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Nordic Alliance
for Clinical Genomics

Clinical reporting of NGS data

A systematic Nordic collaborative, peer-reviewed benchmarking

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A systematic Nordic collaborative, peer-reviewed benchmarking

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Abbreviations

ACMG	*The American College of Medical Genetics and Genomics*
HGVS	*Human Genome Variation Society*
HPO	*Human Phenotype Ontology*
HUGO	*Human Genome Organisation*
NACG	*Nordic Alliance for Clinical Genomics*
NGS	*Next-Generation Sequencing*
OECD	*The Organisation for Economic Co-operation and Development*
QC	*Quality control*
VUS	*Variant(s) of uncertain significance*
WES	*Whole exome sequencing*
WGS	*Whole genome sequencing*

Contents

Nordic Alliance for Clinical Genomics

Executive summary

Within medical diagnostics, the synthesis and interpretation of vast quantities of genomic data into concise clinical guidance has been identified as a major challenge in the effective use of next-generation sequencing-based (NGS) diagnostics in regular clinical practice. These data have significant clinical utility and represent a primary source of information when diagnosing rare genetic disorders and cancer. The clinical genomics report details key findings, and represents a core hand-off between specialized clinical genomics laboratories and the broader healthcare community. However, the design and procedures for analysing and issuing clinical genetics reports are not standardized. Misunderstanding results, limitations or key findings can lead to incorrect therapeutic decisions, directly impacting patient management.

The Nordic Alliance for Clinical Genomics (NACG) represents stakeholders in clinical genomics from across the Nordics, and operates through an open and transparent model to identify and address emerging challenges to the implementation of precision medicine. Here, we describe this user-driven model and its application to the topic of clinical reporting. Key findings of this work are synthesized from an overview of the myriad guidelines that address clinical reporting, issues and challenges identified through discussion and focus groups, a peer-evaluated survey of the current state of Nordic clinical reports, and a series of in-depth interviews.

By examining these topics in depth and comparing and contrasting processes and systems from across Nordic health institutions, a shared lexicon and understanding can be developed. Organizations can use this shared knowledge to improve the standard of care within their own institutions and national infrastructures, leading to more effective healthcare and better patient management.

1. Introduction

Although the technology and tools for inexpensive, high-quality NGS data are widely available in a research context, the implementation of NGS-based diagnostics in a clinical setting has proven difficult. Moving new technology from a translational research setting into common clinical practice requires a broad array of topics surrounding quality assurance, assay validity, data security, legal and regulatory considerations and the interface with pre-existing hospital infrastructure all to be addressed.

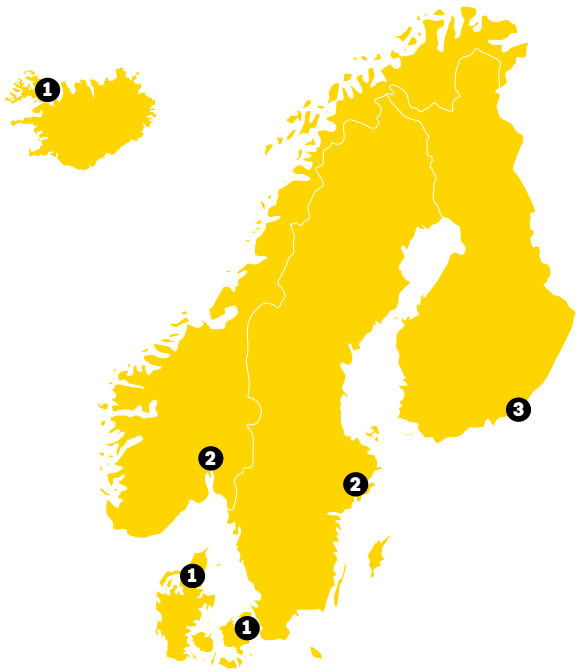
The Nordic countries represent a combined population of 27 million, and share a number of characteristics that make a common approach to addressing these challenges advantageous. The Nordics have well-developed national health systems and digital infrastructure, share similar social and ethical values, and hold similar visions for the role of precision medicine in addressing future healthcare demands and contributing to the economy. With a tradition for transparency and high trust in government, citizens are generally positive towards research and the secondary use of data. This trust and the entrenched ideals of social responsibility and collective welfare have resulted in an extensive network of biobanks and health registries, linked by national ID numbers that allow lifetime traceability of patient data.

Building on Nordic commonalities, advantages and shared challenges, the Nordic Alliance for Clinical Genomics (NACG) is a grass-roots organization that brings together professionals in five Nordic countries (Fig. 1) interested in sharing experiences, data and best practices for the implementation of precision medicine. Founded on the pillars of patient-centric care, open collaboration, innovation, accountability and inclusivity, the organization serves as a transparent and constructive forum to exchange ideas and improve the quality and standard-of-care of precision medicine across the Nordics. In the current NACG organizational structure, topics are continuously nominated, prioritized and voted on for active work within one of four working groups (Fig. 2). Each of the working groups continuously manages topics under ideation, active work, or reporting. Further details about NACG are included in Appendix 1: About NACG, for more information please see www.nordicclinicalgenomics.org or email post@nordicclinicalgenomics.org

NACG working method

At the core of NACG is the emergent method developed to facilitate active collaboration between members. Rather than functioning as an academic conference, NACG is centred around bi-annual interactive workshops. During these, members gather for a series of hands-on discussions and activities. Brief presentations update members on new regulatory and legal developments from each country, but traditional scientific talks are kept to a minimum in favour of active exercises and discussions.

During each meeting, members select and prioritize topics of interest which will form the core of the next workshop (Fig. 2, wheel). These are first exposed to an ideation process, allowing all members the possibility of describing the quality issues associated with the topic. Key stakeholders are identified and a core team is assembled to organize work addressing the topic in depth.



