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Reporting practices for variants of uncertain significance from next generation sequencing technologies

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ABSTRACT

The nature of next generation sequencing technologies (NGS) results in the generation of large amounts of data and the identification of numerous variants, for some of which the clinical significance may be difficult to ascertain based on our current knowledge. These Variants of Uncertain Significance (VUS) may be identified in genes in which the function is known or unknown and which may or may not be related to the original rationale for sequencing the patient. Little is known about whether laboratories report VUS to clinicians and current guidelines issued by some of the most notable professional bodies do not provide specific recommendations on this point. To address this, 26 interviews were conducted with 27 laboratory personnel, representing 24 laboratories in Europe (12), Canada (5) and Australasia (7) in order to explore their reporting practices. Participants highlighted that the classification of variants is a real challenge despite the presence of classification guidelines. We identified variation in the reporting practices of VUS across the laboratories within the study. While some laboratories limit their reporting to variants that are pathogenic and thought to be causative of the phenotype, more commonly laboratories report VUS when they are identified in genes related to the clinical question. Some laboratories will also report VUS in candidate genes. VUS that are secondary findings are generally not reported. While it is unclear whether uniformity in reporting is desirable, exploring the perspectives of laboratory personnel undertaking these analyses are critical to ensure the feasibility of any future reporting recommendations.

1. Introduction

Next generation sequencing technologies (NGS), which encompasses targeted gene panels, exome and genome sequencing, are now well-embedded in the clinical setting. NGS is novel in that it allows numerous genes to be analyzed in a single test (Rabbani et al., 2014). For this reason, NGS has played a critical role in the detection of many new disease-causing genes, particularly in the fields of rare diseases and cancer (Guerreiro et al., 2016; Rotunno et al., 2016; Tetzlaff et al., 2016). However, the nature of the technology means that a large amount of data is generated compared to traditional sequencing methods, and the clinical significance of some of these variants might be difficult to ascertain based on our current knowledge (Ream and Mikati, 2014).

These Variants of Uncertain Significance (VUS), also referred to as Class 3 variants by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP), may be unable to be further classified as either (likely) benign or (likely) pathogenic in two main ways (Richards et al., 2015). First, a variant may be classified as a VUS because, despite being identified in a gene known to be related to the clinical question, there is insufficient evidence of pathogenicity. Second, a variant might be identified in a gene of uncertain significance but where the nature of the variant suggests it may be causative of the patient's phenotype (i.e. de novo or truncating variant). In addition, the term "VUS" may also be used to describe a variant lacking evidence of pathogenicity that is identified in a known disease-causing gene that is unrelated to the phenotype of the patient (i.e. an unsolicited or incidental finding).

The guidelines issued by some of the most notable professional bodies do not provide specific recommendations about whether VUS should be reported to clinicians. Instead, they often state that the laboratories should develop, and clearly document, specific

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protocols for the reporting of VUS but leave the decision about whether or not to report these to clinicians to the discretion of each laboratory (Boycott et al., 2015; Matthijs et al., 2016; Rehm et al., 2013; van El et al., 2013). Given the lack of guidance provided, it is difficult to know which variants are being reported to clinicians and whether these practices are consistent between laboratories.

Although one survey conducted with laboratories in the USA asked participating laboratories whether they reported VUS (O'Daniel et al., 2017), reporting practices for VUS have not yet been explored outside the USA. In order to address this knowledge gap, we aimed to assess the reporting practices of laboratories in Europe, Canada and Australasia for variants of uncertain significance to determine which variants are being reported and how decisions about which VUS to report are made.

2. Methods

Qualitative methods were used to explore the reporting practices of laboratories using NGS. Laboratory personnel were recruited using a purposive sampling strategy which sought to identify and recruit individuals who analyze and report the data generated by NGS technologies, including targeted gene panels, clinical exomes, exome and genome sequencing. Potential participants were identified using two strategies. First, internet searches were used to identify laboratories using NGS and laboratory heads were invited to participate via email. Second, snowball sampling was utilized where participants were asked to nominate potential participants from other laboratories they knew were using NGS in the diagnostic context.

