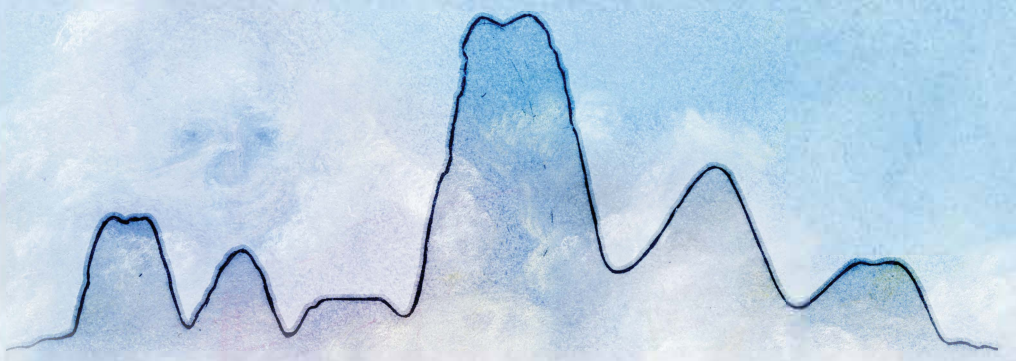
Unsolicited findings in next-generation sequencing: *hide or seek?*



Vyne van der Schoot

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Provided by thesis specialist Ridderprint, ridderprint.nl
Printing: Ridderprint
Cover design: Lisette van Eijndhoven en Gerard van der Schoot, met behulp van GerdySign
Layout and design: Harma Makken, persoonlijkproefschrift.nl

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PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof.dr. Pamela Habibović
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op woensdag 31 mei 2023 om 16.00 uur

door

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Chapter 1

General introduction

Newborn Amélie has multiple congenital anomalies. She is small for gestational age, has facial dysmorphism (hypertelorism, short nose with wide and depressed nasal bridge), and urogenital anomalies. Amélie's parents were referred to the clinical genetics department for genetic counselling. Genetic variants can cause congenital malformations. Sometimes, a causative variant could cause additional health-related problems. Finding a genetic cause for her anomalies will not cure Amélie. It will, however, enable anticipation of potential additional problems, provide an explanation for her condition and enable counselling of family members on their risk of being affected themselves or to have affected offspring. The clinical geneticist told Amélie's parents that they could perform next generation sequencing. This technique allows analysis of the entire exome (whole exome sequencing, WES) or genome (whole genome sequencing, WGS). She told them that the potential outcomes of the next generation sequencing include finding the cause of the condition, finding a possible cause of the condition or finding no genetic variant of clinical relevance. Also, NGS techniques may lead to detection of variants that are not associated with Amélie's anomalies, but rather predict other future medical conditions. Since these findings could be of relevance for the health of Amélie and her family, they were asked whether or not they agree to disclosure of such additional variants. After genetic counselling both parents gave consent for genetic testing. To better interpret potential findings, Amélie's DNA was compared with the DNA of her parents. After two months the genetic test results were disclosed. Unfortunately, the DNA test did not show the cause of Amélie's condition. It did however, reveal a genetic predisposition to heart disease in both Amélie and her father.*

Background

Genetic testing

Genetic testing can be performed for various reasons. Diagnostic genetic testing aims to identify genetic variation underlying a health condition with which someone is already affected. Pre-symptomatic genetic testing is offered to those who are at familial risk of having a disease-causing genetic variant but are not yet clinically affected. Testing individuals who are at risk to be a carrier of a genetic variant that will not affect their own health, but which might affect the health of their offspring, is referred to as carrier testing.

Establishing a genetic diagnosis can be important for timely implementation of precision medicine and optimal health outcomes. For example, someone at risk of developing heart disease might benefit from cardiac screening, medication or a cardiac device, such as a pacemaker. Also, having a genetic diagnosis enables risk calculation of the condition developing in (future) family members, allowing them to have pre-symptomatic testing performed. Additionally, it can provide reproductive options (e.g. prenatal testing, preimplantation genetic

* *In the Netherlands, genetic counseling at the department of clinical genetics, can be performed by certified clinical genetics medical specialists and clinical genetics residents or nurses, specialized in clinical genetics. Throughout the manuscript, we use the term 'healthcare professional' to refer to all clinicians who counsel genetic testing.*

testing) for those who want to prevent affected offspring. Furthermore, a genetic diagnosis provides understanding about why an individual developed a certain condition.

Genetic tests enable detection of structural variation (SV), copy number variation (CNV) and/or single nucleotide variation (SNV)(2). For the latter, conventional tests consist of targeted testing of one or multiple gene(s) of interest. When using targeted tests, genes have to be selected based on the clinical presentation of the patient. Finding a causative variant could be challenging because of genetic heterogeneity, phenotypic variability and/or incomplete knowledge(3). Often, multiple genetic tests have to be performed, making patients endure a diagnostic odyssey(4).

Next-generation sequencing (NGS) techniques allow massive parallel sequencing of multiple genes. This can be a targeted gene panel, the entire exome (all human genes) or the entire genome (all human DNA). Analysis often includes CNVs and SNVs, and sometimes SVs. When exome or genome sequencing has been performed, clinicians may request analysis using an in silico gene panel. This enables analysis of NGS data restricted to genes known to be associated with the patient's condition. Analysis of the entire exome or genome allows analysis without phenotypical or genotypical restrictions.

To identify disease-causing variants, genetic data is compared to data of healthy controls. Over the years, technological innovation has improved NGS, resulting in an increased diagnostic yield, decreased time to diagnosis, and lower sequencing costs(5-7). This has allowed NGS to be incorporated increasingly into clinical care(8), replacing more targeted tests. Extended genetic testing for congenital anomalies, like Amélie's case, exemplifies the numerous and expanding phenotypes for which NGS is being performed (e.g. developmental disorders(9, 10), neurological disease(11), autoinflammatory disease(12)).

Unsolicited findings

Definition

Since NGS techniques have the potential to uncover variants in the entire exome or genome, extended genetic tests allow the detection of variants that are not associated with the clinical question for which the test was performed. These variants could predispose to other health conditions and could be of relevance for the health of an individual and/or of their blood relatives(13).

Such findings are variously described as 'unsolicited findings', 'accidental findings', 'co-incidental findings' or 'incidental findings'. Although none of the terminological suggestions have remained free of objections(14), the term 'unsolicited findings (UFs)' will be used in this

thesis. When actively looking for variants not related to the clinical question, we will refer to these as 'secondary findings (SFs)'.

Genetic variants that predispose to conditions that are considered treatable or preventable health issues are also referred to as being 'medically actionable' findings. Although the concept of 'medical actionability' has been criticized for its inexactness(15), it is broadly accepted as an important criterion for guiding decisions on UF disclosure.

Current perspectives

The issue of UFs is not new in clinical genetics(16). More conventional techniques, enabling detection of genome-wide chromosomal anomalies, have been associated previously with UFs. However, the odds of retrieving UFs has increased through the availability of more sensitive exome and genome wide sequencing techniques. This raised the question of which genetic information should be disclosed to the patient.

A major argument in favour of disclosure is that knowledge about a genetic predisposition could enable prevention or early detection of the condition to which the UF predisposes, potentially resulting in decreased morbidity and/or mortality(17). It has been ethically rejected not to disclose clinically relevant information to patients. This reflects respect for patients' autonomy in the right to know (receiving information) (18). On that note, offering an opt-out from receiving additional information (right not to know) is warranted.

Arguments against disclosure emphasize the distress and anxiety that disclosure might cause in patients(17). Furthermore, since data on disease-related risks are mainly based on study populations affected by the condition a variant predisposes to(19, 20), it has not been elucidated whether variants create the same risk to develop disease when found in an asymptomatic family(19, 20). Thereby, the effectiveness of screening and preventive measures in families with a genetic predisposition but without the associated phenotype has not been studied yet. The complexity and ambiguity of UFs have raised concerns about their utility(21, 22). The value of net benefit has been emphasised: if the perceived negative impact outweighs the potential clinical utility, disclosure is not recommended(13, 17).

Several studies have explored views of different stakeholders (i.e. patients, healthcare practitioners, laboratory personnel, the general public) on UF (and SF) disclosure. They generally agree on the value of UF (or SF) disclosure(23). Patients tend to 'want to know it all', whereas healthcare personnel seems to be more cautious with disclosure. Which results should be generated and made available, however, is less clear.

In ongoing worldwide debates regarding UF policy, no consensus on this matter has been reached(24-27), and many diverse guidelines exist. The American College of Medical Genetics (ACMG) published recommendations to actively look for variants (i.e. SF) in medically actionable disease genes(24, 25). The gene list is evaluated periodically by the ACMG and currently comprises over 70 genes, related mainly to autosomal dominant cardiac and oncological disease(28). After intense debates within the professional society, the ACMG added the option to opt out of receiving these SFs.

The European Society of Human Genetics and the Canadian College of Medical Genetics do not recommend actively seeking SFs(26, 27). Instead, they argue to limit the likelihood of detecting UFs. When a UF happens to be accidentally found, they do agree on recommending disclosure of variants that predispose to autosomal dominant, recessive or X-linked conditions that are considered to be medically actionable. Since disclosing carrier status for autosomal recessive and X-linked conditions would allow reproductive options, recessive disease alleles could be considered for disclosure as well(26). Shared carrier status of recessive disease alleles in a couple will be actionable only when the couple wants to have a child. Disclosure of carrier status in asymptomatic individuals would likely lead to an increase in their partners undergoing testing as well. The low carrier frequency of most autosomal recessive conditions might not justify the substantial additional workload for the laboratory. Therefore, clinical geneticists could consider a risk threshold for disclosure and only disclose carrier status when the allele has a predicted risk greater than or equal to 25%.

Reflecting on patients' autonomy and thus their right (not) to know, current recommendations emphasize broadening patients' choices by offering an 'opt-in' for disclosure of non-actionable diseases and an 'opt-out' to abstain from disclosure of actionable conditions(17, 29).

Aim, relevance and outline of this thesis

In order to evaluate previously proposed recommendations and best practice guidelines, deliberation of potential benefits and risks of UF disclosure is imperative.

Seeking

After the first recommendations regarding SF disclosure by Berg and colleagues(30), numerous studies have presented the prevalence of SFs(31-52). Using large cohorts, participants were screened for variants predisposing to medically actionable conditions. The gene lists used in these studies were mainly based on the ACMG recommendations(24, 25, 28, 50). These studies showed a prevalence of SFs ranging from 1 to 11%. Although most variants were found in genes predisposing either to cardiac or oncological disease, studies showed a variation in the genes in which variants were detected. The differences in prevalence and nature of SFs can be explained largely by variation in experimental design and data analysis, including study population, databases for variant filtering and interpretation, ethnicity-specific variation information and lists of genes extending beyond the ACMG recommendations. Considering these differences, these studies cannot be generalised for the development of generally applicable clinical recommendations.

In ***chapter 2*** of this thesis, we report on anonymized WES data of 1,640 healthy Dutch individuals to establish the frequency of medically actionable disease alleles in the general population.

These data will not be applicable to a clinical setting in which only UFs (and not SFs) are considered for disclosure. Not actively looking for variants, but finding variants accidentally will impact the prevalence and nature of UFs. Also, criteria which have to be met for SFs (e.g. prevalence) can be abandoned in UF disclosure. Both variants in more frequent disease genes and rare disease alleles will be equally of interest. Lastly, when there is no recommended, pre-set list of genes in which variants have to be disclosed, the concept of 'medical actionability' will have to be assessed *ad hoc* and on a case by case basis. Together this broadens the scope of genes in which UFs are uncovered as compared to SFs. Thus, there is a need to systematically assess the frequency and nature of UFs .

Chapter 3 describes UFs identified in 16,482 index patients receiving clinical WES in a 5 year period.

Listening

In order to evaluate the perceived impact of UF or SF disclosure, qualitative studies have addressed patients' perceptions(39, 53). These studies on the impact of SF disclosure report that a minority of patients experience a negative impact due to anxiety and/or difficulties in

conceptualizing the associated risks(39, 53). However, evidence is sparse and the perceived impact of UF disclosure still needs to be studied in more detail.

In ***chapter 4*** we describe insight gained from 20 semi-structured interviews with patients to whom a UF had been disclosed in order to better understand the perceived impact of UFs.

Telling

Genetic counselling is affected by the implementation of NGS and its potential to uncover UFs in daily practice(16). Patients have to be adequately informed pre-test about the possible outcomes to enable informed decision-making and informed consent. Subsequently, UFs (or SFs) have to be considered for disclosure, taking multiple factors into account (e.g. penetrance, expression, actionability). And lastly, healthcare professionals are required to disclose information during their post-test counselling which does not, by definition, concern the main objective of the genetic test. Several studies aimed to gain insight in SF counselling. However, only limited studies provide insight in how clinicians experience counselling UFs(16, 54). Current literature predominantly focusses on hypothetical views, and only limited studies provide insight in actual experience with counselling UF pre-test and with UF disclosure. Further research into healthcare professionals' appreciation for the implications of NGS and UF has been recommended(23).

For ***chapter 5*** we report on 20 semi-structured interviews about UFs with certified clinical genetics medical specialists and clinical genetics residents, working in seven national genetic centres, elucidating their views and experiences regarding UFs.

In ***chapter 6*** we describe the results of the secondary analysis of the interviews from chapters 4 and 5, to better understand the different sources of uncertainty.

Chapter 7 summarizes the findings, elaborates on variant classification and interpretation in the context of UFs and SFs, discusses the concept of medical actionability and explores informed consent in the context of UFs and SFs.

Box 1. Introducing genetics(1)

Individuals' genetic information, or 'the human genome' is organized in chromosomes. In the nuclei of most human body cells, 46 chromosomes can be identified. Most cells contain 22 autosomal pairs and one pair with two sex chromosomes, being either two X chromosomes or an X and a Y chromosome. One of each pair of chromosomes is inherited from each parent. Chromosomes are tightly coiled DNA. The DNA consists of four chemical bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) which pair up (A with T and C with G). Together with a phosphate molecule and a sugar molecule, these bases are called 'nucleotides'. The nucleotides are attached together and form the spiral strands the DNA double helix.

DNA can be subdivided into units corresponding to genes, which carry instructions for building proteins. These proteins allow cells to function and ultimately determine an individual's characteristics. Each gene is represented on two chromosomes ('bi-allelic'). Individuals inherit one copy of a gene (or 'allele') from each parent.

Humans have approximately 20,000 genes. More than 99% of DNA bases are the same in different individuals. The miniscule variation of less than 1% creates a remarkable variation in human beings. Variation in DNA ranges from a single DNA base change (i.e. single nucleotide change, deletion or insertion; monogenic variation) to larger genomic sections involved (i.e. chromosomal anomaly).

Most of the interpersonal genetic variation will have no direct health-related impact. A minority however, is associated with disease. These variants explain why some individuals are affected by certain conditions or why some individuals are predisposed to specific diseases. Genetic variants can either cause disease when in combination with other genetic variants and/or environmental factors ('multifactorial inheritance'). When variation in only one gene will affect an individual's health, this is called 'Mendelian inheritance'.

Mendel described different patterns of inheritance. When disease-causing variation on only one allele is associated with disease, this is called 'autosomal dominant' inheritance. 'Autosomal recessive' inheritance means that both alleles have to have a disease-causing variant to cause disease. When an individual has only one recessive disease-causing variant, this is referred to as being a carrier. 'X-linked dominant' inheritance refers to when a disease-causing variant on one X chromosome causes disease. Persons with only one X chromosome will be affected by conditions with an X-linked recessive inheritance if their one X chromosome harbours a disease-causing variant. Others can only be carriers of X-linked recessive conditions.

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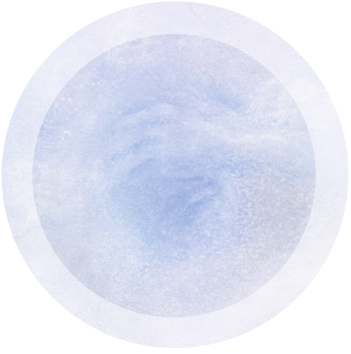
Part I

Seeing



Chapter 2

1 in 38 individuals at risk of a dominant medically actionable disease

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Eur J Hum Genet. 2019; doi: 10.1038/s41431-018-0284-2

Abstract

Clinical genomic sequencing can identify pathogenic variants unrelated to the initial clinical question, but of medical relevance to the patients and their families. With ongoing discussions on the utility of disclosing or searching for such variants, it is of crucial importance to obtain unbiased insight in the prevalence of these incidental or secondary findings, in order to better weigh potential risks and benefits. Previous studies have reported a broad range of secondary findings ranging from 1 to 9%, merely attributable to differences in study design, cohorts tested, sequence technology used and genes analyzed. Here, we analyzed WES data of 1640 anonymized healthy Dutch individuals to establish the frequency of medically actionable disease alleles in an outbred population of European descent. Our study shows that 1 in 38 healthy individuals (2.7%) has a (likely) pathogenic variant in one of 59 medically actionable dominant disease genes for which the American College of Medical Genetics and Genomics (ACMG) recommends disclosure. Additionally, we identified 36 individuals (2.2%) to be a carrier of a recessive pathogenic disease allele. Whereas these frequencies of secondary findings are in line with what has been reported in the East-Asian population, the pathogenic variants are differently distributed across the 59 ACMG genes. Our results contribute to the debate on genetic risk factor screening in healthy individuals and the discussion whether the potential benefits of this knowledge and related preventive options, outweigh the risk of the emotional impact of the test result and possible stigmatization.

Introduction

Clinical genomic sequencing can identify pathogenic variants unrelated to the initial clinical question, that are of medical relevance to the patient and their families(1). To promote standardized reporting of these incidental (unintentionally detected in analysis) and/or secondary findings (deliberate analysis of available data), the American College of Medical Genetics and Genomics (ACMG) published a list of 59 medically actionable genes recommended for return of such findings(2). The potential impact of reporting actionable variants in these genes would be significant and far-reaching as it presents opportunities to prevent disease.

There is an ongoing debate among medical genetic societies worldwide, and the general public, on whether, how, and when, incidental findings and/or secondary findings are to be disclosed or screened for(3). Simultaneously, discussions on obligatory genetic testing of employees and disclosure of the results to their employers are taking place. Some important arguments in favor of routine screening of genomic data are potential improvement of an individual's health, contribution to scientific progress and circumventing expensive treatments. Arguments against routine screening include possible harm to a person by complications of (unnecessary) medical interventions, stigmatization, and negative psychosocial impact(4). Yet, with the decreasing costs for genome sequencing and a growing commercial (direct-to-consumer) market, genetic testing of healthy individuals might eventually be inevitable.

It is of importance to obtain unbiased insight in the potential risks and benefits of opportunistic screening, and to develop adequate education for the general public. To foster such discussions, knowledge on the prevalence of secondary findings in medically actionable genes in the general population is required. Recently, multiple studies have reported frequencies of secondary findings ranging from 1 to 9% in various populations(5,6,7,8,9,10,11,12,13). This broad range of reported frequencies is largely explained by the cohorts tested (e.g., inclusion of individuals more prone to have a pathogenic variant) in combination with differences in sequence technology (e.g., whole-exome sequencing (WES) of inferior quality), classification of variants, and amount of genes for which pathogenic variants are taken into account. To the best of our knowledge, an unbiased prevalence of secondary findings in healthy individuals of European descent identifiable using clinical WES has not yet been described. Here, we analyzed clinical grade WES data of >1500 healthy individuals to establish the frequency of medically actionable disease alleles in the general Dutch population.

Material and methods

In our tertiary clinical genetic center in the Netherlands, 1640 healthy parents (50% males) received family based WES to allow for the interpretation of *de novo* mutations as cause of the intellectual disability observed in their child(14). The parents were predominantly of Caucasian origin and from an outbred, nonconsanguineous population(14). For the purpose of this exploratory and observational study, parental exome data were anonymized. None of these parents carried a known detrimental allele for intellectual disability.

WES was performed following our routine diagnostic procedures(15). In essence, DNA was outsourced to BGI (Copenhagen, Denmark) where exomes were captured using Agilent Sureselect v4 and sequenced to a median coverage of 75-fold on an Illumina HiSeq instrument with 101-bp paired-end reads. Sequence reads were aligned to the hg19 using BWA version 0.5.9-r16. Variants were called in-house using GATK unified genotyper (version 3.2–2) and annotated using custom diagnostic annotation pipeline, using Human Genome Variant Society nomenclature(16). Variant interpretation was limited to high quality variants (GATK quality score≥500) eliminating false-positive calls(17), and to those that occurred in the 59 medically actionable genes(2). Of note, 97.7% of the coding sequence for these genes was covered ≥20-fold. Variants in these genes were prefiltered for truncating, canonical splice sites, insertion deletion and/or missense variants based on frequency of occurrence in dbSNPv137 (<5%), ExACr0.2 (<1%) and our in-house database (<1%) containing exome data of 12,244 exomes. Remaining variants were classified according to the ACMG guidelines for diagnostic variant interpretation(18). Variants classified as pathogenic and likely pathogenic, referring to the potential of the variant to cause disease in a specific context, were considered medically actionable, and percentages referred to in our study are based on these classifications.

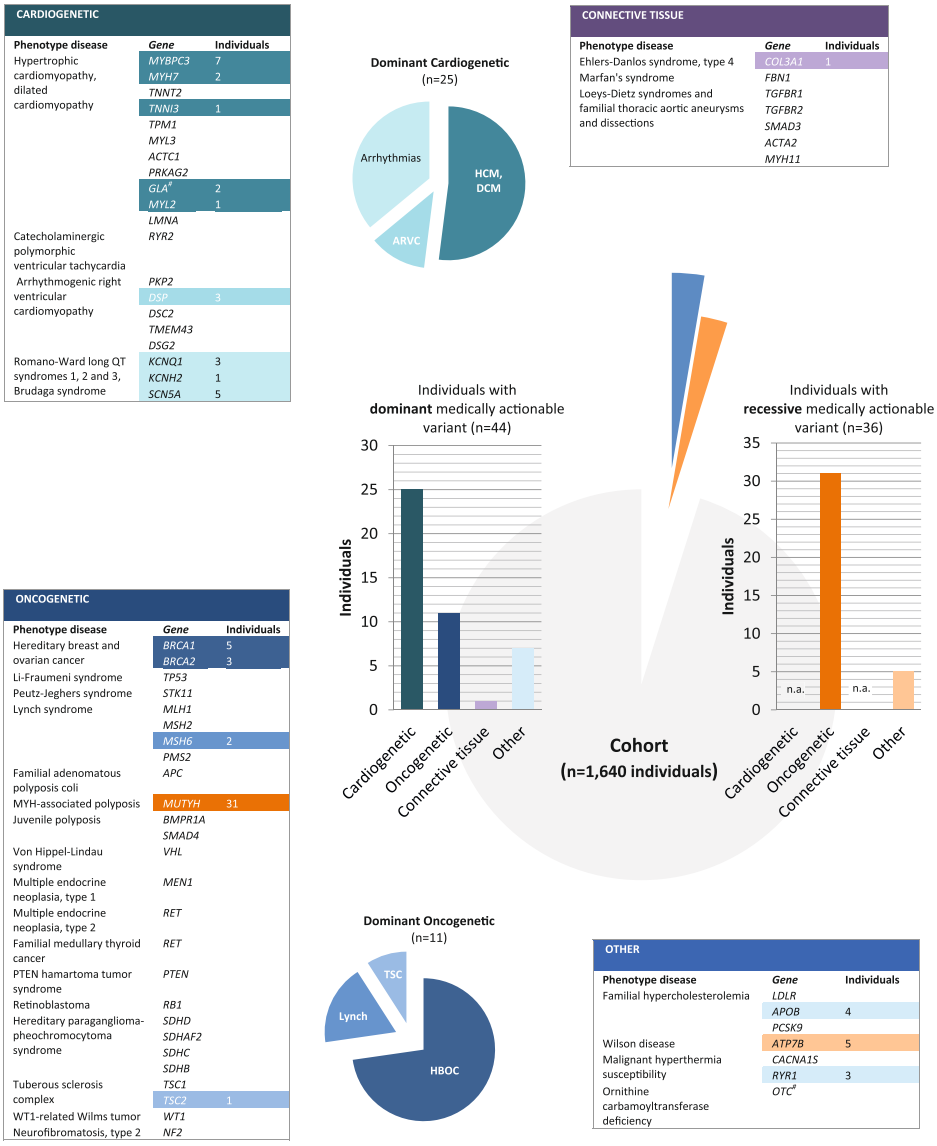

Figure 1. Schematic representation of actionable (likely) pathogenic variants identified in 1640 healthy individuals in the 59 ACMG genes

Data is visualized by type of disease (cardiogenetic, oncogenetic, connective tissue, and other). Mode of inheritance is represented in blue for dominant disease genes and orange for recessive disease genes. X-linked genes are indicated by #. All detected (likely) pathogenic variants and their classification according to HGVS recommendations(16) and ACMG-AMP guidelines(18), respectively, are provided in Supplementary Table 1. Abbreviations: HCM hypertrophic cardiomyopathy; DCM dilated cardiomyopathy; ARVC arrhythmogenic right ventricular cardiomyopathy; TSC tuberous sclerosis complex; HBOC hereditary breast and ovarian cancer; n.a. not applicable

Results

In a cohort of 1640 anonymized healthy individuals, we classified all variants in the 59 ACMG medically actionable genes, including 56 dominant and 3 recessive genes, using the standardized ACMG interpretation and classification variant guidelines(18).

In total, 44 individuals (2.7%) of our cohort had a dominant medically actionable variant, including 33 unique variants, which were detected in 18 out of the 56 dominant actionable genes. Six of 33 variants were detected in more than one individual. Disease alleles in genes for cardiac disease were most frequently observed (24 individuals, 1.5%), with variants in *MYBPC3* (NM_000256.3), responsible for hypertrophic cardiomyopathy, most often reported (seven individuals). Pathogenic variants in genes predisposing to hereditary cancer were detected in 11 individuals (0.7%), including five individuals with a pathogenic variant in *BRCA1* (NM_007300.3) and three others in *BRCA2* (NM_000059.3), both associated with hereditary breast and ovarian cancer. None of the individuals had more than one dominant high-risk disease allele.

In addition to dominant disease alleles, we also identified 36 individuals (2.2%) to be carriers of a high-risk disease allele in two of the three recessive actionable genes (Fig. 1; Supplementary Table S1). Pathogenic variants were observed in *MUTYH* (NM_001128425.1; 31 individuals) and *ATP7B* (NM_000053.2; five individuals), known to cause *MUTYH* Associated Polyposis and Wilson disease, respectively, when present in compound heterozygous or homozygous state. None of the 36 individuals carried homozygous or compound heterozygous recessive high-risk disease alleles. One carrier of a heterozygous recessive high-risk disease allele also had a dominant high-risk disease allele.

Discussion

On March 8 2017, the US House of Representatives approved a Bill that would allow companies to require employees to undergo genetic testing and disclose the results to their employers(19). As a response, the European Society of Human Genetics provided a statement that strongly argued against obligatory genetic testing as decisions on whether or not to undergo genetic testing must be a voluntary choice of the individual(20). For both obligatory and voluntary testing of healthy individuals, it is, however, important to know the prevalence of medically actionable disease alleles in an unbiased population. In this study, we set out to determine this frequency by screening for secondary findings in a healthy population of European descent using existing (anonymized) WES data. From our data we conclude that 2.7% of healthy Dutch individuals has a (likely) pathogenic variant in a medically actionable dominant disease allele

for which returning of secondary findings is indicated by the ACMG. These individuals are predisposed to develop for instance cancer or cardiomyopathy.

Given the wide range of reported secondary findings, we systematically compared our results to studies published previously from other populations(5,6,7,8,9,10,11,12,13) in order to explain the differences in frequencies observed, focussing on (i) the sequence technology used, (ii) the cohort tested, (iii) the certainty of pathogenicity of variants, as well as (iv) the genes for which pathogenic variants were assessed. This comparison yielded three distinct categories: those studies reporting lower(5,7,10,13), higher(6,8,9), or comparable(12) frequencies of secondary findings when compared to our observation of 2.7%.

In comparison to the first studies reporting incidental findings(5,7,10,11), the frequency identified in our cohort is elevated up to twice as high. This could partially be explained by the presence of Dutch founder mutations. From the 33 unique dominant risk alleles detected, two have been reported as founder mutation for the Dutch population (*BRCA1* (c.2685_2686del) and *BRCA2* (c.9672dup))(21). However, both variants were observed in a single individual in our cohort, thus not accounting for the higher frequency observed in our study. We then examined whether the differences are explained by experimental design and data analysis, previous versions of databases for variant filtering and interpretation, absence of ethnicity specific variation information and a shorter list of ACMG medically actionable genes for disclosing secondary findings. However, all variants identified in our study were also present in databases at time of the initial studies, and also, the extension of the ACMG genes with 3 additional genes to 59 as analyzed here, is not sufficient to explain our observed higher frequency. It thus is more likely that the increased sequence coverage in our study allowed more sensitive detection of (likely) pathogenic variants. It is, however, noteworthy that, Thompson et al.(13) also identified a relatively low frequency (1.5%) of secondary findings when based on (likely) pathogenic variants in the 59 ACMG genes, despite using WES at an average sequence depth of 71×, covering 80% of bases at least 20-fold(13,22). Whether this coverage is also achieved for the coding sequencing of the 59 genes is, however, not reported(13), but any deviation from this, could potentially explain the differences observed. Overall, since also other recent publications report higher frequencies than the 1–1.5% previously reported, it seems reasonable to conclude that the frequencies initially reported are too low.

Higher prevalence of secondary findings compared to our 2.7% for the Dutch population have also been reported(6,8,9). For instance, Dewey et al. (2016) reported 49 variants in the 59 ACMG genes in 1415 individuals (3.5%). Their cohort, however, consisted of patients of whom some were affected with conditions likely attributable to the disease alleles in the ACMG genes, thus creating bias towards higher frequency. When excluding this bias, their findings are more in line with our frequency. Interestingly, several papers report frequencies of over 5%(8,9). Since

these studies were conducted at the same time as ours, the difference cannot be explained by the previously mentioned issues like sequence coverage and availability of newer releases of databases for variant filtering. As was also noted by Tang et al.(12), the high frequency reported in these studies is mainly due to improper classification of variants as pathogenic. For instance, the NM_198056.2:c.3575G>A variant in *SCN5A* reported by Lawrence et al.(9) is present in 6% of the Asian population (including homozygotes) and truncating variants in the *RYR1* (NM_000540.2) gene are not causative for malignant hyperthermia susceptibility, as proposed by Jang et al.(8).

Importantly, the frequency of secondary findings observed in our cohort is almost identical to the prevalence of 2.5% published by Tang et al.(12), who tested a cohort of 954 East-Asian individuals using WGS. Interestingly, however, the distribution of pathogenic variants over the genes differs markedly despite the overall the frequency of secondary findings in the cardiogenetic genes and oncogenes being similar; that is, Tang et al.(12) reported 36% of their pathogenic variants in seven of the 59 ACMG genes in which no pathogenic variants were detected in our cohort. Conversely, in our Dutch cohort 48% of the detected pathogenic variants were in nine of the 59 ACMG genes in which no pathogenic variants were detected by Tang et al. Hence, this may indicate that, although the frequency of secondary findings is similar between different ethnicities, different genes contribute to their prevalence.

We also identified 2.2% of the population to be a carrier of a recessive pathogenic disease allele in one of the 59 ACMG genes. Whereas the identification of carriers of recessive disease alleles in the population is not unexpected given our study set up, our unbiased analysis of these alleles in the healthy population elicits discussion on their return. The ACMG recommends to only return bi-allelic pathogenic variants, but one may wonder whether it is not relevant to return carrier status (e.g., for reproductive decisions). Using the carrier frequency determined in our cohort, we can now determine that ~1 in 3000 and ~1 in 100,000 couples are both carrier of a heterozygous disease allele in *MUTYH* or *ATP7B*, respectively. For comparison, in March 2017 the Committee on Genetics of the American College of Obstetricians and Gynecologists stated that cystic fibrosis screening, with a carrier frequency of 1 in 30 in the Caucasian population, should be offered to all pregnant women, or ideally before pregnancy(23). Our data do not only contribute to the discussion on genes that could be selected for preconception carrier screening based on absolute carrier frequency, such as here presented for *MUTYH* (~1:50), but also reopens the discussion whether or not more prevalent diseases, such as cystic fibrosis (~1:30), should be included in secondary screening programs.