10
Sites

5
Nordic countries

23
Participants

Figure 1: NGS professionals from five Nordic countries are active in the NACG. The clinical reporting work performed in this paper was carried out by a total of 23 participants located at the ten sites indicated in black circles on the map.

These core teams typically choose one of two paths. First, they may design and execute an in-depth workshop session or hackathon during the next meeting. These activities are designed with concrete goals in mind, and focus on interactive activities and exercises to discuss and address the topic in detail. Alternatively, the core team may design and conduct benchmarking exercises which are completed by members in their home countries, processed centrally, and reported back during the next workshop.

Regardless of the path followed, topics are concluded by a discussion at the following workshop, summarizing the findings and learnings for adapting laboratories' practices. Larger bodies of work may additionally be summarized in external publications or white papers. Crucially, learnings from these exercises result in increased awareness and the adoption of processes and/or protocols in member institutions.

In the current NACG organizational structure, topics are continuously nominated, prioritized and voted on for active work within one of four working groups: bioinformatics tools development, establishing vehicles for sharing, enhancing quality of data and processes and research. Each of the working groups continuously manages topics under ideation, active work or reporting.

Clinical reporting as focus area

Under the NACG focus area Enhancing data quality and processes, clinical reporting was identified as an area of high interest for further investigation. Amongst NACG members, the clinical reports that summarize genetic findings were identified as the key hand-off between the NGS laboratory and the clinic, representing both a primary output and a critical instrument for informing therapeutic or diagnostic decisions. While other communication streams such as personal contact with physicians, multidisciplinary team meetings, or phone and secure electronic messages are used, the report serves as a static and concrete deliverable, usually integrated into the patient health records. The criticality of this key document is amplified by the complexity of information to be conveyed in the report.

Clinical reporting as a broad topic generates interest as it serves as a wrapper for many issues that are central to the implementation of NGS-based diagnostics such as technical standards, QC, secondary findings, variant classification, interface with hospital IT systems, interface with healthcare ecosystem, reanalysis and reclassification, genetic literacy and patient access to genetic data.

Participants at the workshop recognised the complexity of issues surrounding reporting and acknowledged this was a challenging area where the sharing of experiences, reports and best practices would be beneficial and could lead to improvements within member laboratories. Under this work stream, NACG executed a four-part plan to address clinical reporting, consisting of reviewing existing clinical reporting guidelines, organizing discussions on best practices for clinical reporting, benchmarking in-production reports via peer-review, and conducting in-depth interviews on challenging topics surrounding clinical reporting.

Genomic literacy

The term 'Genomics' was coined back in 1986 during a social event at a meeting held on the mapping of the human genome (1). Today we readily use the word 'genome' when referring to the complete genetic makeup of an organism. As technical advances have enabled genomics to infiltrate nearly all aspects of the Nordic healthcare system, genomics is moving faster than public awareness and understanding. A major challenge now, and in the years to come, is how to educate the general public so everyone understands and benefits from personal health issues that involve genomics. Substantiating the challenges, even health care professionals are not sufficiently trained in using genomics to support and inform patients in regard to personalized risk and treatment (2).

In 2011 a workshop was held by the National Human Genome Research Institute to examine the challenges in how Genomic Literacy could be archived for the general public from kindergarten level to adult education (3). Correspondingly, healthcare professionals should be capable of communicating (conversing, reading and writing) genomic literacy in a non-technical but meaningful way also termed 'functional scientific literacy' (4). Moreover, a recent study examining the level of functional scientific literacy and genomic literacy among medical oncologists concluded that there is still a need to increase the level of genomic literacy in medical school training programs and beyond (5).

Taken together, the workforce behind a genomic laboratory where clinical diagnostic reports are being generated for downstream clinical decision making bears great responsibility to ensure that reports are unambiguous to the medical doctors who are responsible for the clinical course of their patients.