Interviews used a semi-structured interview guide and were conducted by one member of the research team (DV). These interviews explored a range of different topics relating to their use of NGS, including the types of technologies and analysis/filtering strategies used in their laboratory, and their reporting practices. Here we report data from components of the interviews discussing their practices relating to variants of uncertain significance. According to the semi-structured interview guide, participants were asked to respond to the following main questions: Which types of results do you routinely report back to clinicians? How do you make decisions about which results to report? What have you found challenging regarding the reporting of findings from NGS?. Interviews were audio-recorded, transcribed verbatim and analyzed using inductive content analysis, in which content categories were derived from the data, rather than pre-determined (Downe-Wamboldt, 1992; Graneheim and Lundman, 2004; Schamber, 2000). Each transcript was coded into broad content categories. Sections of the data within the broad categories were compared and more specific subcategories were developed. All interviews were coded by DV; KS and PB coded a subset to confirm the coding scheme.

Verbal informed consent was obtained from all participants. This study was approved by the SMEC Review Board (Social and Societal Ethics Committee), KU Leuven and by the Research Ethics Board of the Faculty of Medicine, McGill University.

3. Results

3.1. Participant characteristics

Twenty-six interviews were conducted with 27 laboratory personnel - one interview included two participants from the same institution, but from separate groups. This included participants from 24 different laboratories in Europe (12; the Netherlands, France, Germany, Slovenia, Spain, England, Wales), Canada (5) and Australasia (7; Australia, New Zealand). Participants had a mean of 8.1 years (4 weeks–24 years) experience in their current role and a mean of 17.4 years (6–32 years) in the field of genetics. Of the laboratories, 19/24 laboratories operate in the diagnostic context, although several of them also have research components within their laboratory. Although 5/24 laboratories operate purely on a research basis, they were included in the study because they issue reports to referring clinicians. The sample included laboratories that are integrated within a hospital and also some independent laboratories. Twenty of the laboratories use targeted panels (including five who use a mendeliome-based panel), 22 use exome sequencing, (with or without virtual panels), and 4 use genome sequencing (3/4 are research laboratories).

3.2. Challenges associated with variant classification

The participants identified determining which VUS to report as a real challenge for them, describing the tension between reporting and not reporting uncertain variants. This is partly because they found the classification of variants as (likely) benign, (likely) pathogenic, or uncertain as challenging, despite the ACMG guidelines. One participant commented on the subjective nature of the classification of variants, even when using classification guidelines, and how this could easily result in differences in classification of variants between individuals.

> It still is a challenge, every time you write a letter, especially for the exome sequencing. Because you often do have the feeling like, this is probably not something. But a feeling alone is not enough. […] You do classify those variants based on certain aspects and I find it's sometimes difficult when you have a feeling that this probably, it's nothing. But you have to put a 'class 3' on it, because you don't have enough proof that it is basically not a disease causing mutation.
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> Participant 20

> One of those things that really worries me, it's like that it will depend so on the people. People report different things. For example, my colleagues and I will not always agree on the thing we have to report. But also it could depend on your mood and for me it's always really stressful because when you have time, […] you will spend more time on one specific variant to follow it and really search if it could be or not involved in the disease. And if you have maybe less time […] you will discard it maybe. […] And so for me, what is challenging is to deal with this need to be like, quite objective but also the need to be subjective because it's your experience of molecular biology that can really help you to interpret a variant.
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> Participant 25

3.3. Reporting practices for variants of uncertain significance

The interviews with laboratory personnel indicated that some laboratories, including many of those that operate solely in a research context, do not routinely report VUS to referring clinicians.

> That's obviously a point of ongoing discussion, but I was always pretty keen on reporting less rather than more. Certainly, you know, I've seen reports where there's a whole page of, you know, class 3 variants on the back of the report. I've always been very anti-that. So, we routinely report class 4s and class 5s. Very, very rarely will I actually ever include a class 3 variant on a report.
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> Participant 13

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