Routine screening of healthy individuals for secondary findings in the 59 ACMG medically actionable dominant disease genes will impact at least 1 in 38 individuals. These individuals have an increased risk for life-threatening disease, and could profit from early monitoring

and possible preventive treatment. On the other hand, some of the individuals in whom an incidental findings is identified may spend their lives worrying about a disease that may never manifest itself. This is mostly due to a highly variable disease penetrance for these conditions, ranging from 20 to 100%. Our study design did not allow to link the secondary findings to individuals and their families, but it can be expected that the penetrance is even lower in the absence of a positive family history. In terms of policy decisions about reporting and counseling of individuals in whom an incidental and/or secondary finding is observed, this may lead to a redefinition of what is perceived as a "medically actionable disease allele". Also, individuals at risk may face difficulties—or may even be unable to—acquire job positions, obtain mortgages, and/or health and/or life insurances. These implications not only affect the individuals in whom the incidental or secondary finding was uncovered, but will also directly impact their blood relatives and extended families. Apart from practical implications, such as the impact on the healthcare system to screen healthy individuals, it is presently unclear whether the potential benefits of early monitoring and possible preventive treatment outweigh the risks of the emotional impact of the test result and possible stigmatization.

Taken together, we believe that our conclusion that 2.7% of healthy Dutch individuals has a dominant acting disease allele, is expected to be representative for the European population given the current guidelines on variant interpretation and the limited number of genes studied. Yet, with genetic knowledge still advancing, the number of genes and medically actionable variants for which disclosure could be considered will likely continue to expand. In addition, improvements in sequencing technology will likely allow detection of more variants, and simultaneously, increasing clinical interpretation of the noncoding parts of the genome will allow for the detection of more pathogenic variation. Hence, it may be expected that our estimate that 1 in 38 healthy individuals is genetically affected with a dominant high-risk disease is an underrepresentation for the true prevalence of dominant medically actionable disease alleles in the population.

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Supplementary data

Supplementary Table 1: Actionable (likely) pathogenic variants identified in 1,640 healthy individuals in the 59 ACMG genes

							Pathogenic variants identified [a.]		ACMG-AMP classification [c.]	
Phenotype	**MIM Disorder**	**Typical age of onset**	**Gene**	**Inheritance**	**RefSeq ID**	**cDNA position**	**Protein change**	**Nr of alleles identified**	**Conclusion**	**Categories**
Hereditary breast and ovarian cancer	604370	Adult	*BRCA1*	AD	NM_007300.3	c.5566C>T	p.Arg1856*	3	Pathogenic	PVS1 PS4 PP1
						c.5329dup	p.Gln1777fs	1	Pathogenic	PVS1 PS4 PP1
						c.2685_2686del [b.]	p.Pro897fs	1	Pathogenic	PVS1 PS4 PP1
	612555		*BRCA2*	AD	NM_000059.3	c.5576_5579del	p.Ile1859fs	1	Pathogenic	PVS1 PS4 PP1
						c.7878G>C	p.Trp2626Cys	1	Pathogenic	PS3 PS4 PM1 PM5 PP1 PP3
						c.9672dup [b.]	p.Tyr3225fs	1	Pathogenic	PVS1 PS4 PP1
Li-Fraumeni syndrome	151623	Child/Adult	*TP53*	AD	NM_001126112.2					
Peutz-Jeghers syndrome	175200	Child/Adult	*STK11*	AD	NM_000455.4					
Lynch syndrome	120435	Adult	*MLH1*	AD	NM_001258271.1					
			MSH2	AD	NM_000251.2					
			MSH6	AD	NM_001281493.1	c.1444C>T	p.Arg482*	2	Likely pathogenic	PVS1 PM2
			PMS2	AD	NM_000535.6					
Familial adenomatous polyposis coli	175100	Child/ Adult	*APC*	AD	NM_001127511.2					
MYH--associated polyposis	608456	Adult	*MUTYH*	AR	NM_001128425.1	c.1227_1228dup	p.Glu410fs	1	Likely pathogenic	PVS1 PM2
Adenomas, multiple colorectal, *FAP* type 2	132600					c.1214C>T [b.]	p.Pro405Leu	2	Likely pathogenic	PS3 PM1 PM2 PP1

Supplementary Table 1: (continued)

Phenotype	MIM Disorder	Typical age of onset	Gene	Inheritance	RefSeq ID	cDNA position	Pathogenic variants identified [a.]		ACMG-AMP classification [e.]	
							Protein change	Nr of alleles identified	Conclusion	Categories
Colorectal adenomatous polyposis with pilomatricomas						c.1187G>A [c.]	p.Gly396Asp	13	Pathogenic	PS3 PS4 PM1 PP1 PP3
						c.1147del	p.Ala385fs	3	Pathogenic	PVS1 PS3 PS4 PP1
						c.1105G>T [b.]	p.Glu369* [d.]	1	Likely pathogenic	PVS1 PM2
						c.884C>T	p.Pro295Leu	1	Likely pathogenic	PS3 PM1 PM2 PP3
						c.545G>A	p.Arg182His	1	Likely pathogenic	PS3 PM2 PM5
						c.536A>G [c.]	p.Tyr179Cys	8	Pathogenic	PS3 PS4 PP1
						c.325C>T	p.Arg109Trp	1	Likely pathogenic	PS3 PM2 PP3
Juvenile polyposis	174900	Child/Adult	*BMPR1A*	AD	NM_004329.2					
			SMAD4	AD	NM_005359.5					
Von Hippel-Lindau syndrome	193300	Child/Adult	*VHL*	AD	NM_198156.2					
Multiple endocrine neoplasia, type 1	131100	Child/Adult	*MEN1*	AD	NM_130801.2					
Multiple endocrine neoplasia, type 2	171400	Child/Adult	*RET*	AD	NM_020975.4					
	162300									
Familial medullary thyroid cancer	1552401	Child/Adult	*RET*	AD	NM_020975.4					
PTEN hamartoma tumor syndrome	153480	Child/Adult	*PTEN*	AD	NM_000314.6					
Retinoblastoma	180200	Child	*RB1*	AD	NM_000321.2					
Hereditary paraganglioma-pheochromocytoma syndrome	168000	Child/Adult	*SDHD*	AD	NM_003002.3					
	601650		*SDHAF2*	AD	NM_017841.2					
	605373		*SDHC*	AD	NM_003001.3					
	115310		*SDHB*	AD	NM_003000.2					
Tuberous sclerosis complex	191100	Child	*TSC1*	AD	NM_000368.4					
	613254		*TSC2*	AD	NM_000548.4	c.2903dup	p.Ser969fs [d.]	1	Likely pathogenic	PVS1 PM2

Supplementary Table 1: (continued)

Phenotype	MIM Disorder	Typical age of onset	Gene	Inheritance	RefSeq ID	cDNA position	Pathogenic variants identified [a] Protein change	Nr of alleles identified	ACMG-AMP classification [e] Conclusion	Categories
WT1-related Wilms tumor	194070	Child	*WT1*	AD	NM_001198551.1					
Neurofibromatosis, type 2	101000	Child/Adult	*NF2*	AD	NM_000268.3					
Ehlers-Danlos syndrome, type 4	130050	Child/Adult	*COL3A1*	AD	NM_000090.3	c.1815+2T>C	r.spl? [d]	1	Likely pathogenic	PVS1 PM2
Marfan's syndrome	154700	Child/Adult	*FBN1*	AD	NM_000138.4					
Loeys-Dietz syndromes	609192		*TGFBR1*	AD	NM_001130916.2					
and familial thoracic aortic aneurysms and dissections	608967		*TGFBR2*	AD	NM_003242.5					
	610168		*SMAD3*	AD	NM_001145103.1					
	610380		*ACTA2*	AD	NM_001613.2					
	613795		*MYH11*	AD	NM_001040114.1					
	611788									
Hypertrophic cardiomyopathy,	115197	Child/Adult	*MYBPC3*	AD	NM_000256.3	c.1831G>A	p.Glu611Lys	3	Likely pathogenic	PM1 PM2 PP2 PP3 PP5
dilated cardiomyopathy						c.1468G>A	p.Gly490Arg	3	Likely pathogenic	PM1 PM2 PP2 PP3 PP5
						c.442G>A	p.Gly148Arg	1	Likely pathogenic	PM1 PM2 PP2 PP5
	192600		*MYH7*	AD	NM_000257.3	c.2644C>T	p.Gln882* [d]	1	Likely pathogenic	PVS1 PM2
						c.2389G>A	p.Ala797Thr	1	Pathogenic	PS3 PS4 PM5 PP1 PP2

Supplementary Table 1: (continued)

							Pathogenic variants identified[a.]		ACMG-AMP classification[b.]				
Phenotype						**cDNA position**	**Protein change**	**Nr of alleles identified**	**Conclusion**	**Categories**			
Hypertrophic cardiomyopathy,	601494	Child/Adult	*TNNT2*	AD	NM_001276346.1								
dilated cardiomyopathy	613690		*TNNI3*	AD	NM_000363.4	c.354del	p.Thr119fs [d.]	1	Likely pathogenic	PVS1	PM2		
	115196		*TPM1*	AD	NM_001018008.1								
	608751		*MYL3*	AD	NM_000258.2								
	612098		*ACTC1*	AD	NM_005159.4								
	600858		*PRKAG2*	AD	NM_016203.3								
	301500		*GLA*	XLD	NM_000169.2	c.427G>A	p.Ala143Thr	2	Likely Pathogenic	PS3	PM2	PM5	PP5
	608758		*MYL2*	AD	NM_000432.3	c.403-1G>C	r.spl?	1	Pathogenic	PVS1	PS3	PM2	
	115200		*LMNA*	AD	NM_170707.3								
Catecholaminergic polymorphic ventricular tachycardia	604772	Child/Adult	*RYR2*	AD	NM_001035.2								
Arrhythmogenic right ventricular cardiomyopathy	609040	Child/Adult	*PKP2*	AD	NM_004572.3								
	607450		*DSP*	AD	NM_001008844.2	c.85G>T	p.Glu29* [d.]	1	Likely pathogenic	PVS1	PM2		
						c.4518del	p.Arg1506fs [d.]	1	Likely pathogenic	PVS1	PM2		
						c.6336del	p.Asn2114fs [d.]	1	Likely pathogenic	PVS1	PM2		
	610476		*DSC2*	AD	NM_024422.4								
	604400		*TMEM43*	AD	NM_024334.2								
	610193		*DSG2*	AD	NM_001943.4								
Romano-Ward long QT syndromes 1, 2 and 3	192500	Child/Adult	*KCNQ1*	AD	NM_000218.2	c.961C>T	p.Gln321* [d.]	1	Likely pathogenic	PVS1	PM2		
Brudaga syndrome						c.1066C>T	p.Gln356*	1	Likely pathogenic	PVS1	PM2		
						c.1124_1127del	p.Ile375fs	1	Likely pathogenic	PVS1	PM2		
	613688		*KCNH2*	AD	NM_000238.3	c.2254C>T	p.Arg752Trp	1	Likely pathogenic	PS3	PM1	PM2	PP2
	601144		*SCN5A*	AD	NM_198056.2	c.4999G>A	p.Val1667Ile	1	Likely pathogenic	PM1	PM2	PP1	PP2	PP3
	603830					c.4978A>G	p.Ile1660Val	1	Pathogenic	PS3	PM1	PM2	PP1	PP2
						c.3956G>T	p.Gly1319Val	1	Pathogenic	PS3	PM1	PM2	PP1	PP2
						c.3911C>T	p.Thr1304Met	1	Pathogenic	PS3	PM1	PM2	PP1	PP2
						c.80G>A	p.Arg27His	1	Pathogenic	PS3	PM1	PM2	PP1	PP2

Supplementary Table 1: (continued)

Phenotype						cDNA position	Pathogenic variants identified[a.] Protein change	Nr of alleles identified	ACMG-AMP classification[e.] Conclusion	Categories				
Familial hypercholesterolemia	143890	Child/ Adult	*LDLR*	AD	NM_000527.4									
			APOB	AD	NM_000384.2	c.10580G>A [c.]	p.Arg3527Gln	3	Pathogenic	PS3	PS4	PM5	PP1	PP2
						c.10579C>T	p.Arg3527Trp	1	Likely pathogenic	PS3	PM2	PM5	PP2	
	603776		*PCSK9*	AD	NM_174936.3									
Wilson disease	277900	Child	*ATP7B*	AR	NM_000053.2	c.3955C>T	p.Arg1319*	1	Likely pathogenic	PVS1	PM2			
						c.3008C>T	p.Ala1003Val	1	Likely pathogenic	PM1	PM2	PM3		
						c.2304dup	p.Met769fs	1	Likely pathogenic	PVS1	PM2			
						c.1708-1G>C	r.spl?	1	Likely pathogenic	PVS1	PM2			
						c.19_20del	p.Gln7fs	1	Likely pathogenic	PVS1	PM2			
Malignant hyperthermia susceptibility	145600	Child/ Adult	*CACNA1S*	AD	NM_000069.2									
			RYR1	AD	NM_000540.2	c.1589G>A	p.Arg530His	2	Likely pathogenic	PS3	PM1	PM2	PP2	
						c.14545G>A	p.Val4849Ile	1	Likely pathogenic	PS3	PM1	PM2	PP1	PP2
Ornithine carbamoyltransferase deficiency	311250		*OTC*	XLR	NM_000531.5									

[a.] Mutation nomenclature is provided according to HGVS recommendations (Den Dunnen et al. *Hum Mutat.* 2016, 37: 564-569).
[b.] Dutch founder mutation.
[c.] European founder mutation.
[d.] Variant was not previously described in literature. It is however a loss-of-function variant in a gene exerting its pathogenic effect by haploinsufficiency.
[e.] Variant pathogenicity is evaluated according to ACMG-AMP guidelines (Richards et al. *Genet Med.* 2015, 17:405-424).

Chapter 3

Lessons learned from unsolicited findings in clinical exome sequencing of 16,482 individuals

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Eur J Hum Genet. 2022; doi: 10.1038/s41431-021-00964-0

Abstract

Unsolicited findings (UFs) are uncovered unintentionally and predispose to a disease unrelated to the clinical question. The frequency and nature of UFs uncovered in clinical practice remain largely unexplored. We here evaluated UFs identified during a 5-year period in which 16,482 index patients received clinical whole-exome sequencing (WES). UFs were identified in 0.58% (95/16,482) of index patients, indicating that the overall frequency of UFs in clinical WES is low. Fewer UFs were identified using restricted disease-gene panels (0.03%) than when using whole-exome/Mendeliome analysis (1.03%). The UF was disclosed to 86 of 95 individuals, for reasons of medical actionability. Only 61% of these UFs reside in a gene that is listed on the "ACMG59"-list, representing a list of 59 genes for which the American College of Medical Genetics recommends UF disclosure. The remaining 39% were grouped into four categories: disorders similar to "ACMG59"-listed disorders (25%); disorders for which disease manifestation could be influenced (7%); UFs providing reproductive options (2%); and UFs with pharmacogenetic implications (5%). Hence, our experience shows that UFs predisposing to medically actionable disorders affect a broader range of genes than listed on the "ACMG59", advocating that a pre-defined gene list is too restrictive, and that UFs may require ad hoc evaluation of medical actionability. While both the identification and disclosure of UFs depend on local policy, our lessons learned provide general essential insight into the nature and odds of UFs in clinical exome sequencing.

Introduction

Unsolicited findings (UFs) in clinical genetics are defined as (likely) pathogenic variants not related to the initial clinical question the DNA test was performed for, but that may nonetheless be of medical relevance to the health of the patient and/or his/her family(1)(Box 1).

Box 1. Unsolicited findings

A medical genetic test is aimed to identify (or exclude) genetic disease underlying a persons' health condition. With today's DNA sequencing techniques, an individual's entire exome or genome can be determined in a single experiment. To identify disease-causing variants, the data are compared to data of healthy controls. These techniques allow the detection of variants that are irrelevant to the clinical question but which predispose to another disease. Such unsolicited findings (UFs) may be of medical value for the patient and family. In this latter context, genetic variants imposing a health risk for blood relatives, such as carrier status of autosomal recessive or X-linked conditions, are considered UFs as well.

UFs are variants that are "unsought for", and have variously been described as "accidental findings", "co-incidental findings" or "incidental findings". They differ from "secondary findings" (SFs), which also represent variants not related to the initial clinical question but that are *actively* looked for(2).

Previously, targeted sequence analysis of single genes was performed which made the detection of UFs unlikely. With the implementation of whole exome sequencing (WES) as a first-tier test, analysis is extended to all protein coding genes(3,4), and consequently, the probability of detecting UFs has increased. This has fostered a worldwide debate on the disclosure of UFs – and SFs – on which consensus has yet to be reached(5,6).

The American College of Medical Genetics (ACMG) tightened the recommendations for SFs and created a list of 59 so called "medically actionable disease genes" ("ACMG59")(2).

These genes were selected among the most prevalent monogenic disorders, for which individuals with pathogenic variants remain asymptomatic for a long time, and preventive measures and/or treatment are available(2). The "ACMG59" list has been widely used, and adopted by others(6,7,8,9,10,11,12,13,14,15,16), within total SFs having been reported in over 100 genes(8, 16) (Box 2).

Box 2. What is medically actionable?

Disclosure of UFs and/or SFs depends on whether an individual receiving the information can medically intervene in the process related to the disorder to which the variant predisposes. The term medical actionability has been criticized for its inexactness(33), leading to multiple interpretations and misinterpretations of health-care-related expectations.

Berg et al.(1) were amongst the first to publish recommendations for the disclosure of both UFs and SFs. They recommended disclosure for variants deemed "medically actionable", referring to variants carrying a high likelihood of disease (e.g., monogenic, high penetrant disease), and for which medical interventions could significantly reduce morbidity and mortality(34). Morbidity is defined as "the state of being symptomatic or unhealthy for a disease or condition" and mortality refers to "the number of deaths caused by the health event under investigation"(35). Berg's definition has been adopted by the ACMG and others (e.g., Amendola et al.(6) and Dorschner et al.(7)) for the disclosure of SFs. In contrast, less strict definitions include for example the definition used by Yang et al.(8), which states that variants are considered medically actionable when there are potential therapies or established surveillance protocols available.

In contrast to SFs, the recommendations for disclosing UFs have not been updated since 2011(1). We hypothesize that differences exist in the prevalence and nature of UFs compared to SFs, but to date this has not been systematically assessed. We present a thorough and systematic analysis of UFs identified during clinical WES of 16,482 index patients and compare the results to SFs from the same clinical population to help establish guidelines for decision making for the disclosure of UFs.

Material and methods

Patient, counseling, and informed consent

Between 01 June 2013 and 31 May 2018, 16,482 consecutive index patients received clinical whole-exome sequencing (WES) in the ISO15189 accredited Genome Diagnostic Laboratory of the Radboud university medical center in Nijmegen, the Netherlands. As part of the counseling and consent procedure prior to performing WES, clinicians informed the patients regarding our two-tiered approach for data interpretation, starting with an analysis of in silico disease gene panels, followed by analysis of the entire exome (see "Two-tiered diagnostic exome sequencing procedure"). Especially the second tier is anticipated to involve the possibility of uncovering an UF. As part of the post-test counseling, patients without a conclusive diagnosis are advised to recontact their referring clinician in due time for re-analysis of the existing exome data as the in silico disease gene panels are revised regularly.

Two-tiered diagnostic exome sequencing procedure

WES was performed following our routine diagnostic procedures(17) either on the index patient only, or in a family-based trio strategy (index patient + biological parents). From 2015 onwards, also copy number variants (CNV) were routinely identified from WES data and used for diagnostic interpretation(18).

Analysis of WES data were performed as a two-step process, guided by the consent provided by the patient or guardian. The first step, referred to as tier 1, included the analysis of variants restricted to genes known to be associated with the index's condition by means of an in silico gene panel enrichment (Supplementary Methods). If the patient's symptoms did not allow for selection of (a) disease-specific gene panel(s), the clinician could also request analysis of the Mendeliome, consisting of all 3606 genes with an OMIM-listed disease-gene association. In case no molecular diagnosis was obtained in tier 1, and the patient consented for further analysis, the analysis was followed by tier 2, allowing for prioritization, interpretation and classification of variants in the Mendeliome (if not already performed in tier 1) and those in genes without known disease-gene associations ("open exome analysis").

Variant prioritization, interpretation and classification

DNA from the index patient and parents was often sequenced simultaneously to facilitate detection and interpretation of de novo variants in autosomal dominant disease genes (Supplementary Methods). Filtering steps and prioritization of variants in the gene panel analysis (tier 1) was performed as described(17). In tier 2, rare truncating variants and/or known pathogenic variants were assessed. For trios, assessment also included "de novo" and "compound heterozygous" variants (Supplementary Methods). Trio-based analysis allows to determine the inheritance of all variants identified in the index by comparison the variants in the parental samples. It can show that both parents are carrier of the same pathogenic variant that is detected in heterozygous state in the affected child (also a carrier). It does not, however, detect carrier couples which carry different variants in the same recessive risk allele if the child is not compound heterozygous for these variants. A compound heterozygous state will only be uncovered as UF when the child does not present with a matching phenotype. This information is, however, of relevance in the context of unsolicited findings (UF) evaluation, as the couple has a 25% chance of affected offspring in future pregnancies (see "UF disclosure policy" in the Supplementary Methods).

For diagnostic SNV interpretation and classification, we used the guidelines established by the Association for Clinical Genetic Science (ACGS) and the Dutch Society of Clinical Genetic Laboratory Specialists (VKGL). Their 4-class system (UV1–UV4)(19) was used in 2013–2014, and from 2015 onwards, this was exchanged by a 5-class system (Class 1 to Class 5)(20). CNVs

were classified according to the European guidelines for constitutional cytogenomic analysis (Class 1 to Class 5)(21).

UF evaluation and disclosure policy

During analysis, clinical laboratory geneticists may encounter (likely) pathogenic variants (e.g. UV3/4 or class 4/5), detected in either tier 1 or tier 2, in genes not associated with the disease for which the index was referred. After confirmation of pathogenicity of the variant by a second clinical laboratory geneticist, the variant is subsequently evaluated by an inhouse panel of experts, consisting of a clinical laboratory geneticist, a clinical geneticist, a molecular geneticist, an ethicist, a legal representative and a social worker. The panel assesses whether it is indeed an UF and advises the referring clinician in the disclosure of the UF. Hereto the panel weighs factors such as disease penetrance, severity of disease, the age of onset, the age of the patient, the presumed psycho-social impact, the physical impact of screening program(s) and the time needed to diagnose the genetic disorder without prior knowledge of the UF. The local disclosure policy is provided in the Supplementary Methods.

Of note, this study reflects the first five years of clinical WES at our institute. The default consent option was that medically actionable UFs would be disclosed and non-medically actionable UFs would not be disclosed. Medical actionability was interpreted as 'the potential to change the course of' or 'prevention of' disease by medical interventions in adults or children', or 'when knowledge of the presence of the pathogenic variants allows for early interventions, before or after the first mild symptoms appear', or 'prevention of a diagnostic odyssey'. Carrier status of a recessive disease was also disclosed, provided that the risk to future offspring was at least 25%, as this would allow for reproductive choices.

Defining UFs eligible for analysis in this study

This study aims to provide the incidence of UFs, observed in index patients receiving clinical WES between 2013 and 2018. To overcome interpretation biases introduced over time due to changes in classification, we have systematically reclassified all UFs in June 2020 using ACMG criteria using information known to date(22). UFs in eight individuals (5 variants) were excluded because of reclassification from (likely) pathogenic variants (Class 4/5) to a variant of unknown significance (VUS, class 3) and, in two individuals the UF (2 CNVs) was only observed in a parent of the index but not the index him/herself.

Homozygous or compound heterozygous variants in a gene causing a recessive disease were considered a single UF.

Results

Odds of UF discovery in diagnostic WES cohort

Between 2013 and 2018, a total of 16,482 index patients received WES in our diagnostic laboratory. In total, 97 UFs were identified in 95 patients (two patients had two UFs; Supplementary Table 1). Hence, the odds of detecting an UF in our diagnostic cohort is one in 174 (0.58%; 95/16,482 patients; Fig. 1).

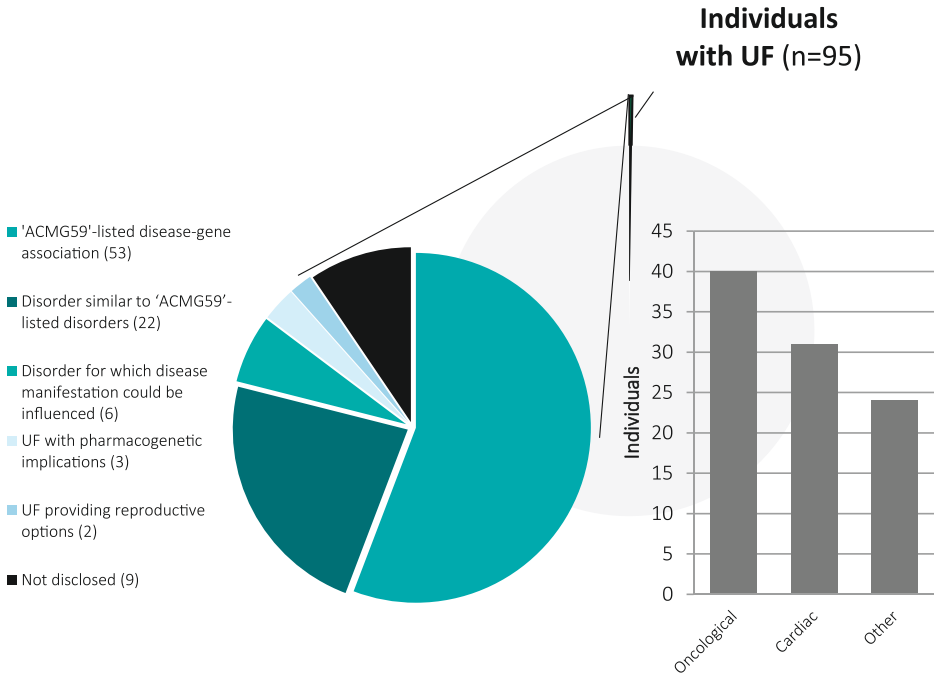

Figure 1. Incidence of UFs in our cohort of 16,482 individuals after clinical exome sequencing and their reasons for disclosure

In 16,482 individuals, UFs were identified in 95 individuals (0.58%). For each gene in which an UF was identified, the medical actionability was evaluated, resulting in six categories (depicted in pie chart on the left). In addition, the disease category to which the UF predisposed was evaluated (depicted in bar chart on the right).

In accordance with our local disclosure policy (Supplementary Methods), the UF was not disclosed to nine of 95 patients because no (national guidelines for) medical intervention would have been applicable (Supplementary Fig. 1). The UF was disclosed to the remaining 86 patients based on the availability of medical interventions. The overall risk of medically actionable UFs in our diagnostic WES cohort is 0.52% (86 of 16,482 patients). For non-medically UFs, it is 0.05% (nine of 16,482 patients). The UFs in 95 individuals were uncovered via various analysis strategies, each resulting in a different odds of UFs (Fig. 2).

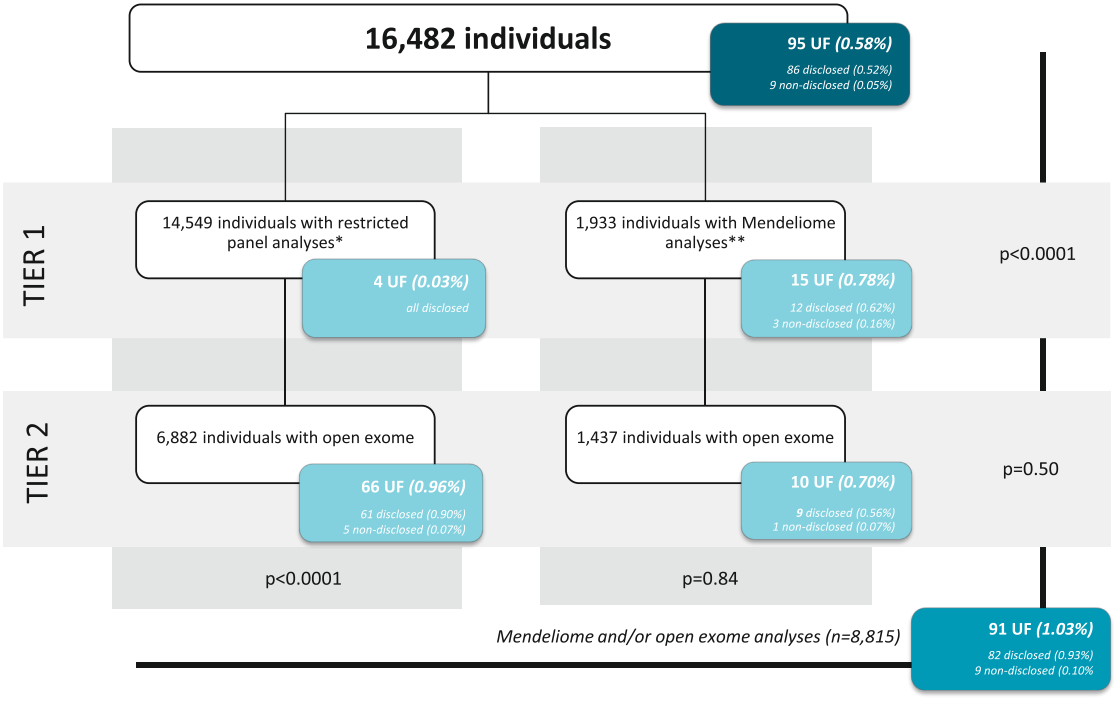

Figure 2. Analysis strategies leading to the disclosure of UFs in 86 of 16,482 individuals after clinical exome sequencing

The odds of UFs after analysis of disease-specific panels were 0.03% (4 of 14,549 individuals), and significantly lower than the odds of UFs in Mendeliome (0.78%; 15 of 1933 individuals; Fisher's exact, p<0.0001). The odds of UFs in an "open exome" strategy performed after either a targeted disease-gene panel analysis (0.96%), or after the Mendeliome (0.70%), are statistically the same (Fisher's exact, p = 0.45). Similarly, the odds of UFs detected in tier 1 Mendeliome analysis (0.78%) did not significantly differ from the incidence detected in an open exome analysis, regardless whether the tier 1 analysis was a disease-specific panel (0.96% UFs in open exome, Fisher's exact p = 0.50) or a Mendeliome analysis (0.70% UFs in open exome, Fisher's exact p = 0.84).

**A panel analysis may consist of the simultaneous interpretation of multiple disease panels, but never includes analysis of the entire Mendeliome; **This analysis consists of at least the Mendeliome, but might include one or more restrictive gene panels.*

For disease-specific panels, this was 0.03% (4 of 14,549 individuals), and for Mendeliome analysis 0.78% (15 of 1933 individuals). The odds of UFs in an "open exome" strategy performed after targeted disease-gene panel analysis were 0.96% (66 of 6882 individuals), and 0.70% after the Mendeliome (10 of 1437 individuals). These results confirm that the probability of uncovering an UF significantly increased when analyzing all genes with proven disease-gene associations (UFs in the Mendeliome and open exome; 91 of 8815 individuals; 1.03%) in comparison to a dedicated disease-gene panel strategy (0.03%; Fishers Exact test $p<0.0001$).

Reasons for disclosure of UFs

In 84 of 86 individuals, the UF was disclosed because of a health risk for the index or family, and in two individuals the UF was relevant for reproductive choices of either the index or relatives (Supplementary Fig. 1). Forty-one of the 84 individuals were aged 12 years and over, and the disease the UF predisposed to would be expected to manifest in adolescence or adulthood. In the 43 minors (<12 years of age), 25 UFs were disclosed because the disease has been reported to have a (possible) manifestation in childhood. The other 18 minors were at risk of a disease with adult onset, and the risk was disclosed because of immediate relevance for family members (Supplementary Table 1).

Comparison to "ACMG59"

The 88 UFs, disclosed to 86 individuals, affected 40 different genes, predominantly predisposing to oncological disease (43%) or cardiac disorders (36%) (Table 1, Supplementary Table 1). Only 20 of these 40 genes (50%) are listed on "ACMG59"(2). These 20 genes harbor 54 UFs (61%) in 53 individuals. In all but one individual, the UF was identified in the Mendeliome or open exome analysis. The odds of an UF in an "ACMG59"-listed gene was thus 0.59% (52/8815 individuals or one in 170 individuals). The 20 non-"ACMG59"-listed genes harbored 34 UFs in 33 patients. These include 11 genes (22 UFs) associated with diseases that are clinically similar to "ACMG59"-listed conditions, such as predisposition to cancer or cardiac disease. Another group consisted of six genes (one UF each) responsible for diseases for which significant treatment options are available to impact disease manifestation by reducing morbidity. Variants in one gene (four UFs) were disclosed because of the risk of serious adverse drug reactions. UFs in two genes (one UF each) were disclosed because of reproductive choices.