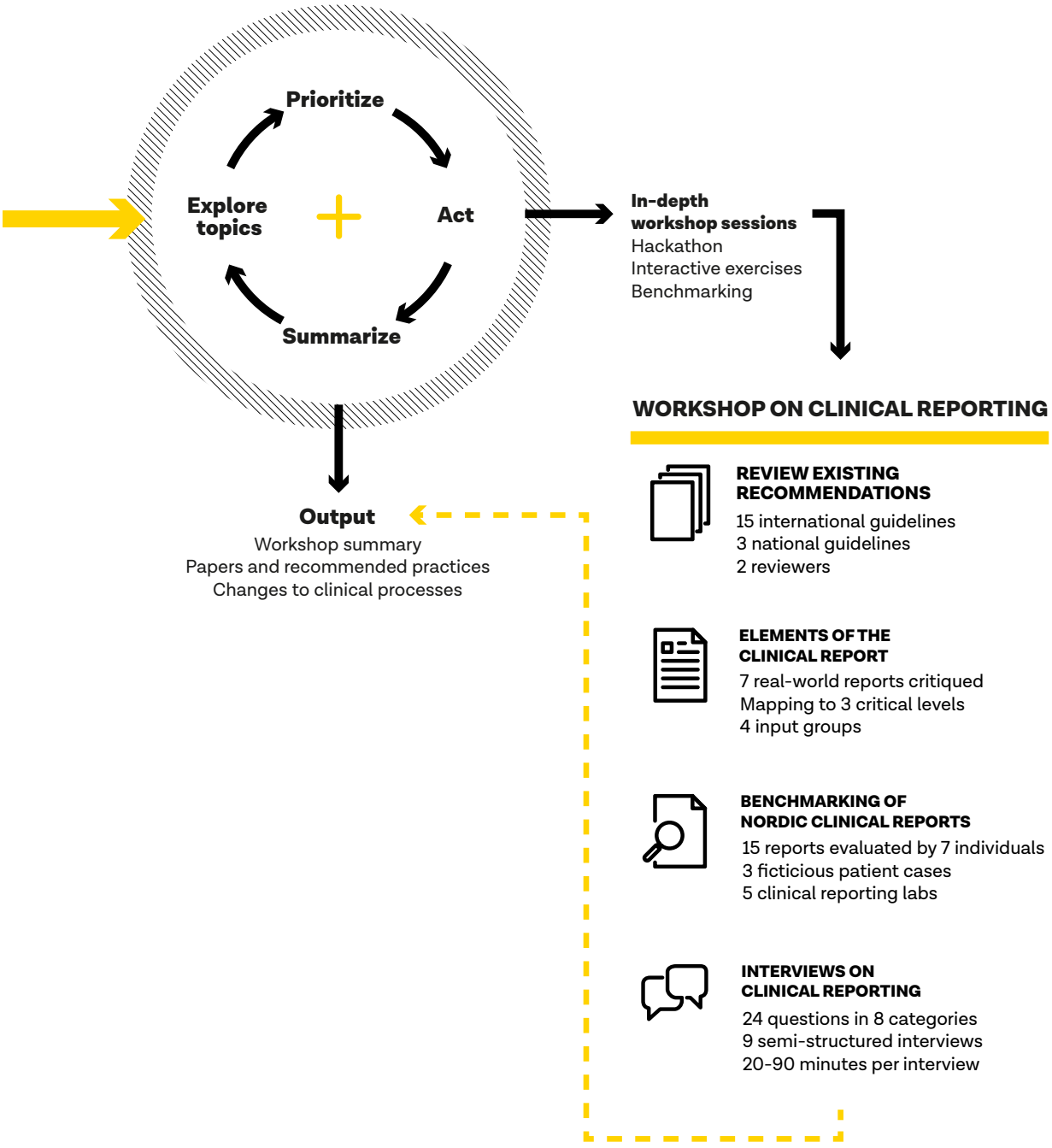


Figure 2. The NACG working method, showing the cycle of topic selection and prioritization, with the act and output arms exemplified by the four activities on clinical reporting presented in this paper.

2. Review of existing recommendations

To better understand existing best practices for clinical reporting, a literature review was performed and identified over 25 recommendations published by academic research groups, medical professional societies and regulatory bodies, which gathered over 400 recommendations that span all aspects of the clinical genomics workflow. These were broadly grouped into 21 categories, such as cybersecurity, quality management and assay validation. Sixteen addressed clinical reporting specifically (see Table 1), and these recommendations are summarized below grouped into 14 topics (Fig. 3). Four topics – variants of uncertain significance (VUS), secondary findings, reanalysis and data delivery to the patient – were recurrently identified as challenging in the subsequent information categorization exercise (Section 6), reporting benchmarking exercise (Section 7) and interviews (Section 8), and as such are discussed in greater depth here.

Main topics

Clarity

There is a consistent focus across recommendations on clarity and understanding: the goal of a clinical report is to communicate test results in a format that is clear and understandable to the clinicians who will be making treatment decisions partially based on the reports (6–9). The guidelines make it clear that medical genetics jargon should be avoided as the report should be clear for both specialists and non-specialists in genetics. The competencies, proficiency testing schemes and level of genomic literacy varies widely between hospitals (10), so the formulation of clear statements of test results will differ in practice. Regardless, reports should be designed to minimize or de-prioritize information which does not aid clinicians in diagnosis, so a clear understanding of the ecosystem surrounding the clinical NGS laboratory is imperative.

The report should cover all essential steps of the test, its indication and limitations. All pages of the report should be marked with a patient ID and page number (8).

Report summary

Most recommendations state that laboratories should issue a report summary, clearly and boldly visible on the front page of the report. This should contain the most important information for providing support to establish a diagnosis. The first page of the report should summarize (6,9,11,12):

- patient ID
- gender
- sample ID
- ethnicity
- availability of a family tree
- the indication for testing
- the methodology and limitations of the test (briefly)
- major findings or the absence of findings
- conclusions

Title	Authors	Published
ACMG clinical laboratory standards for next-generation sequencing	Rehm et al.	2013
College of American Pathologists' laboratory standards for next-generation sequencing clinical tests	Aziz et al.	2015
Guidelines for diagnostic next-generation sequencing	Matthijs et al.	2016
ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing	Green et al.	2013
Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics	Kalia et al.	2017
CLIA program and HIPAA privacy rule; patients' access to test reports. Final rule.	DHHS	2014
General Data Protection Regulation (GDPR)	European Parliament & Council	2016
APPLaUD: access for patients and participants to individual level uninterpreted genomic data	Thorogood et al.	2018
Recommendations for reporting results of diagnostic genetic testing (biochemical, cytogenetic and molecular genetic)	Claustres et al.	2014
Practice guidelines for targeted next generation sequencing analysis and interpretation	Zandra et al.	2015
OECD Guidelines for quality assurance in molecular testing	OECD	2007
Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists	Li et al., 2017;	
Considerations for design, development, and analytical validation of next generation sequencing-based in vitro diagnostics intended to aid in the diagnosis of suspected germline diseases; Guidance for stakeholders and Food and Drug Administration staff	FDA/ CDRH/ CBER	2018
General genetic laboratory reporting recommendations	Smith et al.	2015
Practice guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics	Wallis et al.	2013
Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology	Richards et al.	2015

Table 1. Recommendations and guidelines addressing clinical reporting

Figure 3. The 14 topics identified through review of existing recommendations (boxes), and mapping and categorization of information types identified in the elements of the clinical report exercise to these 14 topics as must have, nice to have, exclude or challenge.

