Unsolicited findings in next-generation sequencing

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.