Table 1. Levels of medical actionability for the 97 UFs detected in 95 individuals

PHENOTYPE	'ACMG59' - listed disease-gene association	Nr.	'ACMG59'-listed disorder	Nr.	Disorder for which disease manifestation can be influenced	Nr.	Pharmaco-genetic implications	Nr.	Reproductive options	Nr.	Not disclosed	Nr.
ONCOLOGICAL												
Hereditary breast and/or ovarian cancer	*BRCA1*	13	*ATM*	1							*CHEK2*	2
	BRCA2	4	*BRIP1*	5								
Lynch syndrome	*MSH6*	2										
	PMS2	3										
Familial adenomatous polyposis coli	*APC*	1										
Multiple endocrine neoplasia, type 2	*RET*	2										
Hereditary paraganglioma-pheochromocytoma syndrome	*SDHD*	1	*SDHA*	3								
Melanoma and neural system tumor syndrome			*CDKN2A*	1								
Leiomyomatosis and renal cancer			*FH*	1								
Basal cell nevus syndrome			*SUFU*	1								
CARDIAC												
Hypertrophic cardiomyopathy, dilated cardiomyopathy	*MYBPC3*	4	*TTN*	2								
	MYH7	1	*CSRP3*	1								
	TNNT2	1	*FLNC*	1								
	GLA	1										
Catecholaminergic polymorphic ventricular tachycardia	*RYR2*	1										
Arrhythmogenic right ventricular cardiomyopathy	*PKP2*	4										
	DSP	1										
Romano-Ward long QT syndromes 1, 2 and 3	*KCNQ1*	2*										
Brugada syndrome	*KCNH2*	1										
Atrial fibrillation	*SCN5A*	6*	*GJA5*	5								
Pulmonal arterial hypertension			*BMPR2*	1								
OTHER												
Familial hypercholesterolemia	*LDLR*	4										
Marfan syndrome	*FBN1*	1										
Malignant hyperthermia susceptibility	*RYR1*	1										
Hailey-Hailey disease					*ATP2C1*	1						

Table 1. *(continued)*

PHENOTYPE	'ACMG59' - listed disease-gene association	Nr.	'ACMG59'-listed disorder	Nr.	Disorder for which disease manifestation can be influenced	Nr.	Pharmaco-genetic implications	Nr.	Reproductive options	Nr.	Not disclosed	Nr.
Complement component 8 deficiency type II					*C8B*	1						
Macular corneal dystrophy					*CHST6*	1						
Von Willebrand disease, type 1					*VWF*	1						
Autosomal Recessive Deafness 1A					*GJB2*	1						
Autosomal Recessive Deafness 49					*MARVELD2*	1						
5-fluorouracil toxicity							*DPYD*	4**				
Oculocutaneous Albinism									*TYR*	1		
X-linked recessive chondrodysplasia punctata									*ARSL*	1		
Cerebral cavernous malformations											*CCM2*	1
Hypobetalipoproteinemia											*APOB*	1
Frontotemporal dementia, aphasia											*GRN*	2
Muscle glycogenesis											*PHKA1*	1
Autosomal dominant spastic paraplegia Type 12											*RTN2*	1
Legius syndrome											*SPRED1*	1

Details per individual are provided in Supplementary Table 1. 'ACMG59'-listed disease-gene association: Gene present on the 'ACMG59'-list, and UFs predispose to the disease listed; 'ACMG59'-listed disorder: gene predisposes to a disorder similar to 'ACMG59'-listed disorders; Disorder for which disease manifestation can be influenced : gene predisposes to a disorder for which disease manifestations can be mitigated; Pharmacogenetic implications: UFs with pharmacogenetic implications; Reproductive options: UFs with a risk of at least 25% of affected offspring. Not disclosed: genes with UFs considered to be not medically actionable.

**One individual had one variant in the KCNQ1 gene and one variant in the SCN5A gene. **One individual had two variants in the DPYD gene.*

Discussion

In total, we identified UFs in 95 out of 16,842 individuals who received WES, and disclosed UFs in 86 individuals, since we considered them medically actionable. The UFs were uncovered via various analysis strategies, each with a different probability of identifying UFs. From our observations, we learned multiple lessons that provide insights into the nature and odds of UFs in clinical exome sequencing.

Lesson 1: The incidence of UFs disclosed after clinical exome sequencing is low and depends on variant prioritization and interpretation strategies

Only in four patients, the UF was detected during the analysis of a restricted gene panel, indicating that the likelihood of UF detection in this diagnostic strategy is low (0.03% or 1 in 3637 individuals). In one of the cases, a collodion baby, the UF (in *GJB2*) was uncovered in the gene panel for skin disorders. *GJB2* was included in this panel because dominant negative variants are associated with keratitis-ichthyosis-deafness syndrome. The compound heterozygous loss-of-function variants that were identified in the neonate are associated with a mild form of autosomal recessive deafness type 1. This exemplifies that gene panels may lead to the identification of UFs predisposing to disorders outside the expertise of the WES requesting physician. In the three other cases, the UF predisposed to a different disease within the disease spectrum analyzed. In an 18-year-old man, the UF in *GLA* predisposed to a later onset disease. In two other index patients, phasing of variants revealed at least 25% risk for their parents of having affected offspring: a heterozygous *ARSL* (X-linked) variant in a female index patient was maternally inherited, and a heterozygous *TYR* variant identified in a 5-year-old girl, was also present in both her parents. These examples highlight the importance of awareness of the gene panel contents to enable adequate counseling of the probability of UFs.

The probability of uncovering an UF in the Mendeliome and/or open exome was significantly higher (1.03% or one in 97 individuals) than in a disease-gene panel, suggesting that the risk of uncovering an UF is related to the number of known disease genes analyzed, as has been postulated before(23). With these odds, one may question whether the probability of detecting UFs exceeds the chance of finding the genetic cause of disease after a negative restricted gene panel analysis. The answer to this question cannot be given unequivocally as this is largely determined by the extent to which the clinical heterogeneity of the primary condition is already captured with laboratory-specific disease gene panels, and will vary between diseases and clinical laboratories.

Lesson 2: UFs can occur during re-analysis of existing data

Patients without a genetic diagnosis are often advised to recontact the clinician for reanalysis of their existing exome data because of increasing knowledge on genes and variants involved in disease, the implementation of new bioinformatic pipelines, and novel sequence technologies. Together this may allow detection of the disease-causing variant (several) years after the initial analysis. The same is true for uncovering UFs: six UFs were identified and disclosed after a request for clinical reanalysis of such existing exome data, performed two to five years after the initial WES analysis. These findings underscore the importance for clinicians to address the possibility of identification and disclosure of so far unidentified UFs, before requesting re-analysis. Moreover, it confirms the notion that not all medically actionable disease-gene variants will be seen upon testing if not actively looked for. Hence, when no UFs are disclosed after clinical exome sequencing, patients should not falsely deduce absence of genetic predisposition for medically actionable diseases.

Lesson 3: The odds of UFs in "ACMG59" are substantially lower than for SFs

The odds of UF discovery depend on variant prioritization and interpretation strategies used in the clinical laboratory. Similarly, the incidence of SFs reported varies because of differences in inclusion criteria, ethnicity, sequencing techniques, and variant interpretation criteria(6,7,8,9,10,11,12,13,14,15,16), which limits the comparison of results between studies. To take away these biases, we compared the data on UFs from this study to our published data on SFs from the same population, for which we reported an incidence of 1:38 individuals (2.7%) for medically actionable dominant diseases listed on the "ACMG59" gene list(2,24).

In 54 individuals UFs in ACMG-listed genes were identified (Supplementary Table 1). One was not disclosed because the variant did not predispose to the ACMG-listed disease (APOB) and another UF was identified through panel-based analysis. We thus identified 52 UFs in 8815 individuals (0.59%) receiving Mendeliome/open exome analysis in genes listed on the "ACMG59". This results in an odds of 1:170, which is fourfold lower than the incidence observed for SFs (1:38)(24). This difference reflects our variant prioritization strategies since variants need to be clearly recognizable as pathogenic in order to be noticed. Truncating, or other loss-of-function variants, are more likely to be noticed than missense variants, because of their more obvious impact on protein function. Hence, variants for diseases caused by haploinsufficiency will be more easily recognized, even if the exact variant has never been reported before. For (rare) missense variants, pathogenicity is less obvious, requiring more extensive analyses. Indeed, we observed more loss-of-function UFs in "ACMG59"-listed genes (57%), than we did for SFs in the same genes (35%)(24). Also, trio-based filter strategies are biased away from inherited autosomal dominant disease-causing variants (i.e., the vast majority of "ACMG59"-listed disorders), as diagnostic prioritization is focused towards de novo and recessive variants.

The four-fold difference between the odds of UFs (1:170) and incidence of SFs (1:38) that we observed, can however not be generalized to other clinical laboratory programs, as it depends on multiple factors, including local variant prioritization strategies. Nonetheless, we expect that other clinical laboratories will also observe a lower odds for UFs than SFs as they prioritize disease-causing variants related to the clinical question.

Lesson 4: Medical actionability for UFs differs from "ACMG59" recommendations

Only 54 of 88 disclosed UFs (61%) involved an "ACMG59"-listed gene. Medical actionability of the diseases listed is based on prevention and reduction of mortality and morbidity. The remaining 34 UFs (39%) were identified in twenty genes not listed on the "ACMG59'. The reported contribution of non-ACMG genes to the incidence of SFs ranges from 13 to 52%(6,7,8,9,10,11,16). Eleven genes we report, however, predispose to the same conditions as listed on "ACMG59", such as breast cancer (*BRIP1*, five UFs) and cardiac disease (*CSRP3*, one UF), or predispose to conditions that fall in the same phenotypic spectrum of diseases, such as renal cancer (*FH*, one UF) and pulmonary hypertension (*BMPR2*, one UF). The low prevalence of these genes in causing these disorders may be a reason why "ACMG59" has not included this well-known extensive genetic heterogeneity(2). Additionally, using a fixed list means that population-specific founder variants may not be taken into account as exemplified in our study by a recurrent and relatively prevalent Dutch founder variant in *SDHA* (three UFs)(25). These findings show the limited applicability of "prevalence" as a universal criterion for disclosing UFs. A proposal to expand the "ACMG59" to over 100 genes to overcome the genetic and clinical heterogeneity of the listed disorders has been made before(8,16).

We disclosed six other variants that may allow individuals to undergo medical interventions, aiming at influencing the course of disease rather than preventing it. For instance, *GJB2* (one UF) and *MARVELD2* (one UF) cause early-onset hearing loss, which itself cannot be prevented, but morbidity associated with the hearing loss, such as speech and language delay, can be mitigated at a young age. Similarly, an UF in *VWF* was disclosed because of potential for intervention with medication to optimize and maintain hemostatic stability. As these interventions reduce morbidity, these UFs fulfill the criteria of being medically actionable and thus represent a category of diseases for which UF disclosure could be considered.

Lesson 5: The odds of UFs depend on (local) disclosure policy

The most prominent reason for disclosure of UFs in our policy has been their medical actionability. The expert panel also discussed nine UFs predisposing to disorders for which medical interventions to reduce mortality or morbidity did not apply. After review, these were not disclosed to the family. One example includes frontotemporal dementia (*GRN*, two UFs), for which worldwide, no medical management is available. We also did not disclose variants

for *CHEK2*-associated susceptibility to breast cancer (two UFs) because no national screening programs have been established for this condition in absence of familial breast cancer(26). Interestingly, we noted that after an initial policy decision not to disclose *CHEK2* variants, our laboratory geneticists refrained from further reporting variants in this gene to the expert panel.

Similar low odds are observed for variants facilitating reproductive and pharmacogenetic options. In total two UFs, in two different genes (*TYR*- autosomal recessive inheritance, and *ARSL*-X-linked inheritance) were disclosed because of the health risk for future offspring. In both cases, parents of the index had at least 25% of having an affected child with a disorder manifesting at birth or early childhood. Only one couple was at risk of an autosomal recessive disorder, which is far less than the empirical ~1% which could have been identified(27). Parents did not receive WES themselves for the purpose of carrier analysis. Our approach only allowed the detection of couples carrying the same pathogenic variant (thus at risk of a homozygous child). Couples at risk of a compound heterozygous child are not detected in our study, whereas they are included in the empirical 1% of couples at risk of an autosomal recessive disease. Notably, should we not have used the threshold of ≥25% risk of affected offspring, the odds of UFs in this category would have significantly increased since it is estimated that every individual is a carrier of at least two pathogenic variants in currently known autosomal recessive genes(27).

We also disclosed four UFs of pharmacogenetic relevance, all identified in *DPYD*, which is low given that many more genes are known to be of importance for management of the optimal dose of medication(28). Most of the variants in these genes are common variants requiring special expertise to recognize these UFs. This assumption was confirmed by the observation that the UFs in *DPYD* were identified by clinical laboratory geneticists with specific expertise in pharmacogenomics. Hence, the odds of UFs in pharmacogenetic genes are not representative of the incidence of pharmacogenetic relevant variants in clinical WES. For both reproductive options and pharmacogenetics, dedicated genetic tests can be performed to assess individual risk of a specific situation such as preconception carrier testing or pharmacogenetic passport.

The default option of our local policy was to only disclosure medically actionable findings. All patients consented as alternative (targeted) diagnostic testing opportunities were offered. With WES becoming a first-tier diagnostic test, offering opt-out options for medically actionable disease and opt-in for non-medically actionable disease has become a matter of intense debate. As a result, opt-out/opt-in options have been implemented in a Dutch national consensus-based guidance in 2021 (https://vkgl.nl/nl/diagnostiek/incidental-findings). The extent to which these options will affect UF disclosure remains to be seen and will allow to register how many patients will choose to “opt-out” from hearing UFs, or opt-in for non-medically actionable disease.

With the ongoing debate on disclosing UFs, we believe that our evaluation of UFs observed in everyday diagnostic practice collected over a five-year period on >15,000 exomes, provides

valuable perspectives on the clinical impact and utility of UFs. Concerns have been raised about the penetrance of genetic variants in the context of UFs/SFs which has led genomic professionals to question their utility(29,30,31). It would be of great value to describe the follow-up of patients to whom an UF was disclosed to evaluate their clinical relevance. For a subset of 20 individuals with appropriate consent for recontact, we have performed qualitative interviews regarding their experiences and preventative measures they have taken(32). Only a minority of our participants experienced symptoms related to the UF. However, it has been beyond the scope of the current manuscript to follow-up on the medical relevance of all of these UFs.

Conclusion

The odds of UFs in our diagnostic workflow are low, ranging from 0.03 to 1.03% for analysis of disease-gene panels and the entire exome, respectively. Our local disclosure policy had a large impact. Our observations that UFs, defined by *ad*-hoc review of medical actionability, affected a broader range of genes than listed on "ACMG59", suggest that pre-defined gene lists may need to be reconsidered.

Acknowledgements

We thank all previous members of the local expert panel on Unsolicited Findings of the Radboudumc, especially Norbert Steinkamp, Frans Cremers, Ineke van der Burgt, and Simone van der Burg for their expertise during discussions. In addition, we would like to express our gratitude for those having contributed to the development and implementation of our local policy, in particular Arjen Mensenkamp, Carlo Marcelis, and Frederike Tesser. In addition, we would like to express our gratitude to Lieve Claes, Arthur van den Wijngaard, Debby Hellebrekers and other clinical genetic laboratory specialists for their contribution to the variant classification.

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Supplementary data

1. Supplementary Methods

Technical details of Whole Exome Sequencing procedure

WES was performed following our routine diagnostic procedures(1) either on the index patient only, or in a family-based trio strategy (index patient + biological parents). In brief, DNA was outsourced to BGI Copenhagen (Denmark) for WES using an Agilent v4 (June 2013 - March 2015) or v5 (April 2015 - June 2018) all Human Exon Enrichment kit, followed by sequencing on HiSeq2000 or HiSeq4000 sequencer (Illumina) to a median sequence depth of at least 75-fold. FASTQ files were subsequently provided to our laboratory, and run through a custom diagnostic bioinformatic pipeline for variant calling (GATK) of single nucleotide variants (SNV) and insertion deletion events. For each variant, annotation allowing prioritization was added, including, amongst others, variant effect prediction, population frequencies and previous reports of pathogenicity. From 2015 onwards, also copy number variants (CNV) were routinely identified from WES data, annotated and used for diagnostic interpretation(2).

Analysis of data

Analysis of WES data was performed in a two-step process, guided by the consent provided by the patient. In the first step, referred to as tier 1, the referring clinician selected the most appropriate *in silico* disease gene panel(s), listing between 56 and 1,159 genes per panel. If the patient's symptoms did not allow for selection of (a) disease-specific gene panel(s), the clinician could also request analysis of the Mendeliome, consisting of all 3,606 genes with an OMIM-listed disease-gene association. In case no molecular diagnosis was obtained in tier 1, and the patient consented for further analysis, the analysis was followed by tier 2. An overview of the number of genes per panel, requested in patients in whom an IF was identified, is listed in the table below:

	Number of genes in panel (v.DG-2.14.0)	
Restricted gene panel	Total	of which 'ACMG59' listed genes
Hereditary cancer	206	25
Skin disorders	611	19
Intellectual disability	1,159	7
Metabolic disorders	625	5
Muscle disorders	157	4
Renal disorders	252	4
Epilepsy	316	2
Movement disorders	304	2
Haemostatic/thrombotic disorders	145	1
Ciliopathies	146	1
Disorders of sex development	56	1
Hearing impairment	168	1
Neuropathies	96	1
Primary immunodeficiencies	386	0
Vision disorders	415	0
Mendelian inherited disorders	3,606	59

Of note, the number of genes as listed is based on the panel content of June 2018 (version DG-2-14.0). A current overview of all (previous) panel releases is presented on-line (https://www.radboudumc.nl/en/patientenzorg/onderzoeken/exome-sequencing-diagnostics/information-for-referrers/exome-panels)

Variant prioritization and interpretation for trio-based analysis

Index patients were frequently sequenced simultaneously with their parental samples ('trio-analysis') to allow identification and interpretation of *de novo* variants in autosomal dominant disease genes. That is, trio-based analysis allows to determine the inheritance of all variants identified in the index by comparison the variants in the parental samples. For SNVs, this strategy precludes the identification of variants solely identified in (one of) the parent(s). It can show for instance that both parents are carrier of the same pathogenic variant that is detected in heterozygous state in the affected child (also a carrier). This information is however of relevance in the context of unsolicited findings (UF) evaluation, as the couple does have a 25% chance of affected offspring in future pregnancies (see 'UF disclosure policy').

For CNVs, analysis is complicated by fragmentation of called segments, and requires additional graphical representation of the data in a genome-wide view to establish inheritance of the variants and interpretation. The latter may thus lead to the visual observation of CNVs only identified in (one of) the parent(s), but which is absent in the index. Whereas these CNVs can be considered an UF for disclosure to the parent(s) if it for instance has clinical implications (see 'UF disclosure policy'), we have excluded these UFs from our analysis here to provide an overview of UFs identified in the index cases receiving clinical exome sequencing.

UF disclosure policy (applicable to clinical WES between 2013-2020)

The policy of the Department of Human Genetics of the Radboudumc applies to UFs, as there is NO active search for disease causing variants in genes that have no relation to the disease for which the patient is referred to by the treating physician. This policy was based on published European points of consideration for the disclosure of UFs(3).

General remarks

A genetic variant for which there is insufficient proof of pathogenicity, is not considered to be an UF. For the 4-class system(4), used until 2015, this refers to variants classified as UV1 and UV2. For the 5-class system(5) of SNVs and indels, used 2015 onwards, this refers to Class 1, Class 2 and Class 3 variants. For CNVs, this refers to Class 1, Class 2 and Class 3 CNVs according to the European guidelines for constitutional cytogenomic analysis(6).

UFs will only be reported during an ongoing clinical consultation. In the event that a variant is reclassified based on novel knowledge gained, it is considered good clinical practice to recontact the patient and send a revised report for variants disclosed as UFs that were wrongly deemed (likely) pathogenic.

Variants with a potential health risk for the patient (or his blood relatives)

In principle, UFs that, at time of discovery, cause a disease which course CANNOT be changed by medical intervention, will NOT be reported.

Mentally competent individuals aged 12 and above will be informed on UFs relevant for their own health (or for that of their blood relatives) when medical intervention is possible.

For minors below the age of 12, UFs related to a childhood-onset disease (manifestation under the age of 16) for which medical intervention is possible will ALWAYS be disclosed.

For minors below the age of 12, UFs increasing the risk of adult-onset diseases WILL NOT be disclosed. Nonetheless, UFs of potential medical relevance to one of the parents WILL BE disclosed if options to medically intervene are available.

Variants with a potential health risk for the patient's unborn progeny (or for the unborn progeny of his blood relatives)

UFs related to genetic carrier status, will - in principle - NOT be disclosed as they, by definition, are NOT of medical relevance to the patient himself. Nonetheless, carrier status exposing the carrier, or couple, at a risk of at least 25% of conceiving a child with a genetic disorder WILL BE disclosed.

References to Supplementary Methods

1. Haer-Wigman L, van Zelst-Stams WA, Pfundt R, van den Born LI, Klaver CC, Verheij JB, et al. Diagnostic exome sequencing in 266 Dutch patients with visual impairment. Eur J Hum Genet. 2017;25(5):591-9.
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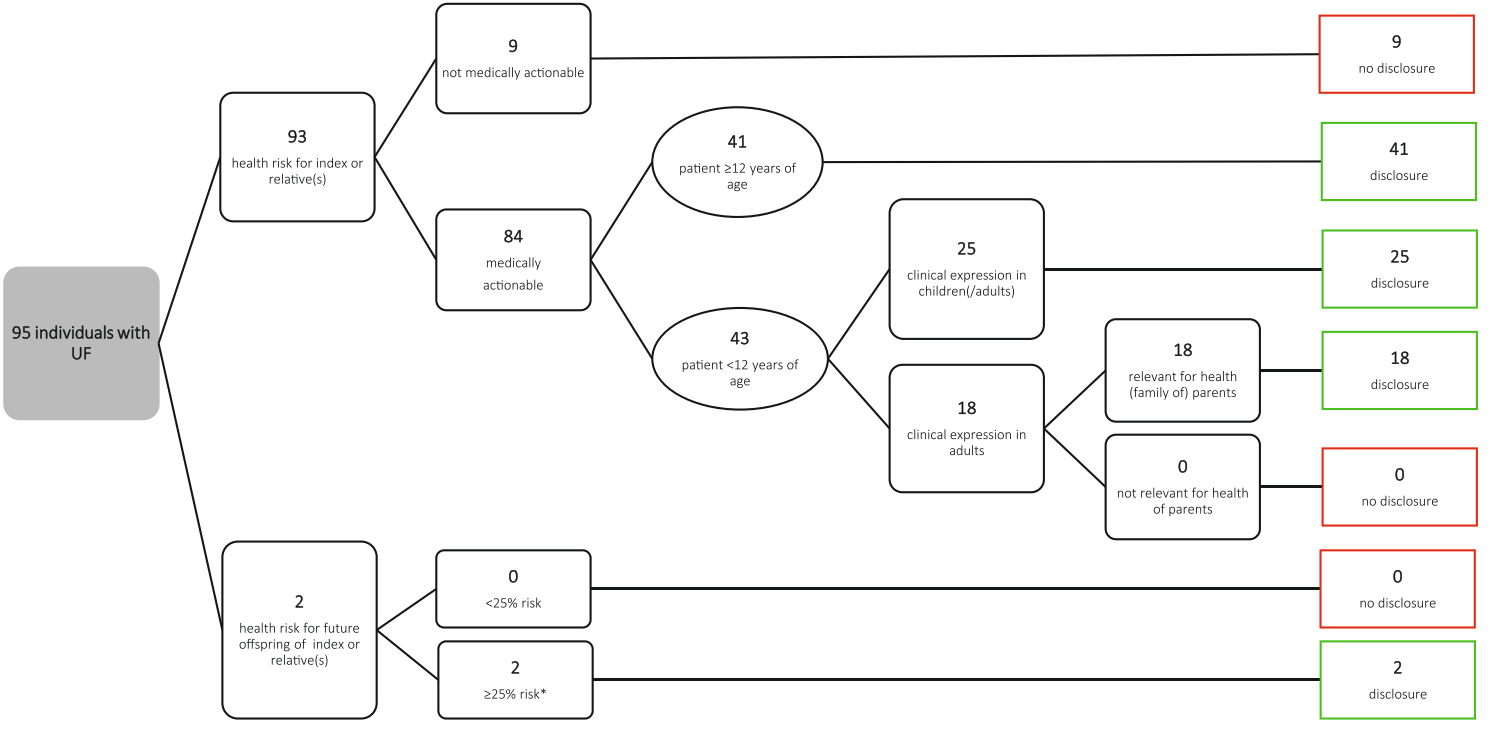
2. Supplementary Figure 1. Dissemination of UFs in 95 individuals



*Flow diagram representing the results of the evaluation process for the disclosure of UFs as guided by our inhouse policy for UF disclosure. Numbers listed in the boxes represent the number of individuals. *: index or parent(s) is are carrier of an X-linked or autosomal recessive condition.*

3. Supplementary Table 1. Detailed overview of UFs identified in 95 individuals

Table 1 is available online via doi: 10.1038/s41431-021-00964-0.

Part II

Listening

Chapter 4

The impact of unsolicited findings in clinical exome sequencing, a qualitative interview study

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Eur J Hum Genet. 2021; doi: 10.1038/s41431-021-00834-9

Abstract

Unsolicited findings (UFs) in clinical exome sequencing are variants that are unrelated to the initial clinical question the DNA test was performed for, but that may nonetheless be of medical relevance to patients and/or their families. There is limited knowledge about the impact of UFs on patients' lives. In order to characterise patient perceptions of the impact of an UF, we conducted 20 semi-structured face-to-face interviews with patients and/or their relatives to whom an UF predisposing to oncological disease (n = 10) or predisposing to a cardiac condition (n = 10) had been disclosed. We have identified a psychological, physical and financial aspect of the perceived impact of UF disclosure in exome sequencing. Actionability, understanding, patients' pre-test health and social context were influencing factors, according to our participants. Although most expressed considerable psychological impact initially, all but one participant would choose to undergo genetic testing again, knowing what they know now. These novel findings provide insight in patients' perspectives on the impact of UF disclosure. Our study highlights the value of incorporating patients' perceptions in UF disclosure policy.

Introduction

Comprehensive genetic testing by next generation sequencing techniques (NGS) is becoming standard care in many clinical settings(1). Sequencing the entire exome or genome allows the detection of unsolicited findings (UFs). These are defined as (likely) pathogenic variants in disease-causing genes which are unrelated to the initial clinical question for which the genetic test was performed but that may nonetheless be of medical value to the patients and/or their family(2). Although throughout the years various terms (i.e. incidental findings, unexpected findings) have been used to describe these findings, UFs is currently considered to be the most appropriate(3,4,5).

For more than a decade, discussions worldwide have weighed arguments in favour and against disclosure of UFs(6). A major argument which has been used in favour of disclosure is that knowledge about genetic predisposition could enable prevention or early detection of the condition to which the UF predisposes, potentially resulting in decreased morbidity and mortality. Potential distress, anxiety, additional costs and overtreatment have been mentioned to weigh against disclosure(6). It has further been argued that if the perceived negative impact of an UF is greater than its potential clinical utility, the UF should not be disclosed(6, 7).

Berg et al. were the first to publish recommendations for the disclosure of UFs(2). The American College of Medical Genetics (ACMG) provided recommendations to promote standardised disclosure of additional findings that should be actively looked for, or so called 'secondary findings' (SF)(8). In contrast, the European Society of Human Genetics (ESHG), as well as the Canadian College of Medical Genetics and EuroGentest argued to limit the identification (and disclosure) of UFs, considering their potential negative implications, which would conflict with the medical maxim "first, do no harm"(9,10,11,12). Both professional societies recommend reporting additional variants which are found unintentionally, only if they predispose to serious, but treatable or preventable health issues, considering both the health of patients (and their family members) as well as patient autonomy(8, 13).

In order to evaluate these recommendations, we believe insight into the perceived impact of UFs in clinical care is essential. The impact of SFs has been evaluated by the Clinical Sequencing Exploratory Research Consortium (CSERC) in both the diagnostic and research setting(14, 15). These studies report that a minority of patients experience a negative impact due to anxiety and/or difficulties in conceptualising the associated risks. To our knowledge, no study to date has evaluated the impact of the disclosure of UFs.

By conducting a semi-structured qualitative interview study among patients and their family members to whom an UF was disclosed, we characterise their perceptions of the impact of UFs in clinical exome sequencing.

Methods

Study design and setting

We used semi-structured interviews to ask participants about the impact of the disclosure of an UF on their lives. We intended to interview index patients (i.e. the persons who initially underwent genetic testing). In case of incompetent or minor index patients, we interviewed family members assigned as their legal guardian in case of incompetent or minor index patients. The Research Ethics Committee Arnhem-Nijmegen (registration number: 2018-4909) and the Research Ethics Committee Maastricht (registration number: 2018-0825) both approved this study.

Participants and recruitment

Between 2013 and 2018, material of 16,482 consecutive index patients was sent to the Genome Diagnostic Laboratory of the Radboud university medical centre for exome sequencing. According to their local policy, which is line with the European recommendations on UF disclosure, UFs were disclosed to 86 patients(16). These concerned mostly variants predisposing to either oncological or cardiac disease(13, 17) (van der Schoot et al., manuscript in preparation).

Using convenience sampling, we recruited participants to whom an UF had been disclosed, predisposing to either oncological or cardiac disease. Eligible index patients had been counselled for DNA testing at the department for clinical genetics at the Radboud university medical centre or at Maastricht University Medical Centre. To ensure a varied sample, we continuously assessed if there was variation in index age (minors, reproductive age), genes, the condition DNA testing was performed for, pathologies the UF was related to and the time since disclosure. Clinical geneticists were contacted to ask patients or their legal guardians for permission to invite participation after which interested potential participants were contacted by a researcher (VS) (Supplementary Figure 1). Interviews were conducted by a resident in clinical genetics (VS) and a trained intern (SV) under supervision of a skilled qualitative interviewer (AO) at a time and place convenient for the participants. Informed consent was obtained prior to each interview. The interviews were held between February and October 2019 and lasted between 32 and 86min. We reached data-saturation after 20 interviews.

Topic guide

We designed a topic guide to chronologically address relevant aspects of the impact of an UF (pre-test counselling, disclosure, follow-up and social context), which was refined after the first interviews to better fit our research questions.

Data-analysis

Interviews were audio-recorded, transcribed verbatim, anonymized and subsequently analysed using ATLAS.ti (version 8.2, Scientific Software Development, GmbH, Berlin, Germany). We used thematic content analysis, a qualitative approach focussing on identification of themes and concepts without predetermined hypotheses or theories(18). The first transcript was analysed by three members of the research team (VS, SV, AO) and all subsequent transcripts were independently analysed by two members (VS, SV). Any discrepancies in the analyses were discussed until consensus was reached. The codes we used emerged from the data and were refined in an iterative process of coding, comparing and refining. They were subsequently grouped into minor categories and major themes by three members of the research team (VS, SV, AO).

Table 1. Participants' characteristics

Variants predisposing to oncological disease (n=10)										
Nr.	Index/ family	Participant age range	IF present in participant	Symptoms of IF in participant	Preventive measures implemented by participant	Moment of disclosure	Indication genetic testing	Main reason genetic testing	Causal variant found	Index incompetent adult or minor
1	Index	21-30	Yes	No	Reproductive options Future periodic follow-up and prophylactic surgery	2 years ago	Congenital anomalies	Reproduction	No	No
2	Index	51-60	Yes	No	Periodic follow-up	2 years ago	Vision disorder	Family	No	No
3	Index	61-70	Yes	No	Not indicated (age-related)	1 year ago	Haemostatic disorder	Understanding	No	No
4	Index	31-40	Yes	No	Periodic follow-up	>2 years ago	Vision disorder	Understanding	Yes	No
5	Index Family	41-50 61-70	Yes Yes	No No	Surgical Surgical	1 year ago	Cardiovascular disease	Understanding	No	No
6	Index Family	21-30 51-60	Yes Yes	No No	Periodic follow-up and future prophylactic surgery Prophylactic surgery	1 year ago	Developmental disorder	Understanding	No	Incompetent adult
7	Family	31-40	Yes	No	Prophylactic surgery	1 year ago	Developmental disorder	Understanding	No	Minor
8	Family	41-50	Yes	No	No periodic follow-up as had been recommended by clinician	1 year ago	Neurological disease	Understanding	Yes	Minor
9	Family	41-50	Yes	Yes	Periodic follow-up and prophylactic surgery	1 year ago	Developmental disorder	Understanding	No	Minor
10	Family	21-30	Unknown	No	n.a.	>2 years ago	Haemostatic disorder	Understanding	No	Minor

Table 1. *(continued)*

Variants predisposing to cardiac disease (n=10)										
Nr.	Index/ family	Participant age range	IF present in participant	Symptoms of IF in participant	Preventive measures implemented by participant	Moment of disclosure	Indication genetic testing	Main reason genetic testing	Causal variant found	Index incompetent adult or minor
1	Family	51-60	Yes	No	Periodic follow-up	<1 years ago	Neurological disorder	Understanding	No	Incompetent adult
2	Family	31-40	Yes	No	Life-style and periodic follow-up	<1 year ago	Developmental disorder	Understanding	No	Minor
3	Family	31-40	No	No	n.a.	1 year ago	Neurological disorder	Understanding	No	Minor
4	Family	31-40	Yes	No	One time follow-up	2 years ago	Congenital anomalies	Understanding	No	Minor
5	Family	41-50	Yes	No	No periodic follow-up as had been recommended by clinician	1 year ago	Neurological disorder	Understanding	Yes	Incompetent adult
6	Family	51-60	Yes	No	Periodic follow-up	2 years ago	Developmental disorder	Understanding	No	Incompetent adult
7	Family* Family* Family*	21-30 31-40 61-70	Unknown Unknown Yes	No No No	n.a. n.a. Periodic follow-up	<1 year ago	Neurological disorder	Family	No	Incompetent adult
8	Index Family	18-20 51-60	Yes No	No No	One-time follow-up n.a.	2 years ago	Immunodeficiency	Reproduction	Yes	Incompetent adult
9	Index	31-40	Yes	No	Periodic follow-up	>2 years ago	Oncological disease	Understanding	No	No
10	Index	31-40	Yes	No	Periodic follow-up	1 year ago	Haemostatic disorder	Future perspective	No	No

**family other than parents*

Results

We conducted 20 semi-structured face-to-face interviews with index patients and/or their family members about the UF that had been disclosed, predisposing to oncological (*n* = 10) or cardiac disease (*n* = 10). In fourteen interviews, we spoke to the family (parents in all but one interview) about the impact of the finding from the perspective of the index and their own experience, since all but two relatives had tested positive for the UF as well. For index patient and participant characteristics see Table 1.