Main topics

	Must have	Nice to have	Exclude	Challenges
Reporting multiple patients				
Compliance with diagnostic standards				
Clarity	· Easy to understand · Little text	· Interactive format	· Lengthy reports · Speculations	
Variant nomenclature	· HGNC gene name, HGVS nomenclature, protein translation, cDNA, ref transcript			· Should genomic coordinates be reported?
Report summary	· Patient and sample information · Guidelines indication · Actionable results · Results	· Detailed phenotype, ideally with HPO terms · Evidence codes and basis for classification · Colour coding · Family studies and co-segregation · Recommendations for follow up		· Negative/no findings · Autosomal recessive carrier
Variant classification	· Classification	· Variants classified according to ACMG guidelines	· Non-treatable variants · Low-penetrance variants	
Assay limitations	· Short disclaimer · Technical limitations · Missed findings			
Anonymity	· Consent information			
Methods	· Description of analysis method · Validation method	· Included genes / gene sets	· Textbook information	
Quality report	· Sequencing quality and coverage · Summary statistics			

Recurrent themes

	Must have	Nice to have	Exclude	Challenges
VUS	· Exclude VUS?			· Avoid unnecessary clinical follow up
Reanalysis				· Inform about possibility for reanalysis and the time frame
Secondary findings	· ACMG secondary findings list		· Exclude incidental findings?	· Definition of what is relevant to return
Data delivery to patient				· Data delivery to patient

Major findings should include pathogenic and likely pathogenic variants with a probable link to the patient's condition (9). The summary of the assay methods should be kept brief and include only the essential information about the test and most important metrics that support validity; if detailed QC metrics are to be included these should be appended later in the report. Information not critical to physician understanding should be relegated to subsequent pages.

Without specifying where in the report it should be included, the OECD Guidelines (2007) also recommends that reports should include:

- information on the referring healthcare professional
- primary sample type
- date of sample receipt
- the identity of the individual approving the report
- recommendations for genetic counselling, follow-up testing and implications for other family members when appropriate

Variant nomenclature

Variants should be described using HGVS nomenclature (7–9,11,13–15). Official gene names approved by the Human Genome Organization (HUGO), RefSeq accession numbers and genome build should be provided somewhere in the report (7,8,14,16,17), although detailed information may be excluded from the front page. Colloquial nomenclature can and should be provided in addition to HGVS nomenclature if it helps clinician understanding (7).

Variant classification

Laboratories should classify variants according to the American College of Medical Genetics and Genomics (ACMG) evidence and classification guidelines (16–18), and should refer to variant categories by their full names, such as "likely pathogenic" rather than category number (e.g. "class 4") to decrease ambiguity. For certain diseases or genes, expert consortiums may maintain variant classification databases, and laboratories should consider where these should be followed and where de novo classification will be performed. These policies should be available to clinicians.

Recommendations on which classes of variants to include on the report vary between organizations. ESHG recommends that laboratories report all pathogenic and likely pathogenic variants, while the reporting of VUS is left to the laboratory's discretion (9). ACMG guidelines indicate that laboratories may report benign and likely benign variants at their discretion, but that these should be clearly distinguishable from VUS or pathogenic variants, and that VUS should be reported if found in genes associated with the indication for testing (13). Other recommendations (16,18) state that laboratories must report all pathogenic, likely pathogenic and VUS, while the reporting of benign or likely benign variants is subject to local policy.

While not necessarily included in the summary, evidence supporting the classification should be included in the report in clear and understandable language. Literature comprising evidence should be summarized and cited, along with other information on the variant (whether supporting or conflicting with the laboratory's classification) from other clinical databases (13,17). The laboratory should assess if phenotypes associated with the detected mutations are similar to the patient's phenotype (13), and this should be a significant factor in deciding whether a variant should be reported or not.

Methods

This section should have a brief description of the procedure for the assay starting from obtaining

nucleic acid (10,17). The report should contain details about the capture technology (if any) and library preparation, sequencing, application type (amplicon, whole exome sequencing (WES) or whole genome sequencing (WGS), and whether the analysis is restricted to certain genes (6,9) either by method design or through an in silico panel (14). It should also contain information about the sequencing platform and the data analysis pipeline including reference genome version, versions and settings for software used (9). The methodology, systems and logic used to filter and prioritize variants should also be included (11,13,17). Finally, it is recommended that pathogenic and likely pathogenic variants are confirmed by an independent test. If the laboratory is also responsible for confirmatory testing, this section should also include information on the orthogonal methods used (9,14).

Quality report
Due to the technical nature of quality metrics, guidelines frequently recommend against including this in detail on the summary page. However, indicators of test validity may be included to indicate to the clinician that quality metrics described later in the report were met (14).

Assay limitations
These limitations may include coverage gaps, the limit of detection, the inability to address specific variant types such as structural variants or copy number variants, bioinformatics limitations, limitations imposed by the structure of the genome or the sequencing method such as the detection of SNPs in GC-rich regions, expected diagnostic yield, and assay sensitivity and specificity (10,13,14). The laboratory may also include a disclaimer that addresses pitfalls in laboratory testing in general, and in particular addresses the issue of negative findings/false negatives, especially for WES and WGS (17) .

Compliance with diagnostic standards
Most guidelines indicate that reports should conform to relevant international diagnostic standards (e.g. ISO 15189 Medical laboratories - Requirements for quality and competence, ISO 17025 General requirements for the competence of testing and calibration laboratories), as well as applicable national standards and regulations where possible (9–11,14).

Reporting multiple patients
If other family members are sequenced as part of a pedigree, secondary findings or genetic risks for these individuals may be discovered. In such cases separate reports should be issued, with a note that findings were uncovered as part of trio or pedigree sequencing (8,11). One exception to this is that it may be appropriate to report a couple's risk of having affected children in the case of recessive disorders on a single report (11).

Anonymity
For tests that require trios, laboratories should avoid including names and relationships (for example, should reference 'parent' instead of 'mother' unless that fact is relevant for treatment), and only include information necessary for the understanding of findings in a clinical context. This extends to larger pedigrees in cases where sequencing additional relatives is necessary (13).

Recurrent themes

VUS

Most variants discovered through NGS-based diagnostics, especially with WES and WGS, lack sufficient evidence to be classified as either pathogenic or benign.

