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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.

1. 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, 2010]. 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 [Alkuraya, 2013; Makrythanasis et al., 2014; Sheridan et al., 2014]. 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 [Alkuraya, 2013] have a high sensitivity; [Bamshad et al., 2011] produce few ambiguous results and

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preferably a low number of unsolicited findings; and [Bell et al., 2011] 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.

2. Methods

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

2.1. 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 counseled 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.

2.2. Analysis

Exome sequencing was performed as described previously [Wolf et al., 2014] and according to standard protocols. The procedure consisted of three steps: [Alkuraya, 2013] specific capture of the exome using SeqCap EZ Human Exome Library v3.0 kit (Nimblegen); [Bamshad et al., 2011] sequencing performed with 100 bp paired-end reads on a Hiseq2000 (Illumina, San Diego, CA); and [Bell et al., 2011] 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 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. [Bell et al., 2011] were not identified during the analysis.

Table 1
Participating couples with their offspring's inbreeding coefficient, phenotypes and genotype and parental genotypes and the interpretation resulting from this study. Q2

Identification	Pre-existing knowledge			Results of exome sequencing	
Couple #	Inbreeding coefficient in children (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))# (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
		n/a	n/a	NPHS1 gene c.2398C > T p.(Arg800Cys) (NM_004646)	Pathogenic, leading to congenital nephrotic syndrome in homozygous state

n/a: not applicable; # mutations not revealed before sampling.

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