Psychological, physical and financial impact

Describing the impact of UF disclosure, participants mentioned aspects within three different dimensions: the psychological, physical and financial impact. Participants interrelated these themes and described four mediating factors, namely actionability, understanding, pre-test health and social context. Interviews with index cases yielded the same themes as those which emerged from interviews with family members.

The psychological impact was highlighted in all interviews. Both short- and long term impact were addressed frequently. Most participants indicated they were at first overwhelmed and some were even 'shocked' by the news of the UF.

> *"Actually, hearing the news was a shock; you don't expect it, certainly not at a young age. It was quite intense." (Oncological/Patient)*

They acknowledged this initial feeling to fade with developing a better understanding of the meaning of the UF and the consequences for their well-being. Most participants said that, after a while, they would think no more of the UF. One participant said:

> *"But as soon as you get back to your normal life, and you pick up your daily routines, you quickly forget about it." (Cardiac/Family)*

Patients attributed the physical impact to the different invasive (i.e. prophylactic surgery, colonoscopies) and non-invasive (i.e. imaging by CT, X-ray, ultrasound or MRI, ECG) preventive measures, lifestyle changes and reproductive choices.

Participants with an UF predisposing to oncological disease all said they were offered periodic follow-up (i.e. colonoscopies or non-invasive imaging) or prophylactic surgery, depending on their age. They expressed that these measures would enable timely diagnosis or prevent the development of malignancies. Invasive measures were described as to be unpleasant but acceptable considering their purpose.

The majority of participants with an UF predisposing to cardiac disease said they were offered periodic follow-up, according to their age and the condition to which the UF predisposes.

This allowed them to assess their current health status and could make them feel reassured no therapy was needed yet. Some were seen once by a cardiologist who told them no further assessments were indicated.

A few participants with an UF predisposing to cardiac disease talked about lifestyle changes: they reduced their workload in order to reduce their stress level or tried to become fitter by going to the gym.

One participant with an UF predisposing to oncological disease mentioned she had received counselling regarding reproductive consequences, namely timely starting a family and the option to try to prevent the condition in offspring.

Most participants were aware of possible consequences for taking out insurance (NB. In the Netherlands, results of genetic testing can might be requested by the insurance company before approval of the request to take out life insurance over a certain threshold for the insured sum). While none of the participants talked about having experienced actual adverse financial effects, they did mention worrying about future financial plans and indicated having reservations about testing children or informing family members because of this. A father said:

> *"They're young, they want a mortgage and then it would be like: 'are you under treatment, do you have an illness or anything?' So I told them: 'If I were you I would not get myself tested.'" (Cardiac/Family)*

A few participants mentioned contemplating not to undergo preventive assessments because of the costs of these treatments. (NB. In the Netherlands, health insurance covers these costs after patients have paid a deductible).

Actionability

All participants underscored the importance of the actionability of the UF, meaning to what extent preventive measures are available. Most participants said that the availability of preventive measures made them value disclosure. A guardian said:

> *"But I can say: okay, now I know and they can do something." (Cardiac/Family)*

Participants described that learning about interventions provided them with more insight in the actual consequences of the UF for their health. Those who underwent more definitive medical interventions to prevent the development of oncological conditions (i.e. prophylactic surgery), said to feel relieved from their fear of becoming ill. Most participants who underwent (periodic) screening to detect disease early mentioned to feel reassured as well. Some participants with

an UF predisposing to cardiac disease indicated that they were aware they could develop the condition in question in the interval between cardiac assessments.

Several participants with an UF predisposing to cardiac disease questioned the knowledge and experience of the cardiologist to whom they were referred. For example, one participant does not undergo cardiac screening because the cardiologist told him this was not necessary:

> *"(The doctor) asked me: 'How did you end up here?' I told him that genetic tests showed that there was a gene missing or wrong or I don't know what exactly. And he said: 'That's a load of nonsense. That's still in its infancy, they're just crying wolf.'" (Cardiac/Family)*

Some participants said that they felt insecure about their health before being seen for medical interventions. Several participants experienced the time they had to wait for their first workup as unpleasantly long. Multiple participants told us that their follow-up consults had ended. They indicated feeling uncertain about their current health status, not knowing if since their final assessment, they might have developed the condition.

None of the participants who underwent periodic workups had been diagnosed with the condition and no participants had required cardiac therapy or curative surgery.

Understanding

Participants frequently addressed their ability to comprehend the consequences of the UF. They said to feel less occupied by worries once they had developed a better understanding. During the interviews, we heard of multiple factors enabling participants to better understand these consequences: the pre- and post-test counselling; the disclosure; associations with (family) medical history; gathering information and follow-up.

All participants indicated that before consenting to the DNA test, they were informed about the possibility of detecting an UF. Some said they told their counsellors explicitly they wanted to know if a genetic variant related to another condition was found. Most participants mentioned that no genetic testing could have been performed had they not consented to UF disclosure. Participants mentioned the return of the DNA test results took a few months to a year. Most participants told us they had forgotten about the possibility of potential UF disclosure when receiving the DNA test result. They said to be surprised or even distressed. Some talked about how this diminished their ability to absorb further information about the UF. The mother of a patient:

> *"It's about your baby. It's not something you ever want to hear. At that moment, everything they tell you just goes past you." (Oncological/Family)*

Most participants indicated they felt able to understand the information provided. Several participants told us they did not fully comprehend the finding. For example, some mentioned

they only truly realised the implications for family members at a later stage. Participants sometimes said that they had been focussing on learning whether exome sequencing revealed a causal variant rather than learning about an UF, especially when hearing about the outcome via a telephone call.

Only one participant said to have had experienced symptoms of the condition the UF was related to at the time of UF disclosure (Oncological/Family).

Several participants with an UF predisposing to a cardiac condition said to be struggling with the answer to the question '*Am I sick or am I healthy?*'. We found some of them conceptualised their health status regarding the UF (affected by the condition the UF predisposes to, not affected, or something in between?) differently, even within the same interview.

Multiple participants immediately related the UF to conditions that were already known to run in the family. A woman to whom an UF predisposing to ovarian cancer was disclosed:

> *"I know my mom had cervical cancer, and my second cousin had cancer before her. So you can kind of assume that something like that would be running in the family." (Oncological/Patient)*

Participants who related the UF to their family's medical history, would conclude that in a way it made sense that the UF was found, even if their clinicians did not confirm that the conditions that ran in the family could be caused by the UF.

Several participants said that they had have tried to learn more about the UF by looking for information online. Others indicated they did not use any other resources than those provided by their clinician, to avoid being informed incorrectly.

Most participants mentioned that they had been contacted by their clinical geneticist after a period of time. The majority of participants who had not heard from their geneticist after the disclosure, expressed being uncertain about the consequences of the UF. A woman with an UF predisposing to heritable breast cancer told us she did not know if this variant could be related to her thrombotic disorder:

> *"We hoped to find the explanation for my complaints but we did not. Unless…I don't know… Maybe if you have one gene you can get very mild complaints. I don't know. It is not clear to me." (Oncological/Patient)*

Overall, most participants indicated feeling that they had developed a comprehension of the nature and implications of the UF. However, when discussing facts such as risks during the interviews, we regularly found their knowledge to be inconsistent with current literature and clinical guidelines, particularly in interviews about UFs predisposing to cardiac disease.

Pre-test health

During the interviews, participants often compared the severity of the condition the DNA test was initially performed for, with the perceived severity of the condition the UF predisposes to (e.g. the burden of untreatable epilepsy compared to a predisposition to an actionable cardiomyopathy). Many expressed worries about their own health or, in case of family, about the health of their child. They would qualify the condition the UF predisposes to as being relatively less severe than the initial condition. Also, most participants said that they accepted the possibility of disclosure of an UF and the consequences of an UF for the sake of finding a diagnosis. Family of a patient with a severe neurological disorder told us:

> *"On the one hand it's a shock, because it's yet another thing to deal with. On the other hand it's an absolute pain to still not have a diagnosis. That is just unacceptable." (Cardiac/Family)*

They indicated to be urgently looking for a way to understand and/or find proper treatment for the health condition of the index patient which they said motivated them to undergo genetic testing. All but one participant answered '*yes*' to the question '*would you have chosen to undergo the DNA test, knowing what you know now?'*. The father of a girl with epilepsy and a developmental disorder who had a cardiac UF disclosed, was not sure whether he would have chosen to undergo genetic testing. He questioned whether the clinical utility could outweigh the resulting financial consequences.

Social context

Participants discussed sharing the news of the UF with relatives in order to inform them about their risks and/or hoping to find comfort. They said to feel burdened by having to be the bearer of the bad news, especially when they experienced poor intrafamilial communication, vulnerability of family members or fear of negative consequences for their relationships. Some participants mentioned their clinical geneticist requested them to inform family members and told them whom to inform and how. They said this made them more comfortable when confronting their family.

With few exceptions, participants said that family members' reactions were mostly understanding and calm. They mentioned that when they shared the news with relatives, friends or colleagues to seek comfort, those people generally reacted compassionately.

Discussion

Over the course of 20 in-depth interviews, we encountered a psychological, physical and financial aspect of the perceived impact of UF disclosure in exome sequencing. Actionability, understanding, patients' pre-test health and social context were influencing factors for these three aspects, according to our participants.

Although most expressed considerable psychological impact initially, all but one participant would choose to undergo genetic testing again, knowing what they know now. This finding is in line with previous qualitative studies about UFs across different clinical settings, as well as for SFs in genetic testing(14, 15, 19). As in our study, the consequences of the UF are generally considered to be more beneficial than adverse, which would argue in favour of UF disclosure(6).

Actionability was a major theme throughout all interviews, similar to studies on the impact of SFs in DNA testing(20, 21). The majority of the participants valued disclosure as they were offered measures that would enable early detection or prevention. This finding affirms current policy guidelines in which actionability is a prerequisite for UF disclosure(13).

Even though all variants disclosed were deemed 'medically actionable' by an expert panel(17), the experienced effectiveness differed among participants. Generally, preventive measures offered for cardiac disease were perceived to be less effective than those to prevent oncological conditions. In this context, it has been suggested that patients value "more concrete" interventions(22). Effectivity of preventive measures has been an acknowledged criterion for UF disclosure, but it is subject to personal judgments of genetic professionals(23). It would be of added value to incorporate patients' perceptions of which interventions are effective and their views on the perceived importance of this criterion.

Only one participant (Oncological/Family) indicated being symptomatic, which reflects the low prevalence of phenotypic expression of UFs(24). Reduced penetrance of both cardiac as well as oncological variants in the context of UFs/SFs previously has led genomic professionals to question their utility(25,26,27). In our study, the value of the UF was mainly attributed to its utility. Potential limited utility of UFs should be embedded in disclosure policy and clinical studies on expression and penetrance of UFs would be of added value(27).

Participants frequently addressed the value of being able to *understand* the finding. They mentioned the relevance of being provided with adequate and timely information through thorough pre- and post-test counselling and follow-up consultations, which has been previously emphasised for delivering bad news in genetic testing and in other medical procedures(22, 28,29,30). Understanding allows patients to develop disease conceptualisation,

contributing to their empowerment. Feelings of empowerment could suppress the initial negative feelings regarding the UF as has been seen in the context of secondary findings(15).

Some of our participants still expressed uncertainty about gene associated risks. Notably, we regularly found participants' knowledge to be inconsistent with current literature and clinical guidelines (e.g. no genetic testing of first degree relatives was recommended in case of an autosomal dominant predisposition for cardiomyopathy in the index with a known low de novo occurrence(31)).

Whether this was due to a lack of understanding or inadequate counselling, is unclear. We saw the extent to which the finding was understood differed between cardiac and oncological variants. Variants predisposing to cardiac disease make up a substantial portion of UFs (van der Schoot et al., manuscript in preparation) and SFs(32), and – compared to variants predisposing to oncological disease – they are known to display reduced penetrance and phenotypic variability(31, 33). In our study, neither participants with a cardiac UF, nor their family members were known to have experienced any UF-related symptoms. The complex relationship between genetic variants and the associated phenotypes are a challenge to the genetic counselling process, and potentially limit healthcare professionals in enabling patients' understanding. Counselling UFs influences patients' behavioural responses(19, 34). Inadequate information and guidance by healthcare professionals due to the complexity of UFs could endanger the fulfilment of UFs' actionability. This further emphasizes the need to critically consider if adequate counselling and follow-up can be ensured before UF disclosure(34).

The *pre-test health* was the third major theme. The urge to find a diagnosis for the index patient was highlighted in all interviews and has previously been noted for genetic testing in general(35). Participants told us no genetic testing would be performed when they would not consent for UFs. In our centre, targeted panel analysis is offered first, which carries a very low probability of UFs. Thus, a requirement to consent to disclosure of UFs applied only to those in whom genetic testing of the entire exome was performed, as this carries a higher yield of UFs (van der Schoot et al., manuscript in preparation). Over the 2013–2018 period in which our participants were counselled for genetic testing, a specific opt-out option for UFs was not available when analysing the complete exome. This has been a matter of intense debate. An opt-out option will be implemented in national consensus-based guidance for UFs. The majority of our participants stated however, that they needed to consent for IF disclosure to have genetic testing performed, rather than mentioning having had the option to restrict genetic testing to a targeted panel or discussing the possibility of an opt-out.

For them, the imperative to find an explanation for their own or their child's complaints seemed to overrule the impact of the UF. Most participants qualified the impact of the UF as less severe than the impact of the condition genetic testing was performed for, which were generally

conditions that were poorly understood and/or for which proper treatment options were lacking. This in contrast to the medically actionable conditions to which UFs by definition predispose. Although the importance of the context in which genetic testing is performed has been highlighted previously(15, 20), understanding how it can relate to experiencing genetic testing provides a new perspective of embedding contextual factors in counselling for DNA testing.

A minority of the participants addressed the *social context* to be of influence on the impact of UF disclosure. Participants particularly acknowledged not fully grasping implications for family members when consenting to genetic testing. As has been pointed out before, this aspect requires attention before deciding to undergo genetic testing(36). Overall, implications of sharing the news of the UF with relatives did not appear to differ from what we know from studies about sharing results of genetic testing in general(37).

The *financial impact* was another minor theme. Possible financial consequences were a main reason to have reservations about sharing the news with family. The perceived financial burden showed similarities with what was found in previous studies on presymptomatic genetic testing(38). At the time of the interview, none of the participants had experienced any actual financial consequences. Of note, the financial impact largely depends on the nature of the healthcare system.

Overall, participants did not experience a great *physical impact* of preventive measures. This is an important finding, as burdening patients with unnecessary interventions has been put forward as a reason to critically consider disclosure of UFs(6). Offering more invasive measures (i.e. prophylactic surgery, ICD) should be carefully considered(26, 39).

Strengths and limitations

Our study investigated patient experiences with the impact of an UF following clinical exome sequencing. These results provide valuable insight for both clinical genetics practice as well as policymaking.

Limitations of our study include the risk of bias, given its relatively small sample size and the recruitment which was restricted to one genetic centre and was not limited to index patient inclusion. This study assessed the impact of an UF as perceived and described by index patients or their guardian family members. Recall bias and choice-supportive bias might have impacted participants' descriptions of their experiences. Although the absolute number of participants was relatively small, this sample size is common for qualitative research, considering its labour-intensiveness and the amount of information each interview yields. In addition, since UFs are a relatively rare occurrence, our sample constitutes 22% (20 of 89) of the total number of UFs detected in our hospitals over a 5-year period. We did not address the impact of UFs other than cardiac and oncological variants. However, UFs related to these two disease entities are the

most frequent additional findings in exome sequencing(32). Since participants were recruited from one genetic centre, our results might not be representative of practice in the Netherlands overall. The provided participant characteristics' and access to the local policy guidelines enable readers to assess whether these data are applicable to other genetic centres. We did interview both index patients as well as their family (i.e. parents). However, since most family members had tested positive for the UF as well, we believe their contribution to this study to be valuable. We found that the themes that were brought up by family members generally mirrored those which emerged from interviews with index patients.

Time since disclosure has been less than five years for all of our participants. Only one of our participants had presented with symptoms related to the UF when conducting the interviews, meaning for the others, no prevention or early detection had yet occurred. The extent to which the potential treatability or prevention has been fulfilled might influence participants' appreciation of the actionability. Therefore, long term evaluation would be needed to address this aspect.

The reporting of this study generally follows recent qualitative research standards (ref. COREQ).

Conclusion

In conclusion, patients and their family members express a psychological, physical and financial impact of UF disclosure. Overall, the perceived impact would not keep patients from undergoing genetic testing again, knowing what they know now. To ensure informed consent in pre-test counselling, counsellors should encourage consideration of all potential outcomes of genetic testing, since the desire for a diagnosis potentially lessens the receptiveness for information on UFs. Post-test counselling should enable understanding of the finding, contributing to fulfilling its actionability. The importance of the actionability criterion suggests the need for critical consideration of the perceived effectiveness of interventions and the clinical utility of disclosure of variants in the context of UFs.

Acknowledgements

We would like to thank all participants for sharing their experiences and thoughts. Also, we thank the clinicians who contacted their patients to ask for participation in this study. Finally, we express our gratitude for the work of the local expert committee on unsolicited findings in genetic testing.

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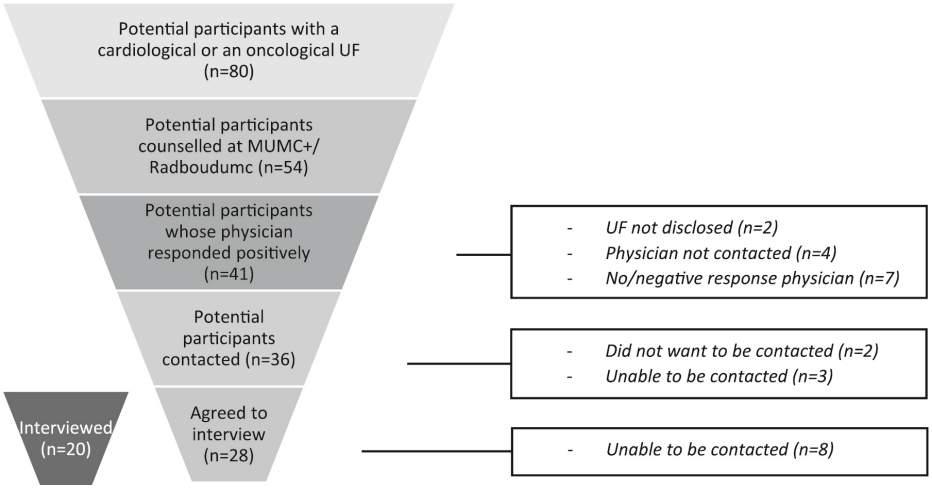
Supplementary data

1. Supplementary figure 1. Study consort diagram



2. Consolidated criteria for reporting qualitative studies (COREQ): 32-item checklist

Developed from:

Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *International Journal for Quality in Health Care*. 2007. Volume 19, Number 6: pp. 349 – 357

No. Item	Guide questions/description	Our study
Domain 1: Research team and reflexivity		
Personal Characteristics		
1. Interviewer/facilitator	Which author/s conducted the interview or focus group?	Vyne van der Schoot, Simone Viellevoije, Anke Oerlemans
2. Credentials	What were the researcher's credentials? E.g. PhD, MD	MD (VS), BSc (SV),PhD (AO)
3. Occupation	What was their occupation at the time of the study?	Clinical genetic counselor in training (VS), student (SV), assistant professor (AO)
4. Gender	Was the researcher male or female?	All female
5. Experience and training	What experience or training did the researcher have?	Short interview instruction, clinical counseling / communications training (VS), interview training (SV) experienced interviewer (15+ years) (AO)
Relationship with participants		
6. Relationship established	Was a relationship established prior to study commencement?	No
7. Participant knowledge of the interviewer	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research	Reasons for doing the research
8. Interviewer characteristics	What characteristics were reported about the inter viewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic	None
Domain 2: study design		
Theoretical framework		
9. Methodological orientation and Theory	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis	Thematic content analysis

No. Item	Guide questions/description	Our study
Participant selection		
10. Sampling	How were participants selected? e.g. purposive, convenience, consecutive, snowball	Convenience sampling
11. Method of approach	How were participants approached? e.g. face-to-face, telephone, mail, email	Telephoned by their physician, subsequently further informed via letter
12. Sample size	How many participants were in the study?	20
13. Non-participation	How many people refused to participate or dropped out? Reasons?	One participant was not at the place we agreed to meet. We unsuccessfully tried to contact this participant afterwards
Setting		
14. Setting of data collection	Where was the data collected? e.g. home, clinic, workplace	At the participants' home
15. Presence of non-participants	Was anyone else present besides the participants and researchers?	In some cases the partner or family
16. Description of sample	What are the important characteristics of the sample? e.g. demographic data, date	All participants had an IF disclosed to them or to a family member. All index patients had been counseled at the Maastricht UMC or Radboudumc
Data collection		
17. Interview guide	Were questions, prompts, guides provided by the authors? Was it pilot tested?	The authors designed an interview guide. The interview guide was modified with minor revisions of a few questions after the first interviews. No pilot study was performed
18. Repeat interviews	Were repeat interviews carried out? If yes, how many?	No
19. Audio/visual recording	Did the research use audio or visual recording to collect the data?	Audio
20. Field notes	Were field notes made during and/or after the interview or focus group?	Yes
21. Duration	What was the duration of the inter views or focus group?	Between 32 and 86 minutes
22. Data saturation	Was data saturation discussed?	Yes, data saturation was achieved
23. Transcripts returned	Were transcripts returned to participants for comment and/or correction?	No

No. Item	Guide questions/description	Our study
Domain 3: analysis and findings		
Data analysis		
24. Number of data coders	How many data coders coded the data?	First interview: 3 (AO, SV, VS), subsequent interviews: 2 (VS and SV).
25. Description of the coding tree	Did authors provide a description of the coding tree?	No
26. Derivation of themes	Were themes identified in advance or derived from the data?	Derived from the data
27. Software	What software, if applicable, was used to manage the data?	ATLAS.ti
28. Participant checking	Did participants provide feedback on the findings?	No
Reporting		
29. Quotations presented	Were participant quotations presented to illustrate the themes/findings? Was each quotation identified? e.g. participant number	Participant quotations are present, and only identified by category (because of anonymity)
30. Data and findings consistent	Was there consistency between the data presented and the findings?	Yes
31. Clarity of major themes	Were major themes clearly presented in the findings?	Yes
32. Clarity of minor themes	Is there a description of diverse cases or discussion of minor themes?	Yes

Part III

Telling

Chapter 5

Clinical geneticists' views on and experiences with unsolicited findings in next-generation sequencing: *"A great technology creating new dilemmas"*

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JOGC; 2022. doi: 10.1002/jgc4.1647. Online ahead of print.

Abstract

Unsolicited findings (UFs) from diagnostic genetic testing are a subject of debate. The emerging consensus is that some UFs from genetic testing should be disclosed, but recommendations on UF disclosure generally leave room for variation in practice. This study aimed to explore clinical geneticists' views on and experiences with UFs during pre-test counseling and UF disclosure. We interviewed twenty certified clinical genetics medical specialists and clinical genetics residents, working in seven Dutch genetic centers. Participants indicated that discussing the probability of detecting UFs is an integral part of pre-test counseling and informed consent. However, they expressed doubts about the degree to which this discussion should occur and about what information they should share with patients. They argued that the contents of their counseling should depend on the individual patient's capacity to understand information. These results endorse the importance of tailored pre-test counseling alongside informed consent for optimal genetic consultations. While 'medical actionability' is broadly accepted as an important criterion for the disclosure of UFs, participants experienced substantial uncertainty regarding this concept. This study underscores the need for further demarcation of what exactly constitutes medical actionability. Installation of an expert panel to help healthcare professionals decide what variants to disclose, will support them when facing the dilemmas presented by UFs.

Introduction

DNA testing with next-generation sequencing (NGS) techniques enables analysis of the entire exome or genome. Over the past decade, NGS has increasingly been incorporated into clinical care(1). Technological innovation has resulted in an improved diagnostic yield, a reduced time to diagnosis, and lower sequencing costs, improving patient care overall(2-4).

One challenge in implementing NGS for diagnostic genetic testing is that the test can find other (likely) pathogenic variants in disease-causing genes which are unrelated to the clinical question for which the genetic test was initially performed(5). Unsolicited findings (UFs) are variants in disease-causing genes that are unrelated to the clinical question for testing and that are identified inadvertently(6, 7). UFs are differentiated from secondary findings (SFs), which refer to variants in disease-causing genes that are unrelated to the clinical question for testing but that are actively sought during the analysis(6-8).

UF and SF disclosure is the subject of a worldwide debate(6, 8-10). The ongoing debate carefully considers the proposed benefits and potential harms of UF and SF disclosure to patients. The American College of Medical Genetics (ACMG) recommends pursuing SFs in over 70 genes predisposing to medically actionable conditions(11). In contrast, the European Society of Human Genetics (ESHG) and the Canadian College of Medical Genetics (CCMG) do not recommend SF disclosure and argue for a more cautious approach when it comes to disclosing UFs. They emphasize potential physical and/or emotional harm(6, 9). They recommend a targeted approach to sequencing, which minimizes the likelihood of detecting UFs. If UFs are uncovered, the ESHG propose limiting disclosure to medically actionable variants. In view of patients' autonomy and their right (not) to know, some centres for medical genetics broaden patients' choices by offering them an 'opt-in' (the disclosure of non-actionable diseases) and an 'opt-out' (the non-disclosure of actionable conditions)(12, 13). This policy allows patients to choose between wanting to learn a genetic predisposition for non-actionable diseases (e.g. hereditary ataxia) and not wanting to learn their risk of developing actionable diseases (e.g. breast cancer).

A literature review by Mackley et al. showed that disclosure of medically actionable SFs is generally supported by both patients and healthcare professionals in genetics(14). Healthcare professionals argue that the potential health benefits (e.g., preventative measures) of both SFs and UFs outweigh the possible burdens (e.g., the psychological burden of knowing)(12). Additionally, they aim to foster patients' autonomy by providing them with access to personal health information(12).

Box 1. National policy regarding UFs

Until June 2021, the eight Dutch genetic centers each had a local policy regarding UFs, which was based on recommendations provided by the ESHG. The old policy recommended that these variants (i.e., 'secondary findings') should not be actively tested, but when inadvertently found, variants should be considered for disclosure *if* medically actionable (Vears et al., 2018). Depending on local policy, UFs were reported to either the clinical geneticist or a local expert panel, followed by the decision to disclose the UF to the patient.

In June 2021, national consensus guidelines were published considering three important principles. First, valuable information should be disclosed, leading to a default disclosure of variants in medically actionable disease genes. Second, the principle is the right to know and not to know, which has led to the implementation of an option to opt-in for non-medically actionable diseases and to opt-out of actionable diseases. Third, in the Netherlands, the clinician ordering the test is legally responsible for all test results. Although a multidisciplinary meeting is recommended, in the end it is the clinician's responsibility to decide on disclosure.

Policy rule	**Local policy (n=8)**	**National consensus**
Expert panel	7/8 default 1/8 upon request	Yes
Attending panel meeting	8/8 molecular geneticist, clinical geneticist 5/8 ethicist 4/8 legal representative 3/8 social worker 1/8 patient representative	Default molecular geneticist, clinical geneticist Consider ethicist, legal representative, social worker and/or psychologist
Clinician attending panel meeting	4/8	Yes
Opt-in	3/8	Yes
Opt-out	3/8	Yes
Disclosure of SFs	0/8	No

The emerging consensus is that UFs from diagnostic genetic testing should – to some extent – be disclosed to patients(5, 6, 9). This has an impact on multiple aspects of pre- and post-test counseling. First, patients need to be adequately informed about the possible outcomes prior to testing, which will enable them to make an informed decision and give their informed consent. Subsequently, a decision needs to be made whether or not to disclose UFs, taking into account multiple factors (e.g., penetrance, expression, actionability). Lastly, the disclosure of information during post-test counseling requires healthcare professionals to disclose information which does not, by definition, concern the main objective of the genetic test.

The number of studies that provide insight into how healthcare professionals experience these aspects of genetic counselling is limited(15, 16). They raise various issues, such as, how,

and to what extent to inform patients about the probability of detecting UFs, how to obtain meaningful consent, and which UFs should be disclosed and in what manner(15, 16).

The implementation of recommendations(5, 6, 9) on UF disclosure generally leaves room for variation in practice (Box 1). For example, the question of how to define medical actionability has proven to be a difficult matter(5, 9, 17, 18). UFs likely require an *ad hoc* evaluation of medical actionability(19-21). Moreover, it remains unclear who should determine medical actionability of variants. Can the treating healthcare professional decide which variants should be considered medically actionable (and therefore eligible for disclosure)? Or should we set up a (local) committee? And should the treating healthcare professional participate in this committee?

Healthcare professionals' views on and experiences with counseling UFs pre-test and UF disclosure might contribute to the evaluation and further delineation of current UF disclosure recommendations (14, 15, 22). Our aim with this study was to obtain insight into the experiences of medical specialists and residents in clinical genetics with UF counseling pre-test and UF disclosure.

Methods

Study design and setting

Using semistructured interviews, we asked certified clinical genetics medical specialists (MS) and clinical genetics residents (R) about their experiences regarding UF counseling pre-test and UF disclosure. The Research Ethics Committee Arnhem-Nijmegen (registration number: 2019-6035) gave permission to conduct this study.

Participants and recruitment

We asked representatives of all eight Dutch genetic centers, who worked together on national recommendations regarding UF disclosure, to recruit eligible peers to participate in this study. The representatives sent the contact details of potential participants to a member of the research team (VS; resident in clinical genetics), who contacted eligible participants. We considered certified clinical genetics medical specialists or clinical genetics residents eligible for participation when they had prior experience in addressing UFs in pre-test counselling. We ensured that the majority of our participants had experience with UF disclosure. All centers disclosed medically actionable UFs in accordance with European standards(6) (Box 1). Most centers held a multidisciplinary deliberation about variant disclosure at their department, which was attended by clinical geneticists, molecular geneticists, ethicists, social workers and psychologists (Table 1; Box 1).

We applied convenience sampling to select participants whilst continuously assessing the diversity of our sample with regard to qualification (i.e. MS or R), years of experience, experiences with UFs and genetic center, thus ensuring a varied sample.

Data collection

To explore participants' experiences with counseling UFs (informing and disclosing) and their views on UF disclosure, two senior researchers(VS and AO) designed an interview guide, with help from a clinical geneticist and senior researcher (HB) and a laboratory geneticist and senior researcher (HY). Our research questions formed the starting point for our interview guide. The guide's focus and wording were chosen based on the authors' clinical experience and literature research. To provide structure to the interviews, the questions were ordered to follow the chronology of the counseling and the disclosure process.

We reassessed and slightly modified this guide after the first interviews to better reflect the aim of our study (see the interview guide in the supplemental content for more details).

Interviews were held between June and August 2020. They took place at a time convenient for participants and were conducted by an experienced interviewer (VS). We used Microsoft Teams (version 1.0, Microsoft Corporation) to conduct and record the interviews. We obtained informed consent prior to each interview.