Including these in a clinical report can be important if no clear, causative pathogenic variants were found, and there is a potential link to a patient's phenotype. Advances in medical knowledge in the future may result in reclassification of these variants. However, reporting VUS can represent a risk to patients if they are misunderstood and acted upon, and including VUS alongside meaningful findings may distract readers.

Most guidelines recognize that data management and reporting of VUS is a nuanced topic, and broadly recommend the development of an appropriate VUS policy. For small panels with high sequencing depth, VUS and benign variants may have clinical utility, and laboratories may choose to report these. However, WES and WGS may identify thousands to millions of VUS, and the broad-scale reporting of all VUS is likely to decrease physician understanding and may lead to negative outcomes if VUS are misunderstood by clinical staff and acted upon. Some guidelines recommend that for these applications, VUS should be reported if they are in genes associated with the primary indication for testing or patient phenotype (13), particularly if no likely causative variants were found. Regardless of if the VUS are reported or not, guidelines generally suggest that laboratories store these variants for the eventuality that they are discovered to be clinically actionable in future.

Secondary findings

Secondary findings are variants of clinical significance that are unrelated to the primary indication for genetic testing. How and under what circumstances secondary findings should be reported remains a contentious issue, as policies can have significant external and social impacts.

The laboratory should have a defined protocol for addressing secondary findings (9,13,16). This protocol should address how patients and clinicians can request secondary findings, consent and genetic counselling requirements, information on which variants can be systematically searched for and how these were chosen, and whether these will be confirmed by an orthogonal method before being reported to the requesting clinician or patient or whether follow-up testing is to be ordered separately (13). The ACMG secondary findings working group maintains a list of selected clinically relevant secondary findings which laboratories can choose to follow (12,19), and this list is referred to by other recommendations.

Reanalysis

The systematic reanalysis of unsolved cases on a regular schedule has been shown to increase diagnostic yield due to rapid increases in medical knowledge (20,21). This practice is contrary to traditional medical testing, which sees a diagnostic as a result fixed in time, and policies can be difficult to institute due to practical bioinformatic and infrastructure limitations.

Guidelines generally indicate the laboratory should have a clear policy on data reanalysis, and include this in the clinical report. The policy should address whether the data will be put on a regular reanalysis schedule, whether physicians must request reanalysis, and what circumstances could trigger reanalysis (whether automated or manual).

Recommendations are conflicted on whether systematic data reanalysis is expected. Recommendations from the ESHG, for example (9), suggest that the systematic reanalysis of data is not required, but at the same time indicates that the reclassification of variants should trigger reanalysis

for all patients previously found to have those variants. ACMG (13) notes the utility of reanalysing unsolved cases, and indicate that whatever the laboratories' stance, this policy is clearly stated to clinicians.

Other studies examining the effects of systematic reanalysis of unresolved cases indicate that the periodic review and reanalysis can increase diagnostic yield, and can be beneficial in some settings (20–22). If laboratories do institute a policy for reanalyzing samples, this should be codified and the reanalysis schedule, along with whether this will be conducted starting with .vcf, .bam, or .fastq files, should be included (13).

Data delivery to patient

Medical professionals and patients generally hold a strong belief that patients have the right to access their diagnostic results, and in many jurisdictions this is legally protected. However, the delivery of NGS data directly to patients poses both practical (e.g., should the patient receive a report or raw data?) and ethical (e.g., how to mitigate risks due to misinterpretation?) questions.

Both US (23) and EU legislation (24) address the right of patients to receive access to reports, their health journal and raw data from genetic tests. While anecdotally such requests are rare, laboratories should develop a policy for delivering reports and/or raw data to patients. Technical aspects for this policy should include data format, encryption and secure delivery, mechanisms to ensure party identity, and mechanisms for the patient to ensure data authenticity and completeness. Social and ethical topics within this policy should include how to mitigate risks due to the misinterpretation of these results by second or third parties, the need for confirmatory testing, and recommendations for genetic counselling (25). General guidelines on clinical NGS fail to address these topics in any appreciable depth, other than to advise laboratories to refer to and conform with local law and institution policies.

Summary of Nordic guidelines for genetic analysis

Within the Nordic region, guidelines for clinical genetics analysis have been published in Denmark (26) and Sweden (27). Norway is in the process of developing guidelines and a draft has been submitted for public consultation at the time of writing (28). Finland aims to start drafting similar guidelines in the second half of 2018, while no such guidelines exist in Iceland to date.

The guidelines from the Nordic countries vary widely in scope and depth. The absence or presence of recommendations specifically for clinical reporting of genetic analysis, and the subsections as discussed in Section 3 Review of Existing Recommendations, are summarized in Table 2 on next page.

	COUNTRY	DENMARK	NORWAY	SWEDEN
	PUBLISHING BODY	Dansk Selskab for Medicinsk Genetik	Helse-direktoratet	Svensk Förening for Medicinsk Genetik
Clarity of findings addressed			✔	✔
Summary to include	Patient ID			✔
	Gender			
	Sample ID			✔
	Ethnicity			
	Availability of a family tree			
	Indication for testing		✔	✔
	Methodology and limitations of the test		✔	✔
	Major findings or the absence of findings			
	Conclusions		✔	✔
Results and interpretation	Multiple patients on separate reports			
	Variant nomenclature		✔	✔
	Anonymity			
	ACMG classification: P, LP, VUS	✔	✔	✔
	Clear VUS policy	✔	✔	✔
	Secondary findings	✔	✔	✔
Variant reanalysis			✔	✔

Table 2. Nordic guidelines for clinical genetics analysis

3. Elements of the clinical report

To share best practices between NACG members, a workshop was conducted to identify and discuss the contents of clinical reports. Members identified and categorized information that could be included in a clinical report based on their own best practices and experience into four categories: essential, nice to have but not critical, topics to be excluded from the report, and topics posing significant challenges.

