find its way to clinical practice.