Data analysis

Recordings were transcribed verbatim and anonymized. We used ATLAS.ti (version 8.2, Scientific Software Development, GmbH, Berlin, Germany) to conduct a content analysis following an inductive approach. Rather than using a predefined hypothesis or codebook, this approach follows an iterative process in which codes, categories and themes are constructed from the data. All transcripts were independently coded by a skilled trainee (CD) and VS. Discrepancies in the analyses were discussed (with AO) until consensus was reached. No relevant differences were observed between certified medical specialists and residents, apart from the fact that clinical geneticists had more experience with disclosing UFs than residents had. This made us decide to combine the data of the two groups for the analysis and reporting. We continued interviewing until we reached data saturation (i.e. when no relevant information emerges and codes only show small variations)(23).

For additional details about the research process, see the COREQ checklist in the supplemental content.

Table 1. Participants' characteristics

		n (20)
Qualification	*certified medical specialist (MS)*	14
	resident (R)	6
Years of experience (in qualification)	1-3	8
	4-9	5
	>10	7
Subspeciality	*DD, MCA**	7
	prenatal	3
	other	3
	no subspecialty yet	5
Number of UFs disclosed	*0*	3
	1-2	12
	3-5	4
	>10	1
Experience with disease category of UF disclosed	*cardiac and oncological*	5
	cardiac	2
	oncological	6
	other	4
	no UFs disclosed	3
Department of Human Genetics	*1*	5
	2	4
	3	3
	4	3
	5	2
	6	2
	7	1
Multidisciplinary deliberation for variant disclosure at department	*default*	16
	upon request	3
	no	1
Direct involvement of healthcare professional in multidisciplinary gathering at department	*yes*	5
	no	15
Policy with offering opt in and opt out at department	*yes*	7
	no	13

Results

We conducted semistructured interviews with 20 participants from 7 genetic centers. We did not include eight further potential participants, since we reached data saturation prior to their participation. We interviewed 14 certified clinical genetics medical specialists and 6 clinical genetics residents in clinical genetics via teleconference. The interviews lasted between 30 and 76min. All but three participants had experience with disclosing UFs (see Table 1 for participants' characteristics).

Pretest counseling: Informing and obtaining consent

All participants reported addressing UFs during pretest counseling. The majority expressed ambivalence regarding informing patients about UFs. On one hand, they considered informing patients about UFs to be an integral part of their job. Those who were asked, denied feeling burdened in doing so. On the other hand, participants mentioned they refrained from elaborating on the topic. Their aim was not to unnecessarily burden patients with information, knowing that the probability of UFs occurring is low. Also, most participants believed that emphasizing this topic would divert attention from aspects of their counseling they considered to be more relevant (i.e., a potential diagnosis, the probability of finding a causal variant). Finally, participants questioned patients' capacity to fully comprehend information regarding this topic and their ability to oversee the potential implications. They felt reluctant to elaborate on UFs, as they felt this effort would be in vain.

> *"But I also think it's impossible to give people a full understanding of what it all means. What are the norms and values you associate with that? You know, you will then have to discuss your views on life, and nobody wants to get into that. It's simply not done." (Medical Specialist; MS; no.17)*

A low educational level or language barrier increased participants' reluctance to engage patients in this complex discussion. A minority of participants said that the religious views of patients sometimes also complicated pretest counseling. In their experience, patients with a strong religious background have a different outlook on the concept of genetics and DNA, which hampers genetic counseling in general.

Additionally, participants questioned patients' ability to comprehend opt-in and/or opt-out options. A majority of participants who work at centers that offer opt-ins and opt-outs, acknowledged that they adopted a more directive method of counseling.

> *"I think that when it comes to pre-test counseling I …quite frankly say, 'Hey, if something's actionable, we'll tell you'. Because with the knowledge we have today, you can actually make a difference. But if there's no possible treatment or nothing else we can do, then we won't*

tell you. And that … I convey this in a pretty directive way—I think—and people accept this." (MS; no.15)

In contrast, only a minority mentioned that they explicitly emphasize opt-in and opt-out options to encourage patients to freely express their preferences.

"It doesn't happen that often, but I really do give people the option [opt-out] because I understand it. I can imagine you'd say okay, I want to know what's causing my heart defect, but I don't want to know if it turns out that I have an increased risk of developing breast cancer, I just don't want to know." (MS; no.13)

In the case of patients leaning toward an opt-in or opt-out, participants said they would further elaborate on the subject. Some consulted their colleagues to ask them for their views on what advice to give.

Participants mentioned that various factors influence the degree of emphasis on UFs and their directiveness in pretest counseling. Most participants considered the clinical value of exome sequencing to have a major influence. When the likelihood of finding a diagnosis was perceived as substantial, participants indicated they were more likely to counsel patients toward the decision to have exome sequencing performed. They said that, in those cases, they would not emphasize the likelihood of detecting UFs, in order to prevent patients from refraining from genetic testing because of this possible outcome. Some participants mentioned that addressing UFs during counseling felt "inappropriate" in high-care settings (neonatal or pediatric intensive care units [ICU]) because they felt that families had a limited ability to cope with additional information in times of great mental stress.

"I don't think it's right to keep people who're already very concerned about a seriously ill child on the ICU occupied for an hour with the ins and outs of our diagnostics. You shouldn't do that." (MS; no.13)

Conversely, when they questioned the value of the genetic test, participants said they were more inclined to elaborate on UFs and/or to counsel toward refraining from (extended) genetic testing. Either way, they acknowledged that the perceived clinical value of the genetic test increased their directiveness in genetic counseling.

Most participants experienced differences between counseling parents of minors or guardians of intellectually disabled patients and competent index patients. They strongly expressed awareness of children's right not to know and their inability to make an autonomous choice at the present time. They wanted to respect the child's incipient autonomy by preventing parents/legal guardians from making a decision that might adversely impact their child, if such a decision could be postponed until that child could decide for himself or herself.

Participants felt more comfortable counseling toward accepting the probability of UFs when they counseled (guardians of) intellectually disabled patients on UFs, compared to counseling parents of minors without an intellectual disability, since the moment when persons in that first group would be able to make a decision autonomously would never come.

Some said they took a more directive approach toward performing DNA testing and accepted the probability of uncovering an UF more easily.

> *"If there will be a time in the future when people can choose at will, I would be very reluctant to deny them that choice. But if that choice isn't going to be there anyway, it would bother me less." (resident; R; no.20)*

Others did not experience differences between counseling minors or intellectually disabled patients regarding UFs.

Most participants emphasized how parents' attitudes affected pretest counseling. They said they provided reassuring information on UFs to parents who felt reluctant to have genetic testing performed because of the possibility of uncovering UFs. Furthermore, participants mentioned they discussed the matter extensively with parents who did not seem to have critically considered the possibility that UFs could be uncovered. Participants felt that parents' urge to find a diagnosis for their children outweighed other implications of genetic testing, such as detecting an UF.

> *"They really wanted to know what was going on, but they were very afraid of any unsolicited findings. In my view that seemed rather unrealistic, which is why I was able to help them with their question. I couldn't take away their fear, but since that fear had increased so dramatically in their minds, I was able to deal with their other question, probably without stumbling over the hurdles they were so afraid of. And that balance needed to be—yes, I probably changed that balance somewhat by providing them with more information, but it only started to tip when a diagnosis was required in school." (R; no.18)*

Participants indicated that engaging both parents in the process was important but challenging, especially in the case of parents who were divorced.

UF disclosure: Deciding to disclose and posttest counseling

A multidisciplinary decision-making process regarding UF disclosure was generally highly valued. Most participants appreciated sharing each other's expertise in and experience with UFs. The degree to which participants felt involved in the decision-making process varied. Some mentioned that, on rare occasions, they diverged from the advice on UF disclosure that had been given.

Most participants did not attend multidisciplinary meetings when an UF found in their patient was discussed (Table 1). Those who did attend, appreciated doing so. It enabled them to provide information about the patient's context, which could be taken into consideration during decision making.

A majority of participants who did not attend mentioned they expected to feel uncomfortable attending the meetings. They anticipated a potential conflict of duties if they were to be involved. These participants imagined finding it difficult having to withhold information that might be clinically relevant. This feeling was articulated by a minority of participants who had attended a meeting during which an UF in their patient was discussed. An opt-out by the patient was thought to complicate this position further. When imagining this situation, one medical specialist said:

> *"I also find it quite hard when I'm aware that a patient doesn't want to know about any unsolicited findings, not even actionable ones, and a BRCA2 mutation has been found and I know that she should get screened. I find that a very difficult position. I'd rather not know." (MS; no. 5)*

The concept of medical actionability was mentioned as one of the most, and for some the most, important factor(s) for consideration when deciding whether or not to disclose an UF. Most participants used this concept in their pretest counseling to indicate what a patient could expect if an UF would be uncovered. However, they found it difficult to apply the term when actually confronted with an UF.

Some participants described situations in which the UF was considered medically actionable by multidisciplinary review, while they themselves perceived this differently (i.e., a variant in *COL3A1*, predisposing to cardiovascular Ehlers-Danlos syndrome).

> *"I found that very difficult in this case and the committee did agree on considering this a treatable condition. But I still think it is, well, there are also plenty of aneurysms that you cannot treat or that rupture between checkups. […] So then, how treatable is it really?" (R; no.2)*

Some participants indicated that these posttest experiences affected their perception of the definition of "medically actionable." It made them realize how ambiguous medical actionability could be; something they did not address in their pretest counseling. Overall, participants expressed a need for a national policy on UF disclosure, including a clear definition of medical actionability.

Other factors mentioned for consideration when deciding on UF disclosure were the severity of adverse health outcomes, the physical impact of screening, and the psychological impact of knowledge of the UF. A minority mentioned taking into consideration potential consequences for patients' families (i.e., potential health benefits and/or the psychosocial impact).

Uncertainty about the expression, penetrance, and age of onset of the disease was said to complicate the weighing of the abovementioned factors when considering UF disclosure. Participants particularly questioned the clinical relevance of UFs in the absence of phenotypic expression in their patient or in the patient's family.

Conversely, participants frequently gave examples of conditions about which they had no doubts when considering disclosure. These mainly concerned variants predisposing to inherited breast and ovarian cancer.

> *"I found it relatively easy because it concerned a gene [ATM] for which guidelines are in place. You can provide your patient with clear advice regarding preventive measures. That makes it easier." (MS; no.4)*

Participants described that they disclose UFs with great care. They emphasized the potential psychological impact on patients caused by receiving this information. The disclosure of UFs with potentially disputable benefits was considered harmful to patients.

> *"Practice has shown that there are cases where things turn out to be more complicated. In these cases, a healthcare provider like myself would be inclined to, you know, report this, just to be on the safe side as it were, and so that something might be done with it, potentially. But I seriously wonder whether that would actually help these people." (R; no.9)*

Participants' perspectives on UFs changed after having disclosed an UF. The majority of clinical geneticists were less concerned about potential UFs. In their experience, patients to whom they had disclosed an UF eventually appreciated the fact that this knowledge had been shared. This experience was shared by the more experienced residents.

Participants mentioned they appreciated receiving information about the follow-up of their patients. Some said they hoped to learn from these cases, while others felt personally involved. A minority stressed that providing aftercare was essential. They felt responsible for burdening patients with a UF disclosure and offered psychosocial support.

Overall, participants outsourced the clinical work-up (i.e., family testing, clinical follow-up) by referring patients to an expert regarding the condition toward which the UF is predisposed (a geneticist or another medical specialist). They said they did not feel up to the task of providing the required care. Generally, they expressed having great confidence in their colleagues.

Discussion

NGS techniques are now widely implemented in genetic testing, but the potential of these techniques to uncover UFs has an impact on the practice of genetic counseling. To our knowledge, this is the first study focused on healthcare professionals' views and posttest experiences with UFs. Our findings provide unique insight into how medical specialists and residents in clinical genetics experience UFs in clinical care.

Pretest counseling: Informing and obtaining consent

Our results show that experienced geneticists and residents currently agree that discussing the potential of detecting UFs is an integral part of providing diagnostic exome sequencing. Yet, they often chose not to elaborate on the subject during pretest counseling. They questioned the ability of patients to understand the meaning and consequences of UFs, especially when opt-in and opt-out options are offered. Irrespective of the perceived level of understanding, they tended to simplify the information and adopt a more directive approach. This was particularly evident in situations where they felt that a long and complex discussion was beyond the coping ability of the patient (e.g., parents of a child at the NICU), and/or when they presumed that testing would yield major health benefits for the patient.

These results suggest that providing information for the purpose of enabling deliberate decision making and obtaining informed consent is complex, potentially restricting the autonomy of patients(13). This raises questions about the desirability and feasibility of providing the same information to all patients in the context of informed consent, as is usually assumed when guidelines are formulated. Experts have argued that instead of providing every patient with standardized information on UFs, clinicians should offer personalized information regarding UFs(24), balancing comprehensiveness and comprehensibility(16). With this in mind, several alternatives to a fully informed consent have been explored(25-27). These alternatives conclude that at least, patients should be informed about the probability of uncovering UFs. Clinicians ought to provide extended information on UFs based on the patient's wish to receive and ability to process more information, which will partly depend on patients' clinical context(28).

Our study reflects that genetic counseling may require varying degrees of a directive approach when a genetic counselor's primary goal is to support a patient's decision making(22,24). Instead of focusing on the transfer of information, genetic counseling should be thought of as a dialogue(16, 29, 30), aimed at enabling patients to make decisions consistent with their goals, values, and beliefs. This dialogue approach allows counselors to consider the patient's urge to find a genetic diagnosis while guiding patients toward a tailored choice for genetic testing. Exploring patients' values pretest enables an assessment of actionability based on counselees'

perceptions of what would be valuable information to them. Only through personalization of pretest counseling, opt-in and opt-out options might increase patients' autonomy.

UF disclosure: Deciding to disclose and posttest counseling

Participants struggled with the concept of medical actionability and recognized that the concept lacks a uniform definition and interpretation(17, 31-33). Through direct experience with an UF with unclear or limited actionability, such as a predisposition for a less penetrant vessel disease with dubious screening options, participants became aware that the concept of medical actionability was less clear-cut than what they had presented to patients during pretest counseling.

Participants highly valued the installation of an expert panel to help participants decide on actionability. Also, they tremendously appreciated the opportunity to consult peers about providing follow-up for UFs.

Our results underline that, while the concept of "medical actionability" is broadly accepted as an important criterion for feedback of UFs, clinical geneticists experience considerable uncertainty in the actual application of this concept in clinical practice. Based on these findings, we believe that a further debate among healthcare professionals about what exactly constitutes medical actionability is urgently needed in addition to research on how the patients themselves perceive actionability. Pending such information, a possible way forward would be to ask patients how they appreciate medical actionability during the consent process and, based on this conceptualization, withhold or disclose UFs.

Our study strongly endorses the value of an expert panel to relieve clinicians of bearing the sole burden of responsibility for what would and what would not be relevant to patients based on what was discussed during pretest counseling. Participants valued the expertise of other clinical geneticists, molecular geneticists, ethicists and social workers, and/or psychologists. It may be worthwhile to involve clinicians and laboratory staff with expertise in the condition and variant to provide insight into the consequences of the finding and the pathogenicity of the variant. Their involvement might be of special value when no consensus on actionability has been reached. We believe that the patient's clinician should also take part in panel meetings to leverage the knowledge gained during the consent process with the patient. Additionally, as UFs are still relatively rare and experience with UFs is generally sparse, the expertise of such panels will be useful for future consultations and follow-up care on UFs. Nevertheless, empirical studies on UFs(14) and studies on the clinical relevance of UFs are urgently required(32, 33).

Strengths and limitations

Limitations of our study include the risk of selection bias as a result of using a convenience sampling strategy and its relatively small sample size. Participants were asked to take part on

a voluntary basis, which might have caused an unintentional selection of clinical geneticists with affinity for the topic. Even though our study is small, the sample size was nevertheless sufficient to reach data saturation. All participants were recruited from Dutch genetic centers, which might have limited generalizability of our results for settings beyond the Netherlands. To enable readers to assess whether our data are applicable to their practice, we have provided participants' characteristics (Table 1).

Strengths of our study include its in-depth approach, diverse sample, and double- and, on occasion, triple coding of the same content, which improved interpretation and decreased subjectivity. We safeguarded internal validity by assessing interpretations during interviews. The COREQ checklist in the supplementary data provides details about the research process.

Practice implications

Our findings have several implications for counseling UFs pretest and UF disclosure policy. Clinical geneticists were uncertain about how to inform patients and about what information to disclose pretest. Instead of focusing on obtaining a fully informed consent, the emphasis of pretest counseling should be on exploring patients' values and beliefs. With this in mind, seeking consent for tests with the potential for UFs requires a certain level of competency. Consequently, counseling UFs pretest might imply specific training needs. Participants struggled with the concept of medical actionability as well. Our results suggest that a multidisciplinary panel with expertise in UFs may be installed to support clinicians in their decision-making process.

Research recommendations

Participants expressed uncertainty throughout the interviews. Uncertainty in clinical genetics has been studied previously(34-38). This has led to recommendations for future studies and guidance for counselors who face uncertainty(34, 37). Gaining more insight into the role of uncertainty regarding UFs could be of added value to recommendations regarding counseling UFs pretest and UF disclosure.

Conclusion

Medical specialists and residents in clinical genetics agreed that discussing the probability of uncovering UFs in genetic testing as an integral part of pretest counseling for diagnostic exome sequencing. They had doubts about the extent to which patients need to be informed and about what information they should disclose. They argued that the content of their counseling should depend on the individual patient's capacity to understand information. These results point to tailored pretest counseling aimed at optimizing genetic consultations. Furthermore, medical specialists' and residents' uncertainty regarding the concept of "medical actionability" underscores the need for further clarification of this concept. The installation of an expert

panel to help decide what variants to disclose will support healthcare professionals who face the dilemmas presented by UFs.

Acknowledgements

We express our gratitude to all participants for engaging in this study by sharing their experiences and thoughts. We would also like to thank the clinicians who contacted their peers for participation in this study. Our special thanks are extended to Dr. E. A. Loehrer for proofreading our manuscript.

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Supplementary data

Consolidated criteria for reporting qualitative studies (COREQ): 32-item checklist

Developed from:

Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. International Journal for Quality in Health Care. 2007. Volume 19, Number 6: pp. 349 – 357

No. Item	Guide questions/description	Our study
Domain 1: Research team and reflexivity		
Personal Characteristics		
1. Interviewer/ facilitator	Which author/s conducted the interview or focus group?	Vyne van der Schoot
2. Credentials	What were the researcher's credentials? E.g. PhD, MD	MD (VS), BSc (Carlijn Damsté), PhD (Anke Oerlemans)
3. Occupation	What was their occupation at the time of the study?	Clinical geneticist in training (VS), student (CD), assistant professor of medical ethics (AO)
4. Gender	Was the researcher male or female?	All female
5. Experience and training	What experience or training did the researcher have?	Experienced interviewer, clinical geneticist in training (VS), trained and experienced coder (CD) experienced interviewer (15+ years) (AO)
Relationship with participants		
6. Relationship established	Was a relationship established prior to study commencement?	VS is has the same role and works in the same field as the participants and had a collegial relationship with some. The majority of participants worked at another genetic center CD and AO did not have a relationship with the participants.
7. Participant knowledge of the interviewer	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research	Reasons for doing the research and professional background.
8. Interviewer characteristics	What characteristics were reported about the interviewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic	VS was a clinical geneticist in training and might have had assumptions based on own clinical experiences. Having interviewed patients who have had an unsolicited finding (UF) disclosed, could have led to assumptions as well.

Domain 2: study design		
Theoretical framework		
9. Methodological orientation and Theory	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis	We conducted a content analysis following an inductive approach. Rather than using a predefined hypothesis or codebook, this approach follows an iterative process in which codes, categories and themes are constructed from the data.
Participant selection		
10. Sampling	How were participants selected? e.g. purposive, convenience, consecutive, snowball	Convenience sampling
11. Method of approach	How were participants approached? e.g. face-to-face, telephone, mail, email	Contacted by e-mail by a peer
12. Sample size	How many participants were in the study?	20
13. Non-participation	How many people refused to participate or dropped out? Reasons?	None
Setting		
14. Setting of data collection	Where was the data collected? e.g. home, clinic, workplace	At the participants' home or work via Microsoft Teams
15. Presence of non-participants	Was anyone else present besides the participants and researchers?	No
16. Description of sample	What are the important characteristics of the sample? e.g. demographic data, date	All participants were medical specialists or residents at the department of clinical genetics. Mostly female. Variation in age, experience and experience with UFs. Additional details in table 1, Participant characteristics.
Data collection		
17. Interview guide	Were questions, prompts, guides provided by the authors? Was it pilot tested?	The authors designed an interview guide. The interview guide was modified with minor revisions of several questions after the first interviews. No pilot study was performed
18. Repeat interviews	Were repeat interviews carried out? If yes, how many?	No
19. Audio/visual recording	Did the research use audio or visual recording to collect the data?	Audio
20. Field notes	Were field notes made during and/or after the interview or focus group?	Yes
21. Duration	What was the duration of the inter views or focus group?	Between 30 and 76 minutes
22. Data saturation	Was data saturation discussed?	Yes, data saturation was achieved

23. Transcripts returned	Were transcripts returned to participants for comment and/or correction?	No
Domain 3: analysis and findings		
Data analysis		
24. Number of data coders	How many data coders coded the data?	Two (VS and CD)
25. Description of the coding tree	Did authors provide a description of the coding tree?	No
26. Derivation of themes	Were themes identified in advance or derived from the data?	Derived from the data
27. Software	What software, if applicable, was used to manage the data?	ATLAS.ti 8
28. Participant checking	Did participants provide feedback on the findings?	No
Reporting		
29. Quotations presented	Were participant quotations presented to illustrate the themes/findings? Was each quotation identified? e.g. participant number	Participant quotations are present, and only identified by category (because of anonymity)
30. Data and findings consistent	Was there consistency between the data presented and the findings?	Yes
31. Clarity of major themes	Were major themes clearly presented in the findings?	Yes
32. Clarity of minor themes	Is there a description of diverse cases or discussion of minor themes?	Yes

2. Interview guide

Start

- *Introduction interviewer and project*
- *Recording and consent form*
- *Introduction by interviewee (hospital, function, years working in function, relation to UFs (qualitatively, quantitively), policy regarding UFs in hospital*

Experiences with pre-test counselling of UFs and UF disclosure

A. Pre-test counseling

- *How do you frame the possibility of detecting an UF? (risk, chance, …)*
- *How do you experience the possibility of detecting and UF? And what makes you experience this in such way?*
- *What do you discuss during your pre-test counselling? And why?*
- *Has this changed?*

B. Deciding to disclose

- *What is your point of view on how to decide which UFs to disclose?*
- *Which factors whould be taken into account according to you and why?*
- *Who should be involved and why according to you?*

C. Receiving UF and documentation

- *How do you receive an UF?*
- *How do you experience receving UFs? And what makes you experience this in such way?*
- *How do you document the UF?*
- *How do you experience this and why?*

D. Post-test counseling

- *How do you communicate the result?*
- *How do you experience this and why?*
- *Has this experience changed?*

E. Follow-up

- *Do you provide follow-up?*
- *How do you experience this and why?*
- *How do you perceive the impact of UFs on patients?*
- *Has this perception changed?*

Concluding remarks

- *Do you have any things unsaid or questions related to this interview?*
- *Short summary*
- *Acknowledgements*

Chapter 6

Exploring uncertainties regarding unsolicited findings in genetic testing

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In preparation for submission

Abstract

Non-normative uncertainty (uncertainty about empirical facts) and normative uncertainty (uncertainty about moral values or beliefs) regarding 'unsolicited findings' (UFs) might play an important role in clinical genetics. Identifying normative uncertainty is of special interest since it might guide towards novel directions for counselling practice. This study aims to gain insight into the role of non-normative and normative uncertainty regarding UFs, as expressed by counselees and counsellors. We performed a secondary qualitative analysis of 40 interviews with counselees and counsellors who had been confronted with UFs. Following a deductive approach, we used an existing theoretical framework of uncertainty, in which we additionally incorporated normative uncertainty.

Major issues of non-normative uncertainty were practical and personal for counselees, whilst counsellors' uncertainty pertained mainly to scientific issues. Normative uncertainty was a major theme throughout the interviews. We encountered the moral conflicts of autonomy vs. beneficence and non-maleficence and of autonomy vs. truthfulness. Non-normative uncertainty regarding UFs highlights the need to gain more insight in their penetrance and clinical utility. This study suggests an important role for moral conflicts as a source of feelings of uncertainty in clinical genetics.

Introduction

Genetic testing aims to identify genetic variants underlying a person's health condition, or health risk. Conventional genetic tests entail targeted testing of one or multiple gene(s) of interest. Next Generation Sequencing (NGS) enables analysis of an individual's complete set of 20,000 genes (Whole Exome Sequencing; WES) or DNA (Whole Genome Sequencing; WGS) (1). NGS has been integrated rapidly into the practice of medicine, replacing targeted genetic tests(2, 3).

Genetic variants can explain why some people are more likely to be affected by disease or to develop certain conditions. Knowing one is at risk enables timely diagnosis of the condition or measures to prevent disease.

Although genetic testing holds the promise of increasing knowledge, uncertainty seems to be inherent to clinical genetics due to results with uncertain significance, uncertainty about prognostic indicators and uncertainty about pathogenicity of variants(4, 5). Uncertainty can be thought of as the conscious awareness of being unsure, of having doubt, or of not fully knowing(6). Within the field of ethics, two main types of uncertainty have been distinguished: 'non-normative' and 'normative' uncertainty. Non-normative uncertainty refers to uncertainty about matters of empirical fact, such as an uncertain significance of a genetic variant(7). Normative uncertainty refers to uncertainty involving a value(8). It has been defined as the question of "what to do when we don't know what [morally] to do"(7). Normative uncertainty among practical comparisons (i.e. is action A better than action B?) arises from conflicting values or competing moral beliefs(8, 9).

Non-normative uncertainty can cause anxiety and might influence decision-making in both patients and physicians(10-12). Additionally, inadequate management of uncertainty may cause unnecessary concern and distress to patients(10).

Non-normative uncertainties are encompassed in a taxonomy of uncertainty within medicine as proposed by Han *et al.*(13), which distinguises three different dimensions (i.e. source, issue and locus). The source of uncertainty refers to the cause or the reason for a knowledge gap (i.e. probability, ambiguity and complexity). The issues of uncertainty are the topics to which uncertainty applies (i.e. scientific, personal, practical), and the locus of uncertainty refers to the person in whom the uncertainty resides (e.g. counselee, counsellor).

Using Han's taxonomy, previous studies have allowed for a better understanding of non-normative uncertainty in the context of genetic testing in general(14), cancer genetics(15, 16), variants of unknown significance(17), prenatal genetics(18, 19), and unsolicited findings (UFs) in imaging(20). They showed that counselees experience uncertainty, mainly regarding practical

and personal issues (e.g. 'how does the blood test work?' or 'could my children develop cancer?' (16, 17)), whilst counsellors expressed more scientific uncertainty during genetic counselling (e.g. 'what is the meaning of the variant that has been found?')(16, 20, 21).

Within the studies on uncertainty in cancer genetics and prenatal genetics, UFs have been identified as one of the sources of uncertainty(14, 16, 19, 20). UFs in genetic testing are variants that are not associated with the condition the genetic test was performed for, but predispose to another health condition and, as such, could be of relevance for the health of the individual and/or of family members(22). UFs have also been referred to as 'accidental findings', 'co-incidental findings' or 'incidental findings'. When actively looked for, additional findings are referred to as 'secondary findings'.

The *probabilistic* nature of UFs (i.e. when will what be found? what will be uncovered?) has been identified as a source of uncertainty in counsellors(14, 16, 19). In addition, since information on genetic variants is generally perceived to be complex and not all information on genetic variants is applicable in the context of UFs, *complexity* and *ambiguity* regarding UFs could contribute to uncertainty related to UFs(23, 24). For example, disease-causing variants in the *BRCA1* gene are found in pedigrees in which women are affected by breast cancer at a young age. Female relatives who harbour the *BRCA1* variant are advised to have themselves screened for breast cancer from the age of 25 or undergo a mastectomy (25). It has not yet been elucidated whether variants create the same risk to develop disease (i.e. 'penetrance') when found in a family in which breast cancer has not (yet) manifested (2, 26). If such asymptomatic families would have lower risks, it would be questionable whether screening and preventive measures are still effective in reducing the risk.

Adding to these scientific issues of uncertainty, the lack of consensus regarding UF policy has the potential to create practical uncertainty(27-29). For example, although it has been recommended to disclose so called 'medically actionable findings', this concept has been criticized for its inexactness(30). An option to 'opt-in' for disclosure of non-actionable diseases and to 'opt-out' to abstain from disclosure of actionable conditions should be considered(31, 32). However, obtaining valid consent and deciding whether to hold back information, will depend on local/national best practice recommendations(Box 1)(33).

The potential of UFs to create uncertainty was implicitly affirmed when interviewing counselees and counsellors about their views and experiences regarding UFs(34; 35). On one hand, they expressed uncertainty related to empirical issues (i.e. probability, complexity, ambiguity). On the other hand, they seemed to express uncertainty related to their moral values and beliefs. The latter type of uncertainty is of special interest, since it cannot be eliminated by obtaining empirical evidence; it ought to be 'managed' instead of resolved(36). Studies on uncertainty in clinical genetics have not explored normative uncertainty(15-19).

Box 1. Dutch National policy regarding UFs

Before the implementation of national consensus guidelines regarding UF policy mid-2021, the eight Dutch genetic centers each had a local policy regarding disclosure of UFs.

In June 2021, national consensus guidelines were published considering three important principles. First, valuable information ought to be disclosed, leading to a default of disclosing variants in medically actionable disease genes. The second is the right to know and not to know, which has led to the implementation of an option to opt-in for non-medically actionable diseases and to opt-out of actionable diseases. Although a multidisciplinary meeting is recommended, in the end it is the clinician's responsibility to decide on disclosure.

Policy rule	Local policy (n=8)	National consensus
Multidisciplinary team meeting (MDTM)	7/8 default 1/8 upon request	Yes
Attending MDTM	8/8 molecular geneticist, clinical geneticist 5/8 ethicist 4/8 legal representative 3/8 social worker 1/8 patient representative	Default molecular geneticist, clinical geneticist Consider ethicist, legal representative, social worker and/or psychologist
Clinician involved in MDTM	4/8	Yes
Opt-in	3/8	Yes
Opt-out	3/8	Yes

Performing WES or WGS increases the probability of uncovering an UF(37). Reflecting on counselees' and counsellors' uncertainties regarding UF could provide a basis for recommendations for future studies and guidance for other counsellors facing uncertainty (15, 18).

With this study we aim to gain insight into the role of uncertainty in counsellors' and counselees' experiences with UFs in genetic testing.

Methods

Study design and setting

We conducted a secondary qualitative data analysis of 40 semi-structured interviews. The interviews were held in the context of two different qualitative interview studies on the impact of UFs in genetic testing: (1) among patients and their relatives to whom an UF was disclosed (from now on referred to as 'counselees')(34) and (2) among clinical geneticists and residents in clinical genetics (hereafter referred to as 'counsellors') (35)

These studies are summarized briefly here.

- *Study 1: The impact of unsolicited findings in clinical exome sequencing, a qualitative interview study*

This study consisted of 20 interviews with index patients, family members and/or legal guardians (participant characteristics can be found in 'Table 1. Participants' characteristics', chapter 4 (pg. 55-56)). Counselees were counselled at the genetics departments of Radboud university medical center (Nijmegen, the Netherlands) or Maastricht University Medical Centre (Maastricht, the Netherlands). By means of convenience sampling, we included counselees in whom an UF was detected predisposing them to either an oncological or a cardiac disease. The interviews were conducted between February and October 2019 by a resident in clinical genetics and by a trained intern under the supervision of a skilled qualitative interviewer. After conducting 20 interviews data saturation was reached (i.e. when no relevant information emerged and codes only showed small variations)(38).

- *Study 2: Views and experiences of clinical geneticists concerning unsolicited findings in next-generation sequencing: "a great technology creating new dilemmas"*

In this study, fourteen medical specialists (MS) and six residents (R) in clinical genetics were interviewed (participant characteristics can be found in 'Table 1. Participants' characteristics', chapter 5 (pg. 79). They were asked about their experiences with counselling UFs pre-test and UF disclosure. Participants were recruited through representatives from all eight genetic centres in the Netherlands. We applied convenience sampling to select participants whilst continuously assessing the diversity of our sample with regard to qualification (i.e. MS or R), years of experience, experiences with UFs and genetic center, thus ensuring a varied sample. Interviews were conducted by a resident in clinical genetics (VS) between June and August 2020. Data saturation was reached after interviewing 20 participants.

Theoretical framework and coding

Prior to analysis, we created a theoretical framework incorporating non-normative and normative uncertainty. Based on studies by Han and colleagues(13, 39, 40), we further specified the different dimensions of non-normative uncertainty (supplementary Table 1).