All input was collected and collated to avoid duplication, before mapping all information types to the 14 topics the guidelines made recommendations on (Fig. 3). All except two of the 14 topics - compliance with diagnostic standards and reporting multiple patients - were identified as must-haves during this session, indicative of a high level of awareness among participants of crucial categories of information to be conveyed. Importantly, there was not always consensus on which category information belonged to. Notably, VUS and secondary findings were listed as both essential and information to be excluded by different participants.

Four areas were discussed in detail at the workshop, two related to essential information (actionable results, and description of test methods) and two related to challenges (reporting when phenotypic data is not available or useful, and negative and no findings). The various aspects of these discussions are detailed below, concluding with workshop output for the four recurrent challenging topics as previously described.

Main topics

Actionable results

While there is broad agreement that findings which are valuable to the diagnosis or treatment of the patient and are related to the indication for testing should be included in the clinical report in all cases, there is uncertainty in defining these systematically. In the context of rare genetic disorders, it must be recognized that even pathogenic findings related to the indication for testing may not be considered therapeutically actionable in the sense that causal links between the variant and the disease have not been established via clinical trials, and that approved treatments for those variants may not exist.

A strict interpretation of 'actionable' would exclude the reporting of such variants, which are of unquestionable value to the management of the patient. In general, a loose interpretation of 'actionable' should be taken, and variants should be reported if they have the potential to:

- change the disease monitoring or surveillance regimen for the patient
- guide therapeutic decisions, both for established and off-label treatments
- identify relevant clinical trials
- guide future diagnostic efforts
- exclude potential molecular mechanisms for disease
- impact the management of relatives of the patient
- be reclassified due to new knowledge or the availability of new therapies in the future.

Description of test methods

The methods used for the test and data analysis are important to understand the scope and limitations of the assay and report. At the same time, detailed technical information may be not useful for non-specialists, and may distract from understanding key findings. The scope and contents of the methods description and QA/QC metrics, along with how these are displayed in the report constitute an open topic. Minimal items to include are:

- A brief description of test methods
- genes analyzed
- types of aberrations analyzed
- genomic coordinates, transcript, human genome reference build
- methods to confirm variants
- how inheritance is reported
- molecular biology, sequencing and bioinformatics methods
- reanalysis policy/procedures
- ACMG gene list for secondary findings
- family studies, co-segregation

Reporting when phenotypic data is not available or useful

In some clinical settings, phenotypic data is not regularly reported, may be of low quality, or may not follow standardized formats such as the Human Phenotype Ontology (HPO). The lack of high-quality, trustworthy clinical data is a critical issue for both analysis and reporting genetic findings. WES and WGS allow the identification of thousands or millions of variants, and in the absence of a definitive phenotype the prioritization and identification of causal, pathogenic variants is extremely difficult or impossible. In the best-case scenario, laboratories can go back to the requisitioning physician to obtain phenotypic data and then return to prioritize and analyze variants, increasing turn-around time and total cost for the test, but in some cases, this could lead to the reporting of non-causative variants and unnecessary follow-up.

Negative and no findings

While the diagnostic yield of NGS-based tests can be extremely high (>85%) and can match or surpass other diagnostics for some indications (29), diagnosing rare diseases is difficult. Gaps in technology and medical knowledge, a lack of prior cases and the use of NGS-based diagnostics only after all other attempts at diagnosis fail mean that diagnostic yield may remain under 50% (30). Physicians not used to NGS-based diagnostics may be less familiar with receiving no findings and the idea that a test can be successful but does not provide any clear diagnostic value. In some settings, true negatives may be clinically informative. Understanding how to present negative findings (true negatives) and the absence of any definitive findings is an important topic to guide treatment decisions and future reanalysis strategies or alternative diagnostics.

Recurrent themes

VUS

VUS are generally not reported on a broad scale in most laboratories, however members agree that reporting VUS may be critical for certain cases. In small gene panels, where the total number of VUS is lower than with WES or WGS and where genes tested are highly relevant to the patient's condition, some laboratories report all VUS as a general rule. From WES or WGS, VUS in genes associated with the patient's phenotype are often reported, particularly if no causative pathogenic or likely pathogenic variants are found. If VUS are included, language surrounding the ambiguity of a VUS classification, and how these findings differ

from known pathogenic variants, is an essential part of the report.

Arguments not to report VUS on a broad scale include that clinicians and patients may misinterpret the findings, or that findings may be taken out of context in the future. In some laboratories, there is limited graphical freedom in the reporting format, and these laboratories may choose not to report VUS rather than report them without the ability to sufficiently highlight how VUS differ from pathogenic findings.

Secondary findings

The reporting of secondary findings presents complex ethical questions and challenges. The group discussed under which conditions secondary findings could and should be reported, and highlighted the central topics of informed consent prior to testing, resources for genetic literacy, and limiting secondary findings to a set of variants with known modes of pathogenesis. The idea of conducting risk assessments surrounding the reporting of secondary findings at health institutions was proposed, possibly using a bowtie approach.

Reanalysis

As knowledge about the pathogenesis of many disease and the technology and computational methods used to analyze these data are rapidly evolving, reanalysis of data generated in an earlier test, or re-testing of the patient using up-to-date technologies, has the potential to increase diagnostic yield (21). The group discussed how this could be practically instituted given bioinformatic, legal and practical limitations.

Data delivery to patient

Clinical reports are written for physicians, and could be misinterpreted by people without medical genetics knowledge. Nevertheless, the patient has a right to receive both the results of a test and the raw data. Each testing laboratory needs to decide on procedures for data delivery to a patient that are consistent with local laws, hospital policies and their society's ethics.

4. Peer-review benchmarking of Nordic clinical reports

There is no shared clinical report format across the Nordics, and there is significant variation between reports issued by different laboratories, even within the same country. After reviewing guidelines on clinical reporting and discussing the topic in depth, NACG laboratories embarked on a benchmarking exercise as a first step in generating information on how to best present NGS-based diagnostic test results.