We created a codebook using the elements of this framework, to enable identification of verbal expressions of uncertainties. The analysis was performed deductively; distinct verbal expressions of uncertainty were identified and coded. Expressions of non-normative uncertainties were coded according to their source (i.e., probability, ambiguity, complexity and competing moral values) and issue (i.e. scientific, practical and personal). We further specified the issues to which uncertainty applied.

Box 2. Example of a verbal expression of uncertainty of a counselee (nr. 15), coded as 'probability' (source) and 'personal' (issue), specified as 'consequences for family members'.

For my own health I didn't have concerns. I did have concerns for [my daughther's] health. What if she would get ill? What if I wouldn't have [the kidney disease] and she would? At least I would be able to donate my kidney to her. But what if both my children would get ill? I only have one kidney to give…

We used qualitative data analysis software ATLAS.ti (version 8.4.2) to facilitate the analysis. An undergraduate student (EvdM) and a research assistant (IM) independently coded the transcripts under supervision of a senior researcher of medical ethics, experienced in qualitative research (AO). A clinical geneticist experienced in qualitative research (VS) subsequently coded all transcripts. Discrepancies in coding were discussed by AO and VS until consensus was reached. All interviews were double coded, six interviews were triple-coded.

Results

We performed a secondary qualitative analysis of 40 interviews with counselees and counsellors who had been confronted with UFs (see Table 1 for participants' characteristics). In all interviews, verbal expressions of uncertainty could be identified. Overall, uncertainty was less evident in the interviews with counselees. Most aspects within the framework were addressed in both groups (supplementary Table 2), but some were only highlighted by either counselees or counsellors. In the following results sections, we discuss each aspect. Representative quotes can be found in table 1.

Table 1. Participants' characteristics

Counselees (n=20)		***n***	**Counselors (n=20)**		***n***
Family/index	*Index*	6	Qualification	*medical specialist (MS)*	14
	Family	11		*resident (R)*	6
	Both	3	Years in current qualification	*1-3*	8
Disease category of UF disclosed	*Oncological*	10		*4-9*	5
	Cardiac	10		*>10*	7
Symptoms of UF in participant	*No*	19	Number of UFs disclosed	*0*	3
	Yes	1		*1-2*	12
				3-5	4
				>10	1

Non-normative uncertainty

Complexity and ambiguity were the main sources of uncertainty expressed. Counselees and counsellors perceived information about UFs to be complex, imprecise or unavailable.

Probability was identified as a source of uncertainty as well: penetrance of disease genes and the effectivity of preventive measures in the context of UFs were commonly identified as uncertain aspects of UFs.

Counselees

Counselees expressed uncertainty regarding several scientific issues. They mentioned being uncertain about when they would develop the condition the UF was associated with. Particularly, the period between the disclosure of the UF and the first time being screened for symptoms of the condition, caused anxiety (see Q1 in Table). Some counselees even chose to visit a different hospital, accelerating their first screening. After screening, counselees did not feel uncertain about being affected with the condition anymore. Some did wonder however, whether the screening interval was too long. Following UF disclosure, uncertainty about the UF's impact on their health was caused by a lack of knowledge about the UF and its consequences (Q2). After counselling and follow-up consultations, uncertainty was caused by contradictory, complex or ambiguous information provided by their counsellors (Q3). Also, counselees were uncertain about whether the UF could explain parts of their own medical history or the medical history of their family members (Q4).

Moreover, counselees expressed practical uncertainties. For example, they wondered what impact the UF could have on their life insurance, or whether or not the finding would increase their deductible (Q5). They questioned whether the UF would have the same impact on their relatives. These concerns made some counselees wonder if and when family members should get tested.

Some counselees reported uncertainty about the care they had received in the hospital. A few counselees mentioned that the physician they were referred to was not aware of the risks associated with the UF and seemed unaware of the guidelines regarding preventive measures. These counselees questioned whether their genetic counsellor had provided this physician with sufficient information (Q6).

The personal issues to which counselees' uncertainty pertained, were how the UF would impact their lives and which financial consequences they were likely to encounter. This made them question their future plans (Q7).

Counsellors

Counsellors mainly expressed uncertainty related to scientific issues. They mentioned probability as a source of uncertainty, when deciding to offer genetic testing. In pre-test counselling, they questioned whether the odds of finding a causal variant would outweigh the probability of uncovering an UF.

Upon disclosure of an UF, counsellors were uncertain whether or not patients would actually develop the condition the UF is associated with. They wondered about the value of a variant when found in a family without medical history regarding the associated condition (Q8). Many counsellors reported uncertainty regarding the concept of medical actionability. For example, they wondered whether reproductive options ought to be considered as "actions" (Q9).

Most counsellors expressed practical uncertainty regarding counselling UFs prior to testing (Q10). They questioned the extent to which patients can fully grasp the information about UFs during pre-test counselling, for example regarding the potential impact on their relatives. In particular, they thought that opt-in (choosing to have non-medically actionable disease variants disclosed) and opt-out (choosing not to have medically actionable disease variants disclosed) options were complex. They expressed major concerns about the capability of patients to oversee their own choices. Patients' context, such as a language barrier, could affect these concerns (Q11). Those who had experience with counselling UFs pre-test and UF disclosure, generally felt less insecure about counselling UFs. The majority of counsellors questioned the feasibility of the policy regarding UFs (Box 1). For example, they wondered how to document test results in patients' medical files when it was decided not to disclose an UF to the patient.

The personal issue to which counsellors' uncertainty pertained was their own capability to decide whether or not to disclose an UF. Some recognized feeling more insecure after having experienced UFs outside the strict scope of medically actionable variants (Q12).

Normative uncertainty

We identified expressions of normative uncertainty in the interviews with both groups. Overall, expressions of normative uncertainty were less prevalent in the interviews with counselees.

Counselees

Counselees expressed uncertainty regarding their responsibility towards their family members. They reported being uncertain about whether it was up to them to decide if it would be in someone else's best interest to learn about the possibility of having the genetic variant. One counselee mentioned a negative experience when sharing the information with a relative (Q13).

Several counselees mentioned they found it difficult to decide whether or not to have their children tested. The right not to know and the potential financial impact were reasons not to (Q14), whilst the right to know and the risk for future offspring were mentioned in favour of testing (Q15).

Counsellors

The majority of counsellors expressed normative uncertainty. Some were not always sure whether performing a genetic test was the right thing to do when considering the small probability of finding a causative variant versus the odds of uncovering an UF. Counsellors indicated that they struggled with the amount of information to give pre-test. They were uncertain whether the value of enabling informed decision-making outweighed the potential negative impact of burdening patients with knowledge about UFs pre-test. Also, they questioned whether the potential benefits of UF disclosure outweighed the burden of knowing to be at risk of developing a certain condition.

With regard to the opt-out option, many counsellors stated that if they could not disclose an UF based on an opt-out consent, they would feel like they would have to lie (Q16). Also, some said withholding beneficial information would not feel right (Q17).

Counsellors reported uncertainty about what and when they ought to decide for their patients. They were not sure whether a patient's autonomy should outweigh potential benefits of UF disclosure (Q18).

Most participants expressed normative uncertainty regarding opt-in and -out options. They questioned whether there could be situations in which they ought to overrule a patient's choice, because they doubted their patients' ability to actually oversee the implications of their choice during the pre-test counselling session. Others mentioned the importance of the potential benefits of UF disclosure for family members (Q18).

Table 2. Exemplifying quotes

non-normative	Counselee	Scientific	Q1	*"Having an UF disclosed can cause anxiety. Tension. Uncertainty. Which we did experience. But we took action to deal with this uncertainty." (family; nr. 1)*
			Q2	*"I went to the hospital for the genetic test results when they told me that I had the BRIP1 gene. My mother and I were both like 'what is that?'. We asked ourselves how to deal with it; is it something serious? Is it not serious? Can doctors do anything, can they remove my ovaries or not...?" (index patient; nr. 5)*
			Q3	*"Because of the contradictory information about the genetic variant, I had a conversation with the clinical geneticist again to clarify the information that was given. (…) The clinical geneticist told us something different than what we had heard before." (index patient; nr. 3)*
			Q4	*"I read what is associated with the UF. 'Low immunity', I have a low immunity; I have always had respiratory infections, (…) It made me wonder, is it related to the genetic variant in any way?" (index patient; nr. 17)*
		Practical	Q5	*"I asked this question to the doctor; 'how do I need to report this to the life insurance; am I sick or not sick?' This is a conflict. Until one year ago, I could say 'I am a healthy person'. And now I'm still healthy but I have this worry, this concern." (family; nr. 2)*
			Q6	*"'I wouldn't know what other examinations we would have to do.', the doctor said. Well, I had all the diagnostic papers from [academic hospital 1] I had already received at home and could show them to this doctor. […] what if I hadn't done that? Would I then have been risking my life, as well as my daughter's?" (family; nr. 1)*
		Personal	Q7	*"For me it was a disadvantage considering I was planning to buy a house. But as long as they don't know…Can I keep this a secret? Can I keep it out of my papers, this UF?." (family; nr. 16)*
	Counselor	Scientific	Q8	*"It's different for conditions that are not fully penetrant. Especially when no one in the family is affected by breast or ovarian cancer or another condition which would be related. Because then one could wonder whether or not this variant affects this family to a lesser extent, with lower associated risks." (R; nr. 1)*
			Q9	*"I think the distinction between actionable and non-actionable is fine. But what is actionable and what is not actionable? Yes, if WES*, for example, reveals Huntington's disease. There is nothing you can do to prevent the disease, but you can prevent your children from getting it" (MS; nr. 5)*
		Practical	Q10	*"I find it difficult to disclose such abstract information, especially when comparing it with presymptomatic testing for BRCA2 mutations. These counselees have 45 minutes to discuss whether or not they want to know. What are the pros? What are the cons? And regarding UFs, the same result can be obtained. But the odds are so small, so I do understand spending limited time on discussing UFs" (R; nr. 20)*
			Q11	*"I am concerned that they don't oversee it. That I don't actually obtain informed consent because they just don't speak the language and the translator doesn't understand it either." (MS; nr. 6)*
		Personal	Q12	*"I highly value multidisciplinary meetings. I know that my opinion is only one of many. And maybe I forgot something. Maybe I didn't think of a certain detail. That just might happen…" (MS; nr. 11)*

Table 2. *(continued)*

normative	Counselee	Q13	*"[The counselee's relative] also feels that I have burdened her with disclosing the UF to her. And of course that's true in a way. I wonder, should I have kept my mouth shut? But I would find it very difficult to, since I know something." (family; nr. 7)*
		Q14	*"We have never performed [DNA testing in our daughter]. We immediately wondered if it would be ethically right to have the test performed. The results would be in her medical file and she would always have problems with insurances and mortgages." (family, nr. 4)*
		Q15	*"If my daughter is a carrier and she would have children, she could pass it on. I think she has the right to know that this could happen." (family, nr. 12)*
	Counselor	Q16	*"If the concerning patient doesn't ask about it, it still doesn't feel right, because you hold back something that was in fact seen. But we think there is no value in disclosing it and not beneficial for the patient. But still, especially if patients start asking about UFs, you feel like you're lying." (R; nr. 2)*
		Q17	*"It creates a moral dilemma; having certain information that can be very important for someone's health when that person knowingly opted out of receiving such information. It creates a feeling of being burdened with information about the UF." (MS; nr. 8)*
		Q18	*"Suppose you find a random BRCA mutation and you know that this patient has a sister and three daughters. Then I would have a moral conflict, thinking: 'shouldn't we tell the family about this?'. That's the tricky part; if a patient has made a certain choice regarding UF disclosure and when you have information that is potentially important for family members. Where does that leave your responsibility?" (MS; nr. 18)*

**** Whole Exome Sequencing***

Discussion

With this study, we have gained insight into uncertainty associated with UFs in NGS experienced by counselees and counsellors. Major issues of uncertainty were practical and personal for counselees, whilst counsellors' uncertainty pertained mainly to scientific issues. Normative uncertainty was a major theme throughout the interviews with counsellors and, although less evident, present in the interviews with counselees as well.

Non-normative uncertainty

UFs were perceived to be complex and ambiguous, as has been described for genetic information in general(5, 14-16, 18). We identified several issues of non-normative uncertainty which have not been addressed in previous publications on uncertainty in genetic testing(15-19). First, counsellors expressed uncertainty regarding whether the probability of finding a genetic cause underlying a patients' condition would outweigh the probability of receiving an UF. Counsellors could benefit from continuous studies on the yield of NGS(41, 42) and the probability of uncovering UFs(37). Second, we identified evident uncertainty

regarding the concept of medical actionability. In the context of UFs, the efficacy and the burden of interventions, together with the probability and severity of an adverse health outcome due to the UF, are often unclear, causing UFs to be ambiguous(2, 23, 24, 26). Until consensus has been reached about how to decide on actionability, variants with ambiguous actionability (for example, when screening protocols have not proven to enable early detection in order to start treatment) should be disclosed with great caution(43). Third, we saw that the additional condition the UF predisposes to, raised new questions regarding potential financial consequences. None of the counselees we interviewed had experienced any actual financial consequences, reflecting general experiences in clinical genetics in the Netherlands(44). Our study stresses the need for follow-up studies on UFs that elucidate the clinical utility and impact of UF disclosure(2, 23, 24, 26).

Normative uncertainty

Our results suggest normative uncertainty plays an important role in counsellors' and counselees' perspectives on UFs. We encountered the moral conflicts of autonomy vs. beneficence and non-maleficence and of autonomy vs. truthfulness.

Moral conflicts

Counselees struggled with the idea of deciding for family members whether to have certain information disclosed. This reflects awareness of the right not to know and the desire to respect decisional autonomy and/or to protect others from receiving unwanted and potentially harmful information (non-maleficence), while knowing information could be beneficial (beneficence)(45).

Counsellors expressed normative uncertainty regarding the amount of information they should provide prior to testing. In the context of UFs, the emphasis on enabling decisional autonomy lies in obtaining informed consent during pre-test counselling(45, 46). Informed consent refers to the permission granted in full knowledge of the possible consequences and is treated as the core of medical ethics(47). It has been acknowledged that the techniques that are currently used in clinical genetics create a situation in which a patient can become overwhelmed by the complexity and volume of the information given(47). Counsellors' struggle with pre-test counselling reflects the moral conflict of autonomy vs. non-maleficence.

Other authors have described this conflict in the context of UF disclosure before(45). Interestingly, qualitative research showed that although most counselees expressed considerable psychological impact initially, almost all would in hindsight choose to undergo genetic testing again(34, 48). This suggests that presumed potential harm ought not to be a reason to refrain from UF disclosure. However, no non-actionable disease genes were disclosed to these counselees. Also, the potential harm of unnecessarily exposing patients to preventive measures ought to be considered as well. Normative uncertainty was most

frequently expressed when discussing opt-in and opt-out options. Counsellors thought that withholding potential beneficial information or disclosing burdening genetic test results would create tension with the intention to respect patient autonomy.

Managing normative uncertainty

More than non-normative uncertainty – which might partly be resolved by gaining more knowledge - normative uncertainty needs to be managed(36). How to manage normative uncertainty is still a topic of debate(36, 49). Cribb describes that "only some of this [normative] uncertainty is deliberately and self-consciously managed through professional ethics, or other overt ethics discourses, but that much is implicitly managed through forms of social organisation and routine practice (i.e. 'moral settlements')"(36). Exploring the moral settlements of counselees and counsellors, is needed to identify the role of these settlements in navigating counselees' and counsellors' normative uncertainty. Insights in their role might provide guidance to counsellors and their peers on how to manage normative uncertainty regarding UFs more deliberately.

The uncertainty about whether or not to share genetic information with a family is a general issue in clinical genetics(17, 50-54). However, existing recommendations regarding informing relatives at risk are practically focused and do not address normative uncertainty(55). Very few studies have surveyed patients and their family members about the ethical dilemmas they have faced(49, 56). These authors have noticed that although ethics consultations are sought by clinicians, these consultations do no originate from patients' requests(49). They recommended to more closely involve ethics consultants to better guide patients who face ethical dilemmas(49, 57).

Based on our findings, we would recommend a thorough follow-up of counselees to whom an UF is disclosed, bearing the counselees' potential uncertainties in mind. In particular, counsellors' need to explicitly address counselees' insecurities regarding the financial impact and informing and/or testing family members. When counsellors identify uncertainty in their counselees, they could consider to engage ethic consultations as a supportive resource.

Uncertainty regarding informed consent has led to discussions that tend to focus on the content of the information provided(47, 58, 59). However, information transactions depend on various counselee- and counsellor-specific factors (e.g. information can be seen as context- and norm-dependent)(59). Since patients' internalisation of information depends on more than information that has been provided, it has been argued to focus on the quality of information transactions rather than on their content(59). This approach fits the much older view on genetic counselling, according to which decision-making capacity is maximally enhanced by means of a dialogue(60). In order to enable counsellors to engage in this dialogue when counselling UFs

pre-test, Manson and O'Neill propose substantive changes on institutional and governmental levels (i.e. they propose to stop adhering to "ever more exacting informed consent forms" and suggest regulators should judge medical performance by the quality of communication that is achieved)(59).

Regarding UF disclosure, we have previously recommended to have a multidisciplinary team meeting to guide decisions on UF disclosure(35). This multidisciplinary approach relieves the counsellor of bearing the sole responsibility in potential moral conflicts and enables counsellors to reflect on their struggle. This struggle includes decisions on disclosure of potentially unwanted and harmful information and counsellors' potential feeling of being untruthful to their patients.

Strengths and limitations

Our study had several strengths and limitations. For some counselees, the interview took place several years after the UF had been disclosed which could be of influence on how they reflected on uncertainty (recall bias). For this study, we conducted a secondary analysis of interviews that did not specifically address the topic of uncertainty. When the interviewee expressed uncertainty, the interviewer did not necessarily ask in-depth follow-up questions. This might have negatively impacted data quality, since expressions of uncertainty were not always explored in depth. For instance, we did not try to make participants differentiate between complexity and ambiguity as sources of uncertainty. However, we feel this differentiation did not affect the value of our findings regarding these sources of uncertainty.

Strengths of this study include the systematic analysis according to a theoretical framework, which allowed comparison of uncertainty between counselees and counsellors. We performed double and on occasion triple coding of the same content, improving richness of interpretation. The COREQ checklists of both studies in the supplementary material provides additional details about the research process(61) (supplementary material chapter 4 and 5, pg. 69,70 and 91-93)

Conclusion

Normative and non-normative uncertainty regarding UFs are evident in counselees and counsellors who are confronted with UFs. They will benefit from gaining more insight in the prevalence, nature and impact of UFs through further qualitative and quantitative studies on UFs. This study suggests a major role for moral conflicts as a source of uncertainty in clinical genetics in general.

Practice implications

In order to obtain valid informed consent, counsellors should focus more on engaging in a dialogue pre-test, rather than on the content of information transactions. During post-test

counselling, counsellors need to explicitly address counselees' insecurities regarding the financial impact and informing and/or testing family members. Multidisciplinary team meetings to guide decisions on UF disclosure allow counsellors to reflect on the uncertainties they face.

Acknowledgements

We express our gratitude to all participants for engaging in this study by sharing their experiences and thoughts. Our special thanks are extended to Ms. I. Maassen for assisting in the secondary analysis.

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Supplementary table 1. Theoretical framework

Non-normative(39)	**Issue**	*Scientific*	The scientific issue refers to uncertainty about the diagnosis, prognosis, explanation of the causality of the condition and recommendations on treatment options.
		Practical	The practical issue refers to uncertainty resulting from the quality of care and procedures of care.
		Personal	The personal issue refers to uncertainty resulting from personal relationships and life goals.
	Source	*Ambiguity*	Ambiguity is defined as the absence of credibility, reliability or appropriateness. For example, the result of a test can be interpreted in several ways. Another reason for ambiguity may be that the result is vague, incomplete, inconsistent or contradictory.
		Complexity	Understanding the situation or outcome can be difficult because the material is complex. This includes situations where it is difficult to determine the causal causes or effects of a situation. The fact that it is difficult to understand lies in the complex phenomenon itself, but is also subject to the judgment of the person dealing with the phenomenon.
		Probability	Probability means that it is not certain whether a situation will occur in the future. This pillar therefore is related to the chance of getting a disorder. This plays a major role in genetic research because finding a mutation does not necessarily mean that the affected person will actually get the disease.
Normative *(7-9)*			Uncertainty arising from competing values or conflicting moral beliefs among two comparisons (i.e. is action A better than action B?).

Supplementary table 2. Issues regarding which uncertainty exists

Non-normative						Normative	
Scientific		Practical		Personal			
Counselee	*Counsellor*	*Counselee*	*Counsellor*	*Counselee*	*Counsellor*	*Counselee*	*Counsellor*
• Risk of becoming ill • Effectiveness of preventive measures • Impact on family members' health • Relation UF and medical and/or family history	• Odds of finding a causal variant • Odds of uncovering an UF • Expression and penetrance of UFs • Effectiveness of preventive measures in the context of UFs	• Policy regarding UFs (FU) • Impact on daily life • Financial impact • Impact on family members' health	• Policy regarding UFs (pre-test, disclosure, FU) • *[Influence of patient context (e.g. language barrier)]*	• Health impact • Preventive measures • Financial impact	• Medical actionability • One's own expertise • *[Influence of experience with UFs]*	• Responsibility towards family	• Responsibility of informing • Not willing to be untruthful • Not disclosing actionable UFs • Disclosing non-actionable UFs • Burdening

FU: Follow-up; UF: Unsolicited Finding

Chapter 7

Discussion

This thesis aimed to gain more insight in the potential of UF disclosure by assessing the nature of UFs and SFs, the probability of uncovering UFs, and evaluating counselees' and healthcare professionals' views and experiences concerning UFs.

Summary of results

After analysing WES data of 1,640 anonymized healthy Dutch individuals we conclude that 2.7% of these individuals have a (likely) pathogenic variant in a medically actionable dominant disease gene from the ACMG59 list (chapter 2). The majority are variants predisposing to cardiac disease and oncological disease. In addition, we found 2.2% to be a carrier of a recessive disease from the same list. Since these variants are actively looked for, they are considered SFs.

By contrast, UFs are variants that are not actively looked for but are inadvertently found. Among 16,482 index patients receiving clinical WES over a 5 year period, the frequency of UFs in 'ACMG59' was substantially lower than the prevalence of SFs (0.59% vs. 2.7%) (chapter 3). We attribute UFs' lower rate of detection to variant prioritization and interpretation strategies. The odds of UFs in all disease genes (not restricted to the 'ACMG59' list) range from 0.03% to 1.03% for analysis of disease-gene panels and the entire exome, respectively. We observed that UFs, defined by an *ad-hoc*, case by case review of medical actionability, affected a broader range of genes than listed on 'ACMG59'.

In order to assess the perceived impact of UF disclosure, we conducted 20 in-depth interviews with patients and/or their relatives to whom a UF was disclosed. We encountered a psychological, physical and financial aspect of the perceived impact of UF disclosure (chapter 4). Overall, the perceived impact would not keep patients from undergoing genetic testing again, knowing what they know now. The importance of patients' pre-test health, the potential of understanding the finding, and the actionability of the UF were highlighted throughout the interviews.

Also, we evaluated clinical geneticists' and residents' views on and experiences with UFs in clinical genetics. Analysis of 20 semi-structured interviews regarding UFs showed that they regarded discussing the probability of detecting UFs to be an integral part of providing diagnostic exome sequencing (chapter 5). They did express doubts about what, and to what extent, they should inform patients during a pre-test counselling session. Also, they struggled with the concept of 'medical actionability'.

Uncertainty was an important theme throughout the interviews with both groups. We performed a secondary analysis of the interviews from chapter 4 and 5, to better understand the different sources of uncertainty. Whilst patients and their relatives mainly expressed uncertainty about practical and personal issues, for healthcare professionals, main sources of

uncertainty were scientific issues (chapter 6). Besides these 'non-normative' issues, normative uncertainty (i.e. based on values and beliefs) was present throughout the interviews.

In order to further improve the understanding of the relevance and impact of UFs and SFs in the context of clinical genome analysis, three overarching themes deserve attention.

First, variant classification and interpretation in the context of UFs and SFs will be discussed. Second, the concept of medical actionability will be considered. And lastly, informed consent in the context of UFs and SFs will be further explored.

Seeing; classification and penetrance

After Amélie and her parents had their blood drawn for the genetic test (Introduction), Amélie's clinical geneticist sent the material and the request to the laboratory. The laboratory specialist who analysed the exome data of Amélie and her father noticed a variant in the MYBPC3-gene. The variant caught her eye because it was a 'loss-of-function' (LOF; when function of the gene is lost) variant, it was listed as pathogenic by multiple databases and the clinical genetics laboratory had previously classified the variant as disease-causing. Because she did not have any experience with classifying variants in MYBPC3, she asked her colleague with expertise in classifying variants predisposing to cardiac disease for help. He told her this variant has been found in multiple patients with hypertrophic cardiomyopathy co-segregating with the condition in their family. Also, LOF is a known disease-causing mechanism of MYBPC3-variants, associated with hypertrophic cardiomyopathy. Although she did not have any clinical details concerning heart disease in Amélie's family, she decided to classify the variant as 'disease-causing'.

Variant classification

In diagnostic genetic testing, classification of variants is based on several criteria. In order to determine if a variant could be disease-causing, the variant itself is assessed (e.g. based on computational and predictive data, functional data) and it is evaluated in which individuals it has been reported (e.g. population data, segregation data, *de novo* data, phenotypic data of the index)(1, 2). For example, a predicted null variant (e.g. when the function of the gene is lost) in a gene where LOF is a known mechanism for disease is a very strong indication that this variant is disease-causing. In contrast, when the allele frequency of the variant is very high in healthy controls, the variant is unlikely to be disease-causing.

Variant interpretation and prioritization tools are based on variant characteristics. Bioinformatics pipelines enable efficient and systematic variant analysis (supplementary material of chapter 3). Some variant characteristics however, need additional interpretation by reviewing literature or using additional databases. Missense variants (i.e. genetic variants that generate protein variants with a single amino acid variation) may induce structural alterations which compromise protein stability or binding interfaces which impairs protein function(3). Literature on phenotypic expression, functional studies and additional computational tools are often needed to classify a missense variant as disease-causing.

The genetic test that the clinic chooses to perform determines how many genetic variants need to be classified. When a patient presents with an evident phenotype (for example: extremely short long bones, cloverleaf skull, differential diagnosis thanatophoric dysplasia), analysis of the gene of interest (*FGFR3)* might suffice. If the phenotype fits a more heterogenous condition (e.g. when variants in multiple genes could have caused the condition) the clinician likely requests analysis of all genes associated with this condition. For example, when the patient presents with a short neck, pulmonary valve stenosis, and short stature, the differential diagnosis of

Noonan syndrome asks for analysis of all genes associated with Noonan syndrome (*PTPN11, SOS1, RAF1, …).* If a patient presents with a phenotype that the clinician cannot directly relate to one or multiple genes of interest, like Amélie's case, an even broader analysis might be requested, e.g. analysis of all genes known to be associated with congenital anomalies or even analysis of the entire exome or genome. Extended genetic testing implies analysis of hundreds to thousands of genes bearing numerous variants. The odds of uncovering variants that are not directly associated with the condition for which the genetic test was performed will increase when analysing a broader set of genes.

Classification in the context of UFs and SFs

Our results show that the classification criteria enabled classification of variants without a matching phenotype (chapter 2; chapter 3). Regarding UFs, variants in well-known disease genes (i.e. *BRCA1*) and known disease-causing variants (i.e. listed by OMIM, LOF variants) seemed more likely to be noticed by laboratory specialists (chapter 3).

These results have led to three conclusions regarding the analysis of UFs and SFs. Regarding SFs, they argue in favour of analysing a pre-set gene list such as the ACMG suggests when pursuing SFs. This seems to be required to make sure genes that could harbour relevant variants are analysed, and that which genes are analysed does not depend on the person performing the analysis. UFs are generally found outside the disease genes that are most associated with the clinical phenotype of the patient. This implies that laboratory specialists may not have experience with the genes in which UFs are found. In these cases, the assessment of UFs' pathogenicity and relevance by a second specialist, preferably one with expertise in classifying variants in the specific gene, should be considered. Lastly, our findings highlight that not every disease-causing variant will be noted. This is especially true for UFs, when the focus of the analysis will be on finding a causative variant. Counselling about UF disclosure should not suggest that all genetic variants will be seen.

Interestingly, new software has been developed which allows successful diagnosis of genetic disease using computational phenotype analysis of the disease-associated genome. This technique enables variant prioritization based on clinical features provided(4). In this way, which variants ought to be classified does not depend on which analysis is requested, but relies on a description of a patient's phenotype. Theoretically, when using this technique, we would expect fewer UFs to be revealed. The extent to which implementation of this technique will affect the nature of UFs and the probability of uncovering UFs however, is yet to be discovered. Before recommendations on UFs can be adapted accordingly, the nature and frequency of UFs when using computational analysis of the disease-associated genome should be analysed.

Penetrance

Before NGS techniques were used in genetic testing, patients with a certain phenotype underwent (targeted) testing for genes associated with the clinical condition. Phenotypic data are, as such, based on ascertained data.

This applies to penetrance of genetic disease (proportion of affected individuals who harbour the familial variant), which is based on affected families. For example, a large prospective cohort study on the risks of developing breast and/or ovarian cancer for *BRCA1*- and *BRCA2*-carriers included databases of affected families(5). If the same *BRCA1*- and *BRCA2*-variants were to be found in an asymptomatic family, carriers would be told these variants predispose to disease because they had been found in affected individuals previously. They would be given risk estimates based on the number of affected carriers in the affected families, regardless of potential disease-modifiers. The same goes for the potential of medical interventions. Their reported effectiveness is mainly based on the effectiveness in symptomatic individuals with a definitive diagnosis or at-risk asymptomatic individuals(6) and has not been studied in asymptomatic families yet.

Penetrance in the context of UFs and SFs

Concerns have been raised about the interpretation of disease-related risks in the context of UFs and SFs(6-12). Several studies ground the hypothesis that the value of genetic variants found in asymptomatic individuals is disputable. As in our study (chapter 3), Ormondroyd *et al.* showed SFs can be expected to be identified in individuals without a phenotype(13). Special attention has been given to variants predisposing to cardiac disease(9-11, 14, 15), as these variants are known to display reduced penetrance and phenotypic variability(16, 17). The interpretation of variants in genes predisposing to oncological disease in the context of UFs and SFs has been discussed as well(12, 18-20).

Misinterpreting penetrance of variants has the potential to cause downstream harm to patients(8). If, for example, a disease-causing *BRCA1*-variant is disclosed to a 28-year old woman, she is told she has a lifetime risk of 60-80% to develop breast cancer(21). When exploring preventive measures, she might consider having a mastectomy performed. If this risk were to be significantly lower - for example due to the presence or absence of certain disease modifiers - she might decide otherwise. And if this variant would not increase her risk of developing breast cancer at all, no additional medical interventions would even be recommended. In cardiogenetics, invasive measures are often only indicated when a patient presents with symptoms. However, the burden of periodic cardiac screening cannot be justified when there is no increased risk of developing cardiac disease.

Recommendations have been published on how to interpret disease-related risks in the context of UFs and SFs(9, 22). These include incorporating the patient's phenotype, family history and using an interdisciplinary approach with clinicians experienced in the condition. This would allow "contextualization" of risk estimates in the context of UFs and SFs(7, 23). Acknowledging the impact of disease modifiers on penetrance(9-11, 14, 15) has even led to the suggestion that individuals with a cardiac UF should undergo a thorough clinical and laboratory evaluation before the UF can be considered disease-causing in their specific case(9).

These suggestions could be a starting point for best practice guidelines on the clinical interpretation of UFs and SFs. However, the ever changing knowledge of genetics, and thus of UFs and SFs, makes frequent reconsideration essential. For example, polygenic risk scores, aiming to stratify unaffected women for breast cancer risk, lead to large absolute risk differences for *BRCA1*- and *BRCA2*-cariers(24). Perhaps, in the near future, how disease modifiers attribute to an individual's risk to develop a certain condition will be elucidated, allowing for a personalized risk assessment.

Clinical relevance of UFs and SFs

The current thesis did not pursue follow-up on the medical relevance of UFs. We learned about follow-up only from a subset of individuals. We found a single symptomatic participant (i.e. the mother of an index patient in whom a variant predisposing to oncological disease was uncovered).

Performing systematic follow-up in families where a UF or an SF was disclosed would be of great value in order to establish the clinical relevance of UFs and SFs. This follow-up should comprise assessment of the phenotype, the uptake of medical interventions, health outcome and quality of life of these individuals. Since UFs and SFs are rare, there is a need for collaboration to collect these data. In particular, studying the utility of findings other than variants predisposing to cardiac and oncological disease will benefit from sharing knowledge and experiences regarding these UFs and SFs, as they are infrequently uncovered.

Telling; medical actionability

The laboratory specialist told Amélie's clinical geneticist that she had found a disease-causing MYBPC3-variant in Amélie and her father. The variant was discussed in a multidisciplinary setting, involving a clinical geneticist and a laboratory specialist with expertise in cardiogenetics, an ethicist and a psychologist, to advise Amélie's clinical geneticist on disclosure. The cardiogeneticist explained that people with a LOF variant in MYBPC3 have a medical indication to undergo cardiac screening from the age of ten. After careful deliberation, the panel concluded this to be a 'medically actionable' variant and advised the counsellor to disclose the variant to Amélie's parents.

Medical actionability

The concept 'medical actionability' has been a major point of discussion in the context of UFs and SFs. It is highly appreciated as a criterion for UF and SF disclosure by different stakeholders(25-27) and, as such, has been addressed frequently in worldwide debates on this subject. However, the term medical actionability is known for its lack of terminological uniformity and interpersonal variability in interpretation(7, 8, 28-30).

In their recommendations on UF and SF disclosure, Berg *et al.* (2011)(1) recommended disclosure of variants carrying a high likelihood of disease (e.g. monogenic, highly penetrant disease), for which medical interventions could significantly reduce morbidity and mortality(7). Morbidity is defined as "the state of being symptomatic or unhealthy for a disease or condition" and mortality refers to "the number of deaths caused by the health event under investigation"(8). Berg's definition has been adopted by the ACMG and others (e.g. Amendola *et al.* (2015)(9) and Dorschner *et al.* (2013)(10)) for the disclosure of SFs.

In contrast, less restricted definitions include the definition used by Yang *et al.* (2014)(11), which considered variants medically actionable when "there are potential therapies or established surveillance protocols available". In other studies, healthcare providers acknowledge a widened scope for medical actionability, which includes almost any action that can be taken based on the knowledge of bearing a genetic variant, including reproductive decision-making(27, 31).

In order to systematically assess actionability, Hunter *et al.* developed a protocol to assist in determination of actionability(32). Assessing five domains (i.e. severity, likelihood of disease, effectiveness of interventions, nature of interventions, state of the knowledge base) enables prioritization of genes for reporting UFs and SFs and offers a constructive and objective approach to actionability assessment. However, the authors acknowledge this protocol does not address certain important factors, such as personal utility (e.g. reproductive options) and patient perspectives on the (burden of) interventions.

Hunter's methodology partly relies on the inevitable relation between a variant's potential to cause disease and the actions that could be taken to prevent such disease. Since variants with no potential to cause disease are by definition not UFs or SFs, this paragraph will focus solely on medical actionability in the context of (likely) pathogenic variants.

The value of actionability

The importance of medical actionability as a criterion for disclosure was affirmed by counselees who had been confronted with UFs (chapter 4). Patients and their genetic healthcare professionals did seem to have a different understanding of actionability. Generally, patients valued "more concrete" interventions (e.g. drug therapies, prophylactic surgery)(33). In contrast, effectiveness of screening and the burden of preventive measures were not addressed as having impact on how they perceived UFs' actionability.

Importantly, how they perceived the actionability of the condition they were tested for, impacted how they perceived the actionability of the UF that was disclosed. For them, actionability seemed to be a relative concept; they made relative comparisons between the actionability of the conditions to which the UFs predisposed and the actionability of the conditions they were affected with. For example, in a child with drug-resistant epilepsy (a condition for which no medical actions have so far improved or cured symptoms), periodic cardiac screening because of a UF might feel like an action with true utility, thus making this variant seem more actionable in relative terms.

Clinical geneticists (in training) acknowledged the ambiguity of the concept of medical actionability (chapter 5; chapter 6). When deciding whether or not to disclose a UF, they experienced practical difficulties in assessing its actionability. They appreciated a multidisciplinary expert panel to guide them in the decision on disclosure. Our results show how an ad hoc, case by case, multidisciplinary deliberation can widen the scope of actionability; we found 39% of UFs disclosed resided in genes that are not listed by the ACMG59 (chapter 3). Specifically, six variants were disclosed that may allow individuals to undergo medical interventions, aiming at providing guidance during the course of disease rather than disease prevention (e.g. early-onset deafness).

However, some clinical geneticists expressed doubts about the utility of certain interventions offered to patients to whom they had disclosed a UF, disputing the UF's presumed actionability. Healthcare professionals' hesitations could lead to ambiguous counselling and unwarranted practice variation, creating uncertainty in their counselees. Therefore, the uncertainty about the interpretation of UFs and SFs potentially limits healthcare professionals in enabling counselees to understand the potential health consequences of these findings. This was confirmed by counselees' experiences of the ambiguity of how medical specialists provided follow-up and

by the uncertainty that counselees expressed about UFs' associated risks, especially by those who had been confronted with a cardiac UF (chapter 4; chapter 6). Inadequate information and guidance from healthcare professionals due to the complexity of UFs and SFs could endanger the fulfilment of their actionability.

In summary, our findings provide insight in how actionability relates to a patient's specific context, and they show how the fulfilment of actionability depends on the healthcare professional's perception of a variant's actionability.

Personal actionability

More than adhering to the concept of medical actionability as a concrete characteristic of a certain genetic variant, medically actionable findings might be considered as findings with a perceived clinical utility in a specific context. In such an approach, the perceptions of the counselee and the healthcare professional are indispensable when deciding on a variant's actionability. In practice, this would imply that healthcare professionals' decisions on actionability should be grounded on their patient's perception, their own perception, experts' opinion and precedents (figure 1).

Gaining insight into patients' perceptions of actionability might be the most challenging. Kater-Kuipers and colleagues have suggested how to promote deliberate decision-making in genetic testing(34). They propose to first "explore [patients'] values, discussing with them why they do or do not want to know about genetic disorders (…). This might enable [patients] to make their values explicit in the context of this decision." Deeper understanding of patients' life priorities and backgrounds is believed to require competencies in eliciting and analysing patient narratives(35). With the implementation of NGS techniques in genetic testing, training regarding communication might need more focus on achieving these competencies in the context of UFs.

Healthcare professionals' own perception of variants' actionability will partly depend on their experience (with UFs or SFs), as we saw in our study (chapter 5). Multidisciplinary meetings allow them to reflect on their perception, to learn from relevant expertise and to gain experience with variants and their potential actionability. As such, these meetings provide a platform to weigh all important aspects relevant for the case, and then decide on the specific variant's actionability.

While deciding whether or not to disclose a UF, healthcare professionals anticipated that if they did not disclose the UF, they would feel like they were lying to the patient or not acting in the patient's best interest (chapter 5). Incorporation of the healthcare professionals' view on actionability might enable them to feel differently about their involvement in the decisional process of disclosure.

Also, this approach might decrease their feelings of uncertainty when assessing actionability (chapter 6). It would also be of interest to re-evaluate healthcare professionals' views on and experiences with UFs after incorporating this method in UF disclosure policy.

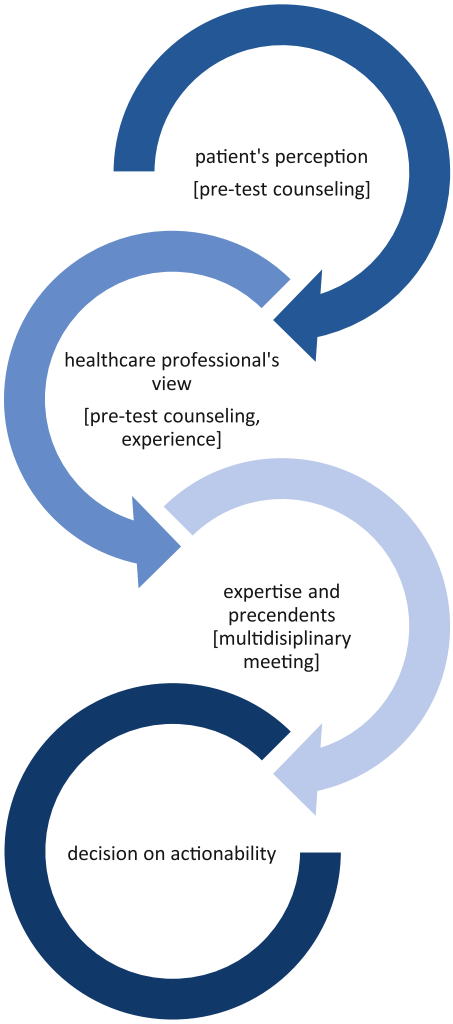

Figure 1. Schematic representation of an approach how to decide on actionability

Listening; informed consent

When hearing about the UF, Amélie's parents were in shock. They had completely forgotten about the possibility of uncovering such a finding. The news came as a surprise, since no one in their families was affected with heart disease. They found it difficult to understand what this finding meant for the health of Amélie and his father. Their clinical geneticist told them that they had been counselled pre-test on the possibility of detecting a UF, whereafter they gave consent to hear findings based on the ability to take clinical action regarding the finding. Amélie's parents said they could now indeed recall this conversation. They did not, however, anticipate such a result. They wondered which actions could be taken, other than "only screening once every few years…". Their clinical geneticist had the same thoughts. Was this actionable 'enough'? What if they would never develop heart disease? What was their risk, as a family without a history of cardiomyopathy? And should she have prepared them better for such a result? Should she have elaborated more on possible UFs during their pre-test counselling? Or maybe she should not have spoken so unequivocally about actionability?

After his first cardiac screening, which fortunately did not show any signs of cardiac disease, Amélie's father felt more at ease. However, sometimes he still wonders "Am I sick or am I healthy…?".

Informed consent

Genetic counselling can be defined as "the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease"(36). Counselling should integrate "interpretation of family and medical histories…", "education about inheritance, testing,…", and promotion of "informed choices and adaptation to the risk or condition"(36).

Informed consent refers to "the permission granted in full knowledge of the possible consequences". It is commonly considered the core of medical ethics(37). Informed consent reflects the ethical principles of autonomy, by allowing for self-determination, and non-maleficence, by protection against harmful situations(38). Legally, in order to obtain valid consent, the patient has to be competent, the implications of a test or treatment have to be discussed and understood, and the consent has to be given voluntarily(38).

Providing information

From an ethical and legal perspective, providing information is essential in order to ensure informed consent. There is international consensus that "At a minimum, prior educational materials/videos should be made available describing and distinguishing the typology of genomic tests including the one proposed to the patient"(39). In the context of UFs, information about the probability of uncovering a UF, its nature, impact, financial consequences and consequences for blood relatives could be of relevance to make a deliberate choice regarding disclosure. Indeed, a study by Wright *et al.* showed that having learned potential consequences,

participants of focus group discussions changed their attitudes towards receiving genetic test results(40).

Clinical geneticists find it difficult to decide when to provide information on UFs, what information to provide and how to provide all information necessary (chapter 5; chapter 6).

First, they indicated that whether or not they would provide information on UFs depended on the type of genetic test they wanted to perform (small panel, large panel, open exome analysis).

Second, they do not aim for a full and explicit consent because of the complexity and volume of information that they then should provide. It has been acknowledged that techniques currently used in clinical genetics create a situation in which a patient can become overwhelmed by the complexity and volume of the information given(37). Additionally, the medical setting influences counselees ability to retain the information provided (chapter 4). Importantly, we saw how information about UFs causes uncertainty regarding UFs (chapter 5; chapter 6). Addressing uncertainty during pre-test counselling is needed in order to enable informed decision-making, but this has been shown to have harmful effects on patients(41). Healthcare professionals tend to provide simple and explicit details about UFs. For example, they often provide examples that are generally known (cancer, dementia)(chapter 5;(42)) to illustrate the nature of UFs. On the other hand, healthcare professionals with experience in disclosure of UFs became aware that the information on UFs they had been providing during pre-test counselling, had been to clear-cut (chapter 5).

Lastly, healthcare professionals indicate they do not have enough time to provide all relevant details about UFs during pre-test counselling (chapter 5; (43)). Healthcare professionals recognize that a lack of time is a limitation that could compromise full, specific informed consent(43).

Exploring alternatives

Although several alternatives to a full, specific informed consent are being explored (e.g. a 'layered consent'; providing information in stages (layers)(38), or a 'broad consent'; restricting details of information (44)), it has been argued to move away from the focus on the disclosure of information in discussions about consent(37, 45, 46).

According to Manson and O'Neill, the focus on disclosure of information and decision-making ignores what is actually needed to think about informed consent(46). They believe that in these discussions, information is seen as discrete units, pre-existing and content-independent ('containers'). Whilst according to them, information is context- and norm-dependent, propositional, audience-sensitive and inferentially fertile (i.e. a statement may convey more than words). Their metaphorical wording makes the view of Samuel *et al.,* who call for

considering informed consent from a relational point of view(45), more concrete. The latter argue that, more than based solely on the information given, decisions are always embedded in a patient's social, cultural, emotional, personal and family context. How patients relate to the nature and severity of the condition and how they relate to their clinicians, is important for decision-making(45).

Manson and O'Neill's viewpoint does not ignore the essential role of information in genetic counselling and in informed consent(36), but it focusses on how to provide this information. Their 'agency model' proposes to look at the act of informing as making 'transactions' instead of providing containers. Successful transactions would be intelligible (i.e. understandable), relevant, adequately accurate (i.e. contextualised) and truthful. The quality of these transactions is secured by the dialogue between patient and healthcare practitioner, who are both accountable for informed consent, according to Manson and O'Neill(46).

Their approach to informed consent very well fits the much older view on genetic counselling in general. White (1998) reconfigured the interpretation of autonomy and suggested a model of genetic counselling by dialogue(47). He proposed a shift from seeing autonomy as a negative right ("a client's right to non-interference in decision-making") to a positive right ("the right to a maximally enhanced decision-making capacity"). The aim of genetic counselling – enabling patients to make decisions consistent with their goals, values and beliefs - in this view requires a dialogue to explore patients' values.

Consent in an agency model would at least respect (some version of) individual autonomy(46). As Bester *et al.* argue(37), Manson and O'Neill agree that autonomy is "only one among a number of important ethical requirements in biomedical practice". Autonomy in itself will not always justify actions. For example, potential harmful effects of uncertainty caused by information about UFs might justify limiting the information disclosed. The agency model distinguishes morally permissible acts from morally unacceptable acts in the disclosure of information, and act upon other ethical obligations as well.

Implications for counselling UFs

Looking at informed consent as informational transactions in a dialogue between a patient and a healthcare practitioner, urges us to focus on the quality of these transactions, rather than solely on the content of disclosure.

Regarding the complexity and volume of information, engaging in a dialogue will allow healthcare professionals to tailor provision of information in such a way that it is understandable and does not overwhelm the counselee. When contextualizing information, special attention should be paid to counselees' medical background. Most patients who are counselled for genetic testing using NGS techniques are individuals who are affected by rare and/or life-

threatening conditions or who have affected offspring. They have an urge to find the diagnosis and opt for genetic testing hoping this adds valuable information about how they can live the rest of their lives (chapter 4). When information is not adequately contextualized, the patient might selectively recall information. Aiming for relevance and truthfulness, the counsellor could limit the information about UFs when only analysing a restricted gene panel because the probability of finding a UF is lower. For example, patients could be informed that there is a low probability (chapter 3) that genetic testing will unintendedly reveal a genetic predisposition unrelated to the clinical question. Specifically, when offering panel analysis, healthcare professionals should be aware of specific panel content (chapter 3).

Redirecting the focus of informed consent practices will require a significant change in the culture that defines current frameworks (e.g. societal, legislative)(box 1). Although acknowledging the substantiveness of these changes, Manson and O'Neill do propose several changes that should be made by either governmental or institutional bodies(46). For example, they propose to stop adhering to "ever more exacting informed consent forms" and suggest regulators should judge medical performance by the quality of communication that is achieved. Their propositions are needed to strive collectively for cultural change.

Uncertainty

Successful transactions might lead to identification of different sources or issues of potential (normative) uncertainty about UFs in patients and/or their family members. This allows healthcare professionals to navigate their patients' uncertainty.

Non-normative uncertainty might be resolved, for example by providing more information. Normative uncertainty ought to be managed, rather than resolved(51). How to manage normative uncertainty has not been clarified(51). Cribb *(2019)* argues "that only some of this [normative] uncertainty is deliberately and self-consciously managed through professional ethics, or other overt ethics discourses, but that much is implicitly managed through forms of social organisation and routine practice". This compels reflection on the "moral settlements" counselees and healthcare professionals find themselves in and the role they have in managing their normative uncertainty. Future studies on normative uncertainty in clinical genetics could provide insight into how patients manage this uncertainty and might identify a potential role for healthcare professionals to offer guidance. In the absence of clear guidance for healthcare professionals' role in managing normative uncertainty, identifying and acknowledging normative uncertainty seems to be essential(52).

Box 1. Ethics of conviction

White's suggestion to approach genetic counselling as a dialogue dates back to as early as 1998. Why do healthcare professionals still seem to struggle with consent when they are unable to provide all information in fullest detail(chapter 5; chapter 6)? And why do interventions tend to focus on the informational part (i.e. when to provide which information)(39)? Instead of appreciating the potential of a dialogue, there seems to be an unspoken desire to define and check what ought to be disclosed.

The work of Max Weber(48) is helpful for understanding why there is this tendency to focus on checklists. It also clarifies why such an approach limits the ability of healthcare professionals to consider their responsibility to their patients' values(49, 50). According to Weber, there are two types of social action: instrumentally rational social action, which is oriented by goals, and value-rational social action, which is oriented by a belief in a value. Someone who acts purely value-based, does not pursue a certain goal. The act is based on a conviction. These two types of social action are reflected in two ethical orientations; ethics of responsibility and ethics of conviction. Although it has been proposed that these two ethics are complementary to each other, the more dominant point of view argues they cannot coincide(50). Moreover, Weber argues we cannot freely choose how to act. Already in 1946, he describes a pluralistic society in which the diversity of perspectives cannot seem to lead to a transcending and connecting point of view. Without a universal conviction to act upon, we are forced to act based on responsibilities.

Implications for mainstreaming and direct-to-consumer testing

Genetic tests are increasingly requested by medical specialties other than clinical geneticists (i.e. 'mainstreaming') and can even be accessed without involving a healthcare professional (direct-to-consumer testing (DTC genetic tests)).

Patients counselled for genetic testing in oncology expressed to be satisfied with the information they received(53). Evaluation of consent in mainstreaming seems to focus predominantly on the informational aspect, whilst the quality of information transactions has been left unexplored. This calls for further studies when genetic testing is being offered by other specialties.

In DTC genetic testing, often no pre-test counselling is performed. Obtaining true informed consent in mainstreaming and DTC does not seem feasible, which urges us to be cautious with UF (or SF) disclosure in these contexts.

Hide or seek?

The results of this thesis argue that disclosure of UFs when using NGS is feasible and could be beneficial for the counselee. However, several conditions have to be met:

- Variant classification and interpretation of disease-related risks in the context of UFs should be approached differently than in the context of a matching phenotype. The clinical context should be taken into account when interpreting UFs.
- When, based on available knowledge and expert opinion, a variant is found to be disease-causing in that specific context, its medical actionability should be evaluated based on the patient-specific actionability and the healthcare professional's perception on actionability, in combination with expertise in and experience with the variant.
- UF disclosure should be guided by the dialogue of pre-test counselling, in which the quality of informational transactions shapes informed consent. This dialogue should continue after UF disclosure, creating awareness about counselees' uncertainties to optimize genetic counselling.

These conditions might apply to disclosure of SFs as well, especially considering the potential reduced penetrance of these variants. Although a pre-set gene list might be required for SF disclosure, a case by case assessment of patient-specific actionability and the required consent dialogue would be of value in the context of SFs as well. Additionally, when deciding to actively look for additional variants, other – societal – values are at stake(54). A public debate on SFs could enable incorporation of societal preferences in SF policy. If society indeed values pursuing SFs, but the field has not elucidated how to interpret SFs, one could argue that healthcare professionals should refrain from actively screening an individual's genome or should restrict to screening a pre-set gene list, while acknowledging the limitation that not all actionable variants will be uncovered.

Recommendations for future studies

This thesis has led to several recommendations for future studies:

- Using computational phenotype analysis of the disease-associated genome will affect nature and frequency of UFs. It would be of interest to assess the nature and frequency of UFs when using this type of analysis. This could be a replica of our study on the nature and frequency of UFs (chapter 3).
- In order to gain insight in the clinical utility of UFs, it would be of great value to follow-up on families in which a UF or SF has been disclosed by assessing the phenotypic expression,

the uptake of medical interventions and eventually, the health outcome of these patients. Preferably, an (inter)national collaboration among genetic centres would be established in order to share knowledge and experiences regarding UFs and SFs.

- Observational studies on whether or not counselees' values can be adequately identified during pre-test counselling and how this could guide UF disclosure, would be of added value when establishing best practice recommendations regarding UF disclosure.
- Future studies on normative uncertainty in clinical genetics could provide insight into how counselees and healthcare professionals manage this uncertainty and might identify a role for healthcare professionals to offer guidance.
- Reflecting on communication skills and ethics education could identify training needs for healthcare professionals who perform genetic counselling in order to be able to optimize informational transactions and manage normative uncertainty.
- The abovementioned conditions could apply to every context in which (extended) genetic testing is performed. It would be of added value to study how and whether the abovementioned conditions can be met in the context of mainstreaming and DTC genetic testing.

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Appendices

Summary

Next-generation sequencing techniques are increasingly incorporated into clinical care. This technique enables analysis of specific regions of interest in the genome (targeted NGS), of all coding regions (Whole Exome Sequencing; WES) or even the entire genome (Whole Genome Sequencing; WGS). With the use of NGS, the probability of uncovering unsolicited findings increases compared to when using more targeted techniques in which less DNA is analysed. Unsolicited findings in DNA testing are (likely) pathogenic variants which are unrelated to the initial clinical question for which the test was performed, but which could be of relevance for patients and/or family members. Unsolicited findings which are 'coincidentally' found ('UFs'), are differentiated from findings that are actively sought for ('secondary findings'; SFs). UF and SF disclosure have been the subject of a worldwide debate. The American College of Medical Genetics (ACMG) recommends to actively look for medically relevant variants in over 70 genes. The European Society of Human Genetics (ESHG) and the Canadian College of Medical Genetics (CCMG) argue not to actively look and to be cautious with disclosure of these variants. In order to reflect on these discussions and to evaluate previously proposed recommendations, potential benefits and burdens of these findings need to be identified. The main aim of this thesis was to report on the nature and frequency of unsolicited findings, and to evaluate their perceived impact on counselees and healthcare professionals. This led to five studies, which are concisely summarized below.

Chapter 2 reports on the frequency of medically actionable disease alleles in the healthy Dutch population following the ACMG recommendations. We analysed 59 genes that were considered medically actionable in 2018 ('ACMG59') in 1,640 individuals. In 2.7% of these individuals we identified a (likely) pathogenic variant in a medically actionable dominant disease gene. The majority had a variant predisposing to a cardiac disease or to oncological disease. In addition, 2.2% carried a recessive disease variant. These results show the potential consequences of actively looking for actionable genetic diseases.

Chapter 3 describes UFs identified in patients receiving clinical WES. Over a 5 year period, 16,482 index patients received clinical WES. The odds of retrieving a UF were 0.03% when analysing a restricted gene panel and 1.03% when analysing the entire exome. The frequency of UFs in 'ACMG59'-genes was substantially lower (0.59% vs. 2.7%) with a large fraction of variants predisposing to oncological disease. A substantial part of the UFs identified in this study was in genes that are not listed in 'ACMG59'. This broadened scope of medical actionability derived from the ad-hoc, case by case review of medical actionability that was applied in our centre.

In **chapter 4** the results from qualitative research on the impact of UFs on patients and/or their family members are described. We conducted 20 semi-structured interviews with patients and/or their family members to whom a UF had been disclosed. Overall, the perceived impact was low; the experience would not deter participants from undergoing genetic testing again,

The perceived actionability played a major role in this assessment. Participants compared the actionability of the UF with the actionability of the condition for which the genetic test was performed. The urgency of finding a genetic diagnosis seemed to affect the perceived impact of the UF. Participants said that once they learnt more about the meaning and consequences of the UF, the worries they had concerning the finding decreased. Lastly, participants' social context played a role in how the impact of the UF was perceived. These findings highlight the value of incorporating patients' perceptions in UF disclosure policy. Particular, attention needs to be paid to patients' pre-test health and their perception of actionability.

Chapter 5 describes the results of 20 interviews about views on and experiences with UFs with certified clinical genetics medical specialists and clinical genetics residents. All were working in seven Dutch centres for genetics. Geneticists indicated that they regarded discussing the probability of detecting UFs to be an integral part of pre-test counselling. They did express doubts about what they should communicate to patients during a pre-test counselling session. This emphasises the importance of tailored pre-test counselling alongside informed consent for optimal genetic consultations. Also, geneticists struggled with the concept of medical actionability. A multidisciplinary panel to reflect on actionability helped them in deciding on UF disclosure. This study underscores the importance of defining what exactly constitutes medical actionability. Based on these results, we recommend a multidisciplinary team to help healthcare professionals face the dilemma's UFs might present.

In **chapter 6** we explore expressions of uncertainty of patients and/or their family members and geneticists. We performed a secondary analysis on the interviews from chapter 4 and 5. Uncertainty was expressed by both groups. In general, the sources of uncertainty differed. Whilst patients and their relatives mainly expressed uncertainty about practical and personal issues (e.g.: what is the financial impact?), for geneticists, the main sources of uncertainty were scientific issues (e.g.: what is the penetrance of this variant in this family?). Besides these 'non-normative' issues, normative uncertainty (i.e. based on values and beliefs) was present throughout the interviews. These results show the importance of exploring uncertainty after UF disclosure.

Based on the findings presented in this thesis we conclude that UFs present a challenge for patients, their family members and healthcare professionals, even if the actual probability of uncovering a UF is low. For UF disclosure, several conditions have to be met.

Chapter 7 elaborates on these conditions: correct variant classification and interpretation in the context of UFs and SFs, clear definition of what constitutes medical actionability and informed consent for UFs and SFs.

First, variant classification and interpretation of disease-related risks in the context of UFs should be approached differently than in the context of a matching phenotype; it has been suggested that in the absence of a clinically affected family member penetrance of pathogenic variants may be lower than in families where the disease has already manifested. UFs' medical actionability should be evaluated based on the patient-specific actionability and the healthcare professional's perception of actionability, in combination with expertise in and experience with the variant. Finally, UF disclosure should be guided by the dialogue of pre-test counselling, in which the quality of informational transactions shapes informed consent. This dialogue should continue after UF disclosure, creating awareness about counselees' uncertainties to optimize genetic counselling.

These conditions might apply to the disclosure of SFs as well. Until the field has achieved consensus on how to interpret SFs, one could argue that healthcare professionals should refrain from actively screening an individual's genome. If society indeed values pursuing SFs, this should be restricted to screening a pre-set gene list. Following such policy, healthcare professionals and patients have to be aware of the fact that not all actionable variants will be uncovered.

In conclusion, we do not have to hide unsolicited findings in next-generation sequencing but we should be cautious with seeking them. For now, we should embrace what is still to be learned about this topic and let the dialogue with the patient be leading in how to approach unsolicited findings.

Samenvatting

Voor DNA-onderzoek wordt steeds meer gebruik gemaakt van next-generation sequencing (NGS). Deze techniek maakt het mogelijk om gericht naar meerdere genen te kijken (targeted NGS), naar alle coderende regio's (Whole Exome Sequencing; WES) of zelfs naar het volledige DNA (Whole Genome Sequencing: WGS). Met het gebruik van NGS neemt de kans toe dat er een nevenbevinding wordt gedaan, ten opzichte van meer gerichte genetische testen, waarbij naar minder DNA wordt gekeken. Nevenbevindingen bij DNA-onderzoek zijn (waarschijnlijk) pathogene varianten die niet gerelateerd zijn aan de reden waarom de genetische test werd gedaan, maar wel relevant kunnen zijn voor de patiënt en diens familieleden. Er wordt onderscheid gemaakt tussen nevenbevindingen die 'per ongeluk' worden gevonden ('unsolicited findings'; UFs) en waar actief naar wordt gezocht ('secondary findings'; SFs). In de afgelopen jaren is er veel discussie geweest over hoe het beste omgegaan kan worden met nevenbevindingen. Het American College of Medical Genetics (ACMG) adviseert om actief te zoeken naar medisch relevante bevindingen in meer dan 70 genen. De European Society of Human Genetics (ESHG) en het Canadian College of Medical Genetics (CCMG) beargumenteren daarentegen om juist terughoudend te zijn in het mededelen van dit soort bevindingen en er niet actief naar te zoeken. Om beter te kunnen reflecteren op deze discussies en beleidsvoorstellen, was meer inzicht nodig in potentiële voor- en nadelen van nevenbevindingen. Het hoofddoel van dit proefschrift was om de aard en frequentie van nevenbevindingen in kaart te brengen en de ervaren impact van nevenbevindingen door patiënten en zorgverleners te verkennen. Met dit doel zijn vijf studies verricht, waarvan een korte samenvatting volgt.

Hoofdstuk 2 beschrijft bij hoeveel procent van de gezonde Nederlandse populatie een genetische variant wordt aangetoond als het beleid van het ACMG zou worden gevolgd. We analyseerden 59 genen die volgens het ACMG in 2018 behandelbaar werden geacht ('ACMG59'), bij 1.640 gezonde individuen. Bij 2.7% van deze gezonde individuen werd een genetische aanleg aangetoond voor een autosomaal dominant overervende aandoening. Voor het merendeel van de geteste personen betrof dit een aanleg voor een cardiale aandoening en voor een ander groot deel een variant predisponerend voor een oncologische aandoening. Daarnaast bleek 2.2% drager te zijn van een recessief overervende aandoening. Door deze studie hebben we inzicht verkregen in het gevolg van actief zoeken naar behandelbare erfelijke aandoeningen.

In **hoofdstuk 3** hebben we in kaart gebracht hoe vaak er tijdens WES-analyses een nevenbevinding wordt gevonden. Gedurende een periode van vijf jaar werd bij 16.482 indexpatiënten WES verricht. De kans op een nevenbevinding was 0.03% bij een gerichte en 1.03% bij een bredere analyse. De kans dat er een nevenbevinding werd gedaan in een ACMG59-gen was kleiner (0.59% vs. 2.7%) en er werden relatief veel varianten gevonden die predisponeren voor oncologische aandoeningen. Een groot deel van de genen die als behandelbaar werden beschouwd, stond niet op de ACMG59-lijst. Onze verklaring voor de

ruimere invulling van het concept behandelbaarheid is de ad-hoc, case-by-case-review van behandelbaarheid.

Hoofdstuk 4 beschrijft de uitkomsten van een kwalitatief onderzoek naar de impact van een nevenbevinding op de patiënt en/of diens familie. We interviewden 20 patiënten en/of diens familieleden die met een nevenbevinding geconfronteerd zijn. Over het algemeen was de ervaren impact laag; bijna alle deelnemers zouden wederom genetisch onderzoek laten uitvoeren, wetende wat ze nu weten. Het feit dat zij de nevenbevinding als behandelbaar beschouwden, speelde daarbij een grote rol. Zij vergeleken de behandelbaarheid van de nevenbevinding met de behandelbaarheid van de aandoening waarvoor het DNA-onderzoek werd uitgevoerd. De grote wens om een genetische diagnose voor de gezondheidsklachten te vinden leek invloed te hebben op de ervaren impact. Ook bleek uit de interviews dat het belangrijk was dat deelnemers de bevinding begrepen om onzekerheden over de bevinding te verminderen. Tot slot speelde de sociale context een rol in de impact die deelnemers ervaarden. Deze uitkomsten benadrukken het belang van het meewegen van de context en het perspectief van de patiënt en/of diens familieleden in het beleid omtrent nevenbevindingen. In het bijzonder dient er aandacht te zijn voor de gezondheid van de patiënt en diens visie op wat behandelbaarheid inhoudt.

Hoofdstuk 5 beschrijft de resultaten van 20 interviews met klinisch genetici (in opleiding) uit zeven Nederlandse genetische centra over hun ervaringen met en visie op nevenbevindingen. De genetici gaven aan dat ze het informeren over nevenbevindingen zien als integraal onderdeel van de counseling voorafgaand aan een genetische test. Ze vertelden dat ze het lastig vinden om te bepalen wat zij over nevenbevindingen moesten vertellen tijdens de pre-test-counseling. Deze resultaten tonen het belang aan van op maat gemaakte pre-test-counseling en informed consent. Daarbij worstelden deelnemers met het thema behandelbaarheid. Een multidisciplinair panel om dit concept per bevinding te toetsen hielp hen bij de besluitvorming tot mededelen. Deze studie toont het belang van een concretere invulling van het concept behandelbaarheid. Op basis van deze resultaten adviseren we het inzetten van een multidisciplinair panel om zorgverleners te helpen met de dilemma's die nevenbevindingen met zich mee kunnen brengen.