In this exercise, three hypothetical clinical cases were provided to NACG laboratories. Each case was supplied with the indication for testing, the background and medical history of the hypothetical patient, details regarding the assay, and a list of findings assigned to the five ACMG classes, along with other relevant information (compete cases can be found in Appendix 2: Clinical Cases):

- Case 1 included a single likely pathogenic variant associated with the patient's phenotype.
- Case 2 included a secondary finding from the ACMG secondary findings working group list (19), along with two heterozygous VUS in a gene associated with the patient's phenotype.
- Case 3 contained a likely benign variant in a gene associated with phenotypes matching the patient.

The three cases were put through the reporting process by five NACG laboratories, which then issued a total of 15 reports. These were assessed by seven evaluators against the criteria identified at the workshop using a survey based on the criteria identified through the review of existing guidelines (Appendix 3: Survey questions). Through peer evaluation, strengths and weaknesses were identified for each laboratory, which could then serve as the basis for internal discussions on improving the reporting process within each health system. The workflow for this benchmarking exercise is shown in Fig. 4, with two illustrative findings, with a summary of key findings below. The full set of plots supporting these findings is available on request.

Main topics

Clarity

In general, evaluators correctly identified pathogenic variants, likely pathogenic variants and VUS within reports, but evaluators reached consensus on which classes of variants were included for only a single report. Evaluators were not clear which classes of variants were reported for four out of five reports.

One report included a likely benign variant in a gene associated with the patient phenotype and indicated this was insufficient to assign a genetic diagnosis, however only three out of seven evaluators stated that the report included a likely benign variant, suggesting that the clarity surrounding this finding was low.

Reports scored generally good or very good with regards to the clarity of findings, where those that

Real-world use of clinical reports

Anecdotal evidence suggests that in many clinical settings, clinicians spend a short amount of time, 30-60 seconds, reviewing clinical NGS reports. Groups were asked to take one of the reports they had not previously seen, and take one minute to draw meaningful conclusions about that hypothetical patient. When faced with a one-minute time limit, NACG workshop members were unable to identify main findings in many reports, which initiated discussions surrounding the importance of visual design, brevity, essential vs. extraneous information, and how their end-users perceive and interact with reports at a first glance.

Participants recognized that if a group of their peers, who are generally experts with a deep understanding of genetics, NGS and the features of a clinical report, has difficulty pulling out main findings, it would be unlikely that this would be possible by clinicians less familiar with the technical aspects and limitations of NGS-based diagnostics.

highlighted the main findings by using tables or colours scored higher (Fig. 4).

Language clarity was generally rated as good or very good. However, despite the perception of clarity in language and key findings, there was significant variability between the information evaluators marked as being included in reports and information that was actually present. This suggests that while readers perceive these reports to be clear, in practice this is not the case.

Confirmatory testing

All reports stated that while primary findings were confirmed by Sanger sequencing, secondary findings were not, but the clarity of this information varied widely between reports. For each sets of reports, between one and six evaluators stated that reports included information about confirmatory testing of secondary findings. Evaluators gave similar responses to a question about confirmatory testing of key findings, indicating that the clarity surrounding orthogonal testing varies substantially between reports.

Report summary

While key findings, the indication for testing and patient ID were included in all reports and clear to evaluators, other information was absent in some reports. One set of reports did not include information on patient gender and none of the evaluated reports stated ethnicity of the patient, both information categories recommended by the reviewed guidelines (see Review of Existing Recommendations).

Test limitations

All laboratories addressed assay limitations to some extent. Methods for the test along with limitations were provided with the cases, and while most laboratories used this as-is, one laboratory extracted only the information they found to be relevant to their current production system.

All laboratories indicated the assay did not address complex structural variants, however this was unclear for evaluators for two sets of reports. Two laboratories added information about limitations due to coverage gaps. Most evaluators correctly identified this in reports that contained this information, but several evaluators stated that this information was also contained in the reports that did not address the topic. Several evaluators stated that some reports contained information on the limit of detection (LOD), yet this was not addressed by any of the reports.

Recurrent themes

VUS

All laboratories included the VUS from Case 2. All reports contained additional information about the potential relevance of VUS to the patient's condition along with qualifying statements about the two VUS, explaining in various ways the difference between VUS and a pathogenic variant and considerations for clinical interpretation. In 89% of evaluations the presence of pathogenic and likely pathogenic variants was correctly identified, and in 86% of evaluations the presence of VUS was correctly identified, indicating that these findings are presented with similar clarity to readers. While all clinical reports included VUS, information regarding the possibility of future reanalysis was not available in any reports.

Secondary findings

One clinical case contained a pathogenic mutation in BRCA2, which was included on all reports along with indications that it was secondary to the primary indication for testing. All laboratories clearly