In **hoofdstuk 6** verkennen we de onzekerheid die patiënten en/of diens familieleden en genetici ten gevolge van nevenbevindingen uitten. We hebben een secundaire analyse uitgevoerd op de interviews uit hoofdstuk 4 en 5. Alle deelnemers uitten onzekerheid in de interviews. Over het algemeen verschilde de aanleiding van de onzekerheid tussen patiënten en/of diens familieleden en genetici; de eerste groep uitte onzekerheid over praktische en persoonlijke zaken (bv. wat is de financiële impact?), terwijl bij genetici de onzekerheid voort leek te komen uit wetenschappelijke aspecten (bv. komt een genetische variant wel tot uiting in deze familie?). Naast deze 'niet-normatieve onzekerheid', uitten beide groepen ook een onzekerheid die voort

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leek te komen uit twijfels over wat het goede is om te doen op basis van normen en waarden ('normatieve onzekerheid'). Deze resultaten tonen het belang aan van het verkennen van de onzekerheden van patiënten na het mededelen van een nevenbevinding. Morele conflicten zijn mogelijk een bron van onzekerheid bij klinisch genetische vraagstukken in het algemeen.

Op basis van dit proefschrift concluderen we dat nevenbevindingen een uitdaging vormen voor de patiënt, diens familieleden en zorgverleners, ook al is de kans op een nevenbevinding klein. We stellen dat er voor het melden van nevenbevindingen aan enkele voorwaarden dient te worden voldaan.

Hoofdstuk 7 beschouwt deze voorwaarden: een juiste interpretatie van genetische varianten in de context van nevenbevindingen, een betere invulling van het concept behandelbaarheid en het kunnen verkrijgen van informed consent voor nevenbevindingen.

Genetische varianten die als nevenbevinding worden aangetroffen zullen anders benaderd moeten worden dan varianten die de klinische verschijnselen van de patiënt kunnen verklaren. Er dient rekening gehouden te worden met het ontbreken van de aandoening bij de patiënt en/of diens familie waarbij de nevenbevinding wordt gedaan; mogelijk liggen de gezondheidsrisico's lager in deze families. Het concept behandelbaarheid lijkt op basis van onze resultaten lastig toe te passen in de praktijk. Het is belangrijk om deze term invulling te geven samen met de patiënt en gebruikmakend van de expertise van een multidisciplinair panel met ervaring met nevenbevindingen. Tot slot lijkt het naar aanleiding van deze studies nodig om informed consent in de context van nevenbevindingen nader te verkennen. In plaats van te focussen op het informatie-aspect, zou de kwaliteit van de dialoog tussen zorgverlener en patiënt meer aandacht moeten krijgen. In het bijzonder dient de potentiële onzekerheid van de patiënt in ogenschouw genomen te worden.

Deze voorwaarden gelden mogelijk ook voor SFs. Zolang het nog niet opgehelderd is hoe SFs geïnterpreteerd moeten worden, dient er terughoudend om te worden gegaan met het actief screenen van iemands genoom. Indien er een maatschappelijk draagvlak blijkt te zijn voor SF-rapportage, zou de rapportage zich moeten beperken tot varianten in een vooraf vastgestelde lijst van genen. Zowel zorgverlener als patiënt dienen zich dan te realiseren dat niet alle behandelbare varianten gezien zullen worden.

Concluderend hoeven we nevenbevindingen bij next-generation sequencing niet te verbergen, maar is terughoudendheid geboden met naar ze te zoeken. Vooralsnog moeten we omarmen wat we nog kunnen leren over dit onderwerp en dient de dialoog met de patiënt leidend te zijn bij hoe we omgaan met nevenbevindingen.

Sommige ziektes komen door een verandering in het erfelijkheidsmateriaal. Met DNA-onderzoek wordt naar deze veranderingen in het erfelijkheidsmateriaal gezocht. Bij DNA-

onderzoek kan het gebeuren dat er toevallig veranderingen worden gevonden die niet de ziekte veroorzaken waarvoor DNA-onderzoek werd gedaan, maar wel kunnen leiden tot een andere ziekte veroorzaken. Deze per toeval gevonden veranderingen noemen we nevenbevindingen.

Nevenbevindingen kunnen belangrijk zijn. Het kan bijvoorbeeld gebeuren dat er een nevenbevinding wordt gevonden waardoor iemand een risico heeft op het krijgen van een hartaandoening. Als iemand dat op tijd weet, dan kan het hart goed in de gaten worden gehouden. Zo kan worden voorkomen dat iemand erg ziek wordt. Als er iets te doen is aan de ziekte noemen we de ziekte 'behandelbaar'. Het kan fijn zijn om te weten dat er een behandelbare verandering in het erfelijkheidsmateriaal is gevonden. Weten dat je zo'n verandering hebt kan je ook bang maken en onzeker.

We weten nog niet goed wat we het beste kunnen doen met nevenbevindingen bij DNA-onderzoek. We weten nog niet alles over deze bevindingen en ook niet over hoe het is om te horen dat er een nevenbevinding is gevonden. Door dit onderzoek te doen, probeerden we meer te weten te komen over nevenbevindingen en de gevolgen ervan.

We hebben in het erfelijkheidsmateriaal van gezonde mensen gezocht naar behandelbare veranderingen. Bij 1 op de 38 mensen vonden we een behandelbare verandering. De meeste mensen hadden een verandering die te maken had met hartaandoeningen of kanker. Ook hebben we gekeken hoe vaak er bij mensen die DNA-onderzoek kregen, per toeval een behandelbare verandering wordt gevonden. Dit was bij veel minder mensen dan wanneer we er naar op zoek gingen (1 op de 170 mensen). Ook vonden we andere veranderingen dan toen we actief zochten. Het was lastig om te bepalen of de nevenbevinding behandelbaar was of niet.

Ook hebben we gepraat met 20 mensen die te maken hebben gehad met een nevenbevinding. Dit waren mensen bij wie een nevenbevinding was gevonden of familieleden van mensen bij wie een nevenbevinding was gevonden. Deze mensen zeiden dat ze na een tijdje niet veel last meer hadden van weten dat de verandering gevonden was. Bijna alle mensen zouden weer DNA-onderzoek laten doen ook al zou er weer een nevenbevinding worden gevonden. Ze vonden het belangrijk dat het behandelbare nevenbevindingen waren. Maar één van de mensen met wie we hebben gepraat had de ziekte die de nevenbevinding veroorzaakt.

Daarnaast hebben we gepraat met 20 erfelijkheidsartsen. Zij vonden het belangrijk om hun patiënten te vertellen dat er misschien een nevenbevinding zou worden gevonden. Maar zij vonden het lastig wat en hoeveel ze zouden moeten vertellen. Ook wisten ze niet goed wat behandelbaar is en wat niet. Nevenbevindingen veroorzaken bij patiënten en artsen onzekerheid. We hebben deze onzekerheid beter proberen te begrijpen. Patiënten en artsen waren onzeker over feiten, bijvoorbeeld: wat is het risico dat de verandering de ziekte gaat

veroorzaken? Maar ook waren ze onzeker over wat goed is om te doen, bijvoorbeeld: moet ik mijn kinderen vertellen over deze verandering?

Onze conclusie is dat de kans op een nevenbevinding klein is, maar dat de gevolgen groot kunnen zijn. We denken dat er meer kennis nodig is over de betekenis van nevenbevindingen. Ook denken we dat de arts en de patiënt samen moeten beslissen wat behandelbaar is voor de patiënt, omdat dit voor iedereen anders kan zijn. Mensen die veel weten over de ziekte kunnen helpen om hierover mee te denken. Tot slot denken we dat het belangrijk is dat de arts goed luistert naar de patiënt voordat het DNA-onderzoek wordt gedaan. Zo kunnen ze er samen voor zorgen dat de uitkomst van het DNA-onderzoek past bij de patiënt.

Impactparagraaf
(impact paragraph)

Achtergrond(1)

In iedere cel van het menselijk lichaam bevindt zich DNA. In het DNA zijn onze erfelijke eigenschappen opgeslagen. Genen zijn kleine stukjes DNA die voor een bepaalde eigenschap coderen. Mensen hebben ca. 20.000 genen. Meer dan 99% van het DNA in deze genen is van mens tot mens gelijk. De minuscule variatie van minder dan 1% veroorzaakt een bijzondere variatie aan mensen. Meestal heeft variatie geen nadelig effect. Soms veroorzaakt een genetische variatie een ziekte, bijvoorbeeld een aangeboren afwijking of een hartaandoening. Door middel van DNA-onderzoek kan de erfelijke oorzaak van een ziekte worden aangetoond.

Door de jaren heen zijn de mogelijkheden van DNA-onderzoek sterk toegenomen. Vroeger werd voornamelijk gericht DNA-onderzoek verricht; er werd dan gezocht naar een specifieke genetische variatie. Met de komst van Next Generation Sequencing (NGS) is het mogelijk om naar variaties in meerdere of zelfs alle genen tegelijkertijd te kijken. Deze techniek is steeds beter, sneller en goedkoper geworden. In de periode 2015-2020 werd deze techniek in het Radboudumc en MUMC+ samen bij 16,482 patiënten toegepast.

Door het kijken naar meer genetische variatie, wordt het waarschijnlijker dat er variaties worden gezien die niet de ziekte van de patiënt verklaren, maar wel een andere ziekte kunnen veroorzaken. Deze bevindingen worden nevenbevindingen genoemd. Nevenbevindingen kunnen relevant zijn voor de patiënt en diens familieleden. Voorgesteld wordt dat het goed is om nevenbevindingen waar de patiënt iets mee kan doen ('behandelbaar') te melden aan de patiënt. Het American College of Medical Genetics (ACMG) stelt zelfs voor om actief te zoeken naar deze bevindingen (zogenoemde 'secondary findings'). In Europa en Canada worden geen secondary findings gezocht. Genetici zijn het eens dat de patiënt voordat een genetische test wordt gedaan, uitleg moet hebben gekregen over nevenbevindingen en bewust toestemming moet hebben gegeven voor het doen van de test ('informed consent'). Ook wordt gedacht dat er een keuze aangeboden moet worden om behandelbare nevenbevindingen niet gemeld te krijgen en niet-behandelbare nevenbevindingen wél gemeld te krijgen. Behoudens deze algemene aanbevelingen, is het tot op heden niet zeker hoe we op een optimale manier om kunnen gaan met nevenbevindingen. Met dit onderzoek hebben we meer inzicht verkregen in nevenbevindingen bij DNA-onderzoek middels NGS, wat heeft geleid tot aanbevelingen met impact op de zorg en op de samenleving.

Resultaten van het onderzoek

Toen we actief zochten naar additionele varianten die een ziekte kunnen veroorzaken ("secondary findings"), bleek 2.7% van gezonde individuen een genetische variant te hebben die door de ACMG als behandelbaar wordt beschouwd (hoofdstuk 2). Als er niet actief naar additionele varianten wordt gezocht, maar deze 'per toeval' worden gevonden

(nevenbevindingen), zagen we dat het onwaarschijnlijker is dat er zo'n ziekte-veroorzakende variant wordt gezien (<1%) (hoofdstuk 3). Bovendien omvatten de nevenbevindingen varianten in andere genen dan op de ACMG-lijst staan, terwijl ze wel relevant zouden kunnen zijn. De kans op een nevenbevinding bleek sterk afhankelijk van hoeveel genetisch materiaal er geanalyseerd wordt en de manier van analyseren door het laboratorium.

Patiënten en familieleden van patiënten die met een nevenbevinding geconfronteerd zijn, gaven tijdens interviews aan dat ze geen grote impact ervaren van de nevenbevinding (hoofdstuk 4). Ze vonden het belangrijk dat ze iets konden doen om te voorkomen dat ze ziek zouden worden. Wel waren ze onzeker over wat de consequenties van de nevenbevinding waren voor de gezondheid en geldzaken van henzelf en van hun familieleden. Slechts in één familie was de ziekte waarop er een risico bestond door de nevenbevinding op het moment van de interviews tot uiting gekomen. Artsen vonden het informeren van patiënten over nevenbevindingen voorafgaand aan het uitvoeren van de genetische test als vanzelfsprekend horen bij hun counseling (hoofdstuk 5). Ze gaven aan dat ze worstelden met wat ze precies zouden moeten vertellen over nevenbevindingen. Ook hadden ze moeite om te bepalen wat 'behandelbaar' precies inhoudt. Onzekerheid speelde bij zowel de ervaring van patiënten als die van artsen een belangrijke rol in hoe zij nevenbevindingen ervaren (hoofdstuk 6). Patiënten waren vooral over praktische en persoonlijke zaken onzeker, terwijl artsen over wetenschappelijke aspecten onzekerheid uitten. Ook werd onzekerheid geuit over wat het beste is om te doen op basis van normen en waarden ('normatieve onzekerheid').

Betekenis van de resultaten

We concluderen dat hoewel de kans dat er een nevenbevinding wordt gedaan klein is, deze bevindingen een uitdaging vormen. Genetische varianten die als nevenbevinding aan het licht komen zullen anders benaderd moeten worden dan wanneer ze gevonden worden als verklaring voor het ziektebeeld van de patiënt. Er dient rekening gehouden te worden met het ontbreken van de aandoening bij de patiënt en/of bij diens familie. Waarschijnlijk liggen de gezondheidsrisico's lager in deze families. Het concept behandelbaarheid blijkt op basis van onze resultaten een lastig te hanteren term. We suggereren om deze term invulling te geven samen met de patiënt en gebruik te maken van expertise van een multidisciplinair panel met ervaring met nevenbevindingen. Ook lijkt het naar aanleiding van onze studies nodig om 'informed consent' in de context van nevenbevindingen in een ander daglicht te plaatsen. In plaats van te focussen op het informatie-aspect, zou de dialoog tussen arts en patiënt meer aandacht moeten krijgen. Tot slot zou er aandacht moeten zijn voor de onzekerheid die na het melden van een nevenbevinding bij de patiënt kan ontstaan. Met name zou er aandacht voor normatieve onzekerheid moeten zijn, zowel bij de patiënt, als bij diens arts.

Impact op de zorg

De hogere mate van efficiëntie van DNA-onderzoek en de steeds lager wordende kosten, zorgen ervoor dat genetisch onderzoek in toenemende mate toegepast kan worden in de gezondheidszorg. ***Steeds meer*** patiënten en zorgverleners zullen geconfronteerd worden met nevenbevindingen.

Lange tijd was er een gebrek aan kennis over nevenbevindingen en was er geen sprake van een uniform beleid. Dit onderzoek heeft geleid tot waardevolle informatie voor zorgverleners die geconfronteerd kunnen worden met nevenbevindingen.

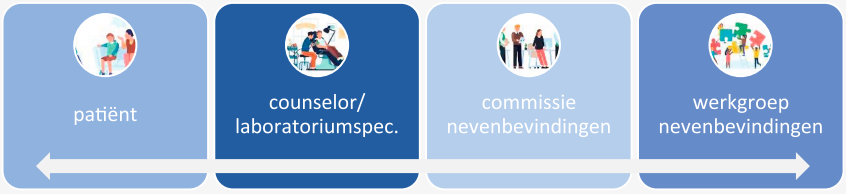
Ook heeft het onderzoek tot inzichten geleid die hebben geholpen bij het formuleren van ***aanbevelingen***. In juni 2021 is een nationale consensus-gebaseerde richtlijn geïmplementeerd(2), die tot stand is gekomen door beraden van de nationale werkgroep nevenbevindingen. Een voorbeeld van een aanbeveling in deze richtlijn die gebaseerd is op resultaten uit het onderzoek in dit proefschrift, is om het besluiten tot het mededelen van een nevenbevinding een gedeelde verantwoordelijkheid te laten zijn van de betrokken counselor en een multidisciplinaire commissie met ervaring met nevenbevindingen (Box 1).

Er werd door de artsen onzekerheid geuit over enkele aspecten van de aanbevelingen (bv.: wat als de patiënt niet wil weten wat ik als arts wel belangrijk acht voor diens gezondheid?). Evaluatie van ervaringen met deze richtlijn is belangrijk om te kunnen reflecteren op het effect ervan en wijzigingen aan te brengen daar waar nodig. Vóór de implementatie van het beleid hebben we reeds een rondvraag (vragenlijst) gedaan bij klinisch genetici (in opleiding) en de commissies nevenbevindingen om hun visie op het beleid te kunnen verkennen. We lazen dat er verschillende uitdagingen werden verwacht door de implementatie van het beleid (ongepubliceerde data). Na de implementatie zal deze evaluatie worden herhaald. Zo kunnen we, uitgaande van daadwerkelijke ervaringen, eventuele aanpassingen bespreken en in praktijk brengen en van ondersteuning voorzien daar waar nodig.

Box 1. Werkgroep nevenbevindingen

In Nederland is sinds enige jaren een landelijke werkgroep nevenbevindingen actief waarin alle genetische centra zijn vertegenwoordigd. Zij hebben een klinisch geneticus en een laboratoriumspecialist afgevaardigd, die ook betrokken zijn in hun lokale commissie nevenbevindingen. Deze werkgroep biedt een platform waarop ervaringen en resultaten kunnen worden gedeeld. Ook kan zij vanuit deze gezamenlijke bron van informatie adviezen uitbrengen richting de lokale commissies nevenbevindingen. De commissies staan in contact met de artsen die nevenbevindingen met patiënten communiceren. Er is door deze structuur sprake van een horizontale communicatie tussen verschillende relevante lagen.

De commissies hebben daarvoor de artsen en laboratoriumspecialisten gevraagd om hun visie te delen. Andersom is een informatieoverdracht ook mogelijk. Wanneer een ervaring van een counselor met een patiënt kan worden besproken met de commissie, kan de afgevaardigde van de commissie bij de landelijke werkgroep de ervaring ten tafel brengen. De inzichten die de onderzoeken hebben opgeleverd en de ervaringen van artsen en patiënten kunnen op deze manier tussen de verschillende lagen uitgewisseld worden.



Daarbij toont dit onderzoek het belang aan van kennis over de ***risico's op ziekte*** door genetische varianten. Deze risico's lijken niet hetzelfde te zijn in de context van nevenbevindingen. De werkgroep biedt een platform om op basis van gedeelde data een beter beeld te krijgen van hoe vaak de aandoeningen die nevenbevindingen zouden veroorzaken, daadwerkelijk voorkomen bij deze patiënten en/of hun familieleden.

De resultaten van de studies en ervaringen die gedeeld zijn, reiken verder dan de beleidsvoering van nationale genetische centra. Met name ***genetische centra*** die hun beleid aan willen laten sluiten bij Europese richtlijnen kunnen baat hebben bij het onderzoek dat wij hebben verricht. Maar ook buiten genetische centra zijn onze onderzoeksresultaten relevant. Medisch specialisten anders dan klinisch genetici vragen in toenemende mate genetische diagnostiek aan. Het onderzoek schetst voorwaarden voor het aanbieden van (uitgebreid) genetisch onderzoek die ook gelden in deze context.

Impact op de samenleving

Technologische ontwikkelingen maken het mogelijk om het DNA te ***screenen***. Steeds vaker wordt de vraag gesteld of dit wenselijk is. Onze resultaten dragen bij aan het kunnen beantwoorden van die vraag. Ten eerste hebben we gezien dat de interpretatie van varianten die niet worden gevonden als antwoord op een diagnostische vraag anders benaderd moet worden. Op dit moment is er nog onvoldoende informatie over hoe de risico's op ziekte van deze varianten geïnterpreteerd dienen te worden. In de context van screening is er ook geen sprake van de ziekte die de bevinding betreft bij de adviesvrager. Het is aannemelijk dat er ook nog niet voldoende informatie beschikbaar is over hoe varianten in de context van screening geïnterpreteerd moeten worden. Ten tweede voorspellen we een grote uitdaging om te bepalen waarop men dan gescreend kan worden. In Amerika screent men mensen die uitgebreid DNA-onderzoek krijgen op een vooraf bepaalde lijst met aandoeningen die 'behandelbaar' zouden zijn. Onze resultaten benadrukken hoe lastig het is om te bepalen wat behandelbaar is. Dit geldt des te meer wanneer er een arts-patiëntrelatie ontbreekt. Het ontbreken van deze relatie lijkt ook voor het verkrijgen van een informed consent voor DNA-screening op basis van dit onderzoek niet opportuun. Concluderend is de haalbaarheid van deze drie voorwaarden voor DNA-screening op basis van onze resultaten te betwijfelen en is onderzoek naar de haalbaarheid wenselijk voordat screening geïmplementeerd wordt.

Op dit moment is er een toenemend aanbod van ***direct-to-consumer*** genetische testen: testen die de patiënt zelf kan laten verrichten zonder tussenkomst van een zorgprofessional. Consumenten worden via onder andere advertenties van Nederlandse en buitenlandse bedrijven uitgenodigd om hiervan gebruik te maken.

Omdat er geen tussenkomst van een zorgprofessional is, lijkt in de context van direct-to-consumer testen niet voldaan te kunnen worden aan de door ons voorgestelde zorgvuldigheidseisen voor het aanbieden van uitgebreide genetische testen. We stellen voor om te onderzoeken hoe deze testen wel zorgvuldig aangeboden kunnen worden.

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Dankwoord

De afgelopen jaren heb ik veel mogen ervaren om dankbaar voor te zijn. Dat was niet zo geweest zonder de geweldige mensen om mij heen. Hen wil ik dan ook allemaal bedanken.

Ik ben dankbaar voor de openheid, de eerlijke woorden en intieme gedachten van de deelnemers van mijn interviewstudies. Ook ben ik dankbaar voor alle hulp die anderen hebben geboden bij het verrichten van deze en de andere onderzoeken in mijn proefschrift.

In het bijzonder wil ik mijn promotieteam bedanken. Prof. Dr. Brunner, beste Han, bedankt voor het delen van je analytische vermogen en 'doorgrondigheid'. Bedankt voor je ongezouten opmerkingen in de kantlijn; de 'dat is onzin'-uitspraken werden ruimschoots gecompenseerd door suggesties en vragen die precies de juiste nuances aanbrachten. Dank voor het voorzien en doorzien. Ik bewonder hoe jij betrokken bent bij zo veel; wetenschap, kliniek, management; de levens van zo veel mensen. Ik kan me niet voorstellen hoeveel partijen jou gaan missen vanaf september. Alvast alle goeds gewenst.

Dr. IJntema, beste Helger, veel dank voor het doorzettingsvermogen en de integriteit die je altijd toonde. Dank voor de maandenlange worsteling met de 'master tabel' waar we onze exceltalenten op botvierden en de bijbehorende les dat 'het lukt ons niet' vaak geen reden kan zijn. Dank voor het vertrouwen dat je gaf en de zekerheid die je me liet ervaren. Ik ben blij dat onze samenwerking met het schrijven van de laatste zinnen van dit boekje niet stopt, maar zich in ieder geval voorlopig nog voort zal zetten via de landelijke werkgroep nevenbevindingen.

Dr. Oerlemans, lieve Anke, heel veel dank voor je waarachtigheid en de voorbeeldrol die je mij gaf. Bedankt voor je gepaste humor over dino's en hamsters met eigen instragramaccounts (want die hamsters bestaan en wij kennen ze) en het menszijn dat je daardoor en door zo veel andere mogelijke voorbeelden liet voelen. Bedankt voor het delen van je kennis over dat waar ik nog niet in thuis was; de ethiek, het kwalitatieve onderzoek en dank voor alle feedback die eindigde met een 😊. Bedankt voor het telefoontje op het moment dat dat nodig was. Ik hoop anderen zo te mogen begeleiden als jij mij hebt begeleid.

Ook een speciaal woord van dank aan Lisenka en Lonneke, voor jullie inspanningen en motiverende enthousiasme. Dank ook aan Carlijn, Simone en Lisa, voor het werk dat jullie hebben verricht en de – misschien onbewuste – stok achter de deur die jullie mij gaven. Ook wil ik de organisatie en mijn mede-deelnemers van Ehtiek in de Zorgsector bedanken; het oprechte en zuivere klankbord dat jullie mij boden heeft mij in vele opzichten gesterkt.

En mijn paranimfen, Kirsten en Margot, aan jullie ook veel dank. Ik ben super trots om in het midden te mogen staan van twee powervrouwen. Beiden in verwachting toen ik jullie vroeg om mijn paranifm te zijn; ik kreeg nare dromen over dat ik alleen voor de corona zou moeten staan. Maar jullie laten mij niet in de steek. Jullie delen hetzelfde verantwoordelijkheidsgevoel en de integriteit die ik bewonder, zoals jullie de naam van jullie inmiddels geboren zoontjes delen.

Bedankt Kirsten voor het geven van een plekje in het Zuiden om thuis te kunnen komen. Voor de geweldige support tijdens mijn samenvattingdebakel. En bedankt Margot voor het worden van een oude nieuwe collega. Heel mooi om weer vooruit te kunnen kijken.

Dank aan mijn andere oude en nieuwe collega's. Bedankt collega's uit Maastricht. Voor de mooie en pittige momenten. Een tijd om op terug te kijken en ook om vanuit verder te kunnen gaan. Bedankt Yvonne, mijn opleidster, voor de ruimte die je me toevertrouwde. Een speciaal woord van dank aan Christine, voor de blijvende inspiratie, gedeelde kennis en ervaring. Ik wens je alle goeds in het nieuwe hoofdstuk van je leven. Ook jij zal enorm gemist worden.

Veel dank aan mijn nieuwe collega's in Rotterdam. Bedankt voor het vertrouwen en het geloof, in wie ik ben en wat ik belangrijk vind. Een speciaal woord van dank aan team prenataal; Marieke, Karin, Kyra, Myrthe, Robert-Jan, Gosia, Diane, Marjolein en ook Mieke en Yvonne. Jullie inspireren me om klinisch een integere arts te zijn, wetenschappelijk een creatieve onderzoeker en professioneel een fijne collega.

Maar er is meer dan werk. Er is ook de fiets. En de roze fiets. Dank aan beide voor jullie bestaan. Welke kleur dan ook, ik houd van jullie zolang er lucht in de bandjes zit en het onderhoud niet achterstallig is. Dat laatste is mijn verantwoordelijkheid, dat geef ik toe. Door jullie kon ik mijn hoofd leegmaken en fris verder gaan. En dank aan de kilometers asfalt die ik onder jullie heb zien wegrollen. Het asfalt in de heuvels, de bergen of lekker langs het kanaal. En dank ook aan de onverharde paden voor uren marathontrainingen, huttentochten en gravel ritten (minder dank aan de single tracks, ooit bedank ik jullie misschien ook).

Maar vooral veel dank aan degenen die naast, voor of achter mij fietsen. Degenen die mijn bandje onderweg fixen, omdat ik nog steeds geen band vervangen kan. Die met mij een taartje willen eten in Malmédy, naar een cyclo willen gaan of een gravel event, op vakantie willen gaan om te trainen en vooral om te genieten. Bedankt Sigrid, Maarten en Eef, voor de allereerste en allerlaatste loodjes in Girona en in het Nederlandse. Dank Dorien, Mieke en Maarten, onze tijd op Tenerife was enorm waardevol en ik kijk uit naar het vervolg (Rwanda 2024?). Bedankt Eric, voor jouw voorbeeldverdediging vóór onze uitdaging in de Alpen - al heb ik geen gelakte schoenen kunnen scoren en bestaat mijn voedselplan nog steeds niet uit slechts blocks. Bedankt aan mijn vaste fietsmaatjes in Limburg; Peter, Jorg, Zita, Rob, Dirk, Michiel, beide Barten, Youri, Rein, Steven, Dominique, Thomas en Sander, voor alle gemaakte ritjes die we hopelijk nog lang kunnen blijven maken. Ook bedankt nieuwe EMC fietsbuddy's, voor de platte pannenkoek ritjes met epische hoogtestage als tegenhanger. En veel dank aan mijn nieuwe fietsmaatjes in Eindhoven, waaronder natuurlijk de GRVLRBLS, voor de maximale hartslagen op woensdagavonden vanaf Strijps bultje, motivatie en inspiratie. Mede dankzij jullie voel ik me enorm thuis in Eindhoven.

En natuurlijk ook heel veel dank ook aan alle niet fietsers die ook met mij een taartje willen eten, wijntje willen drinken, of 2000 hoogtemeters willen rennen in Noorwegen. Jeanne, ondanks dat we dat laatste misschien maar niet meer moeten doen, hoop ik dat je me kan blijven inspireren met je tomeloze enthousiasme. Dank aan alle meisjes van Zoet; Tjen, Manon, Nienke, Tamara, Katinka, Marloes en beide Anne's. Jullie bewondering en acceptatie van de keuzes die ik maakte, heb ik enorm gewaardeerd. Bedankt Anna, voor een verleden van goede gesprekken. Ik hoop dat ze snel weer tot het heden behoren. Bedankt Voramus dames voor de wijze lessen in kaas en wijn. Mooie lessen om samen op terug te kijken Renee, mede BertA'tje, bij voorkeur in Salamanca. Heel fijn dat ik nu zo dichtbij je woon, Madelon en jouw collega mag zijn, Laura. Ook aan mijn andere 'lange Laura' en aan Marleen veel dank. De onvoorwaardelijkheid van onze vriendschap ervaar ik als enorm waardevol en neem ik iedere uitdaging mee.

Dank lieve familie; van der Schoot en familie van Eijndhoven. Voor de vorming en de ervaring, de interesse en de trots. Lieve familie Leijte; Jos, Diny, GuSan en Bobs, BrEmma, dank voor jullie steun en interesse. Bedankt ook voor het begrip toen ik op kerstochtend een artikel aan het submitten was, en het eitje dat jullie toen ondertussen voor mij kookten.

Lieve pap, dank voor het onvoorwaardelijke, oprechte geloof en vertrouwen. Ik weet dat je, zoals tijdens de marathon, altijd achter me staat - of schuin voor me fietst, om me uit de wind te houden. Lieve mam, ook aan jou heel veel dank. Niet alleen voor je enorm waardevolle bijdrage aan de kaft van mijn thesis, maar voor heel veel meer, want ik weet dat je even trots en steunend bent als pap. En de woorden, ach, het zijn maar woorden. Door jullie beklom ik mijn eerste berg, ik had er geen van die volgden willen missen. Lieve zus, lieve Hanne, hoewel dit misschien wel het laatste boekje is dat jij ooit had willen schrijven, steeds vaker worden we geconfronteerd met hoeveel we op elkaar lijken. Bedankt voor het oprechte begrip wanneer dat precies is wat ik nodig heb. Dank ook aan Martijn, Thomas (a.k.a. Lus) en Lise. De vreugde die voortkomt uit jullie gezin kan voor mij alles in het juiste perspectief plaatsen.

Lieve Wouter, lieve puup. Bedankt voor je steun op momenten dat ik dat nodig had, je pragmatische oplossingen voor mijn 'hypothetische problemen'. Soms ziet de wereld er inderdaad anders uit na een goed glas wijn. Ik ben super trots op het leven dat we samen aan het opbouwen zijn en kijk uit naar onze toekomst. Aangekomen bij de laatste zin van dit proefschrift zijn wij nog lang niet klaar; climb every mountain?

Curriculum Vitae

Vyne van der Schoot was born on the 18th of October 1990 in Eindhoven, the Netherlands. She graduated cum laude from the Zwijsen college in Veghel, after which she started studying Medicine at the University of Maastricht in Maastricht in 2009. She obtained her bachelor's degree in 2012 and her Master of Science in Medicine in 2015. During the last year of her master's, Vyne became intrigued by clinical genetics, with a special interest in prenatal genetics. She became a senior intern in clinical genetics at the Maastricht University Medical Centre (MUMC+) and studied the health outcomes of children born after preimplantation genetic diagnosis during her scientific internship at this department.

Following this internship, Vyne started working as a resident (AIOS) at the department of Clinical Genetics of the MUMC+ in 2015. During the second year of her residency, Vyne became a member of the committee for unsolicited findings, a joint initiative of the departments of Genome Diagnostics of the Radboudumc and MUMC+. This was the inspirational starting point of multiple projects under supervision of her promotor Prof. Dr. Han Brunner and her co-promotors Dr. Helger IJntema and Dr. Anke Oerlemans. Vyne presented the results of these studies at various national and international occasions and shared the results with the national working group of unsolicited findings, which allowed formulation of national recommendations. Vyne's special interest in bio-ethics further developed when she followed the 12-month post-academic course 'Ethics in healthcare'. During the last phase of her residency, Vyne specialized in prenatal genetics, the first months of the COVID-19 pandemic excluded, in which she worked as a resident in the 'COVID wards'. She finished her residency in Clinical Genetics at the Erasmus MC in Rotterdam in February 2021. This is where she started working as a clinical geneticist for prenatal genetics and initiated installation of a local committee of unsolicited findings. In order to finish her thesis, she kept on combining scientific and clinical activities.

Vyne likes to be challenged in (trail)running or on her road and gravel bike, on which she yearly covers more kilometers than the human genome has genes. She is in a relationship with Wouter Leijte with whom she lives in her birth town Eindhoven.

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