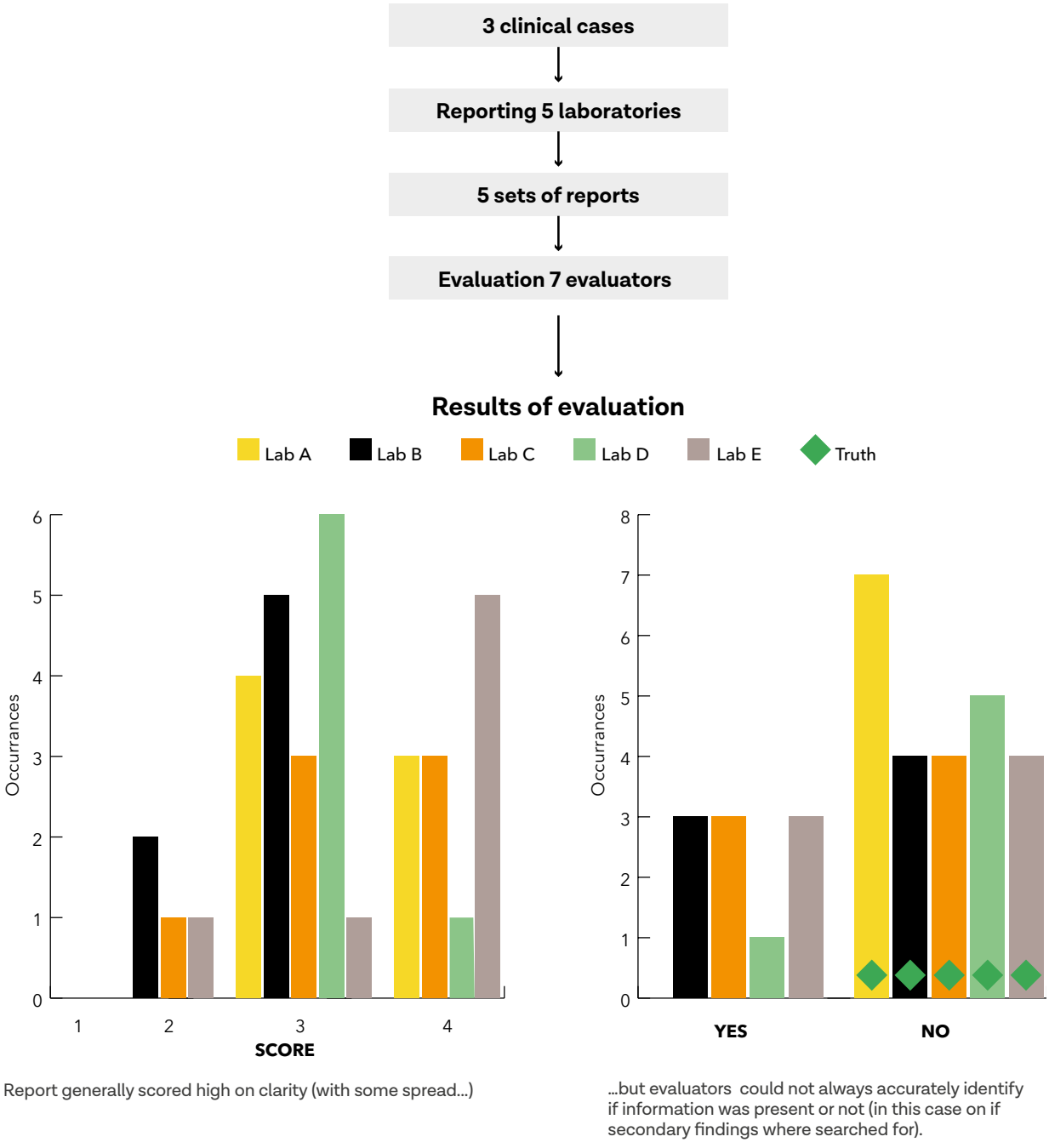


Figure 4. Workflow of the clinical reporting benchmarking exercise, with two resulting findings.

indicated the presence of secondary findings in the reports.

Although secondary findings were reported, no reports provided information on the laboratory's secondary findings policy, such as if secondary findings were systematically searched for (Fig. 4). Only one report provided a list of which variants or genes would be reported. This information was not clear to evaluators: for four out of five sets of reports evaluators gave mixed responses when asked if the reporting laboratory had a systematic secondary findings policy or not. Furthermore, for the single laboratory that indicated ACMG secondary findings were reported, only 29% of evaluators could find this information in the report.

Reanalysis

None of the reports indicated possibility for reanalysis or a reanalysis policy, even for case 2, which included two VUS in a clinically relevant gene. This was clear to all evaluators.

Data delivery to patient

None of the reports addressed delivering raw data or the clinical report directly to the patient.

Suggested improvements

Laboratories received access to the complete survey information, but also a set of specific recommendations from each evaluator. While these were different for each laboratory, in general these are summarized in Fig. 5 and included suggestions to:

Better highlight key findings

Generally, positive feedback on reports related to how clearly and briefly information was presented, while suggestions for improvement surrounded improving the visual design and layout to better highlight critical information. Reports scored higher in overall clarity, and evaluators could more accurately answer questions about the contents of the reports, when they included visual elements that drew attention to specific information. Three out of five reports used only text in the reports and did not highlight the main findings with colour, tables or other visual aids. When discussing these results, some participants indicated this was due to technical limitations, and that improvement would by necessity involve changes to both their process and IT infrastructure.

Add information on test content

Several evaluators believed that reports did not adequately describe the methods and limitations of the test. In particular, the scope of the test was unclear. While for small in silico or amplicon panels laboratories can provide a list of genes analyzed, this is not a tenable solution for WES or WGS assays. This exercise simulated a 60 MB exome, and for similar tests or WGS, the list of genes analyzed are normally limited in some way to pathways relevant to the patient phenotype. Adding information on the total content of the assay or

how pathways, HPO terms or other information was used during variant prioritization, classification or interpretation, could provide transparency and improve understanding of the scope of the test.

Codify and clarify the laboratory's reporting guidelines

Evaluators struggled with many reports to understand the types of variants and secondary findings that would be reported from the assay, and critical feedback highlighted this as an area for improvement. Existing guidelines leave significant room for interpretation for laboratories when deciding when and if to report VUS (13), and

many participants indicated that VUS reporting was a nuanced topic. Laboratories should consider developing criteria for when VUS are to be reported. Including notes on the laboratory's secondary findings policy and the situations under which VUS or other non-pathogenic, but potentially clinically relevant findings would be reported could improve understanding about the methods and limitations of the test.

Include a list of potential secondary findings

While many laboratories reported secondary findings, the laboratory's secondary findings policy was often unclear, and reports rarely included a list of which secondary findings the laboratory examined. Many laboratories considered adhering to the ACMG secondary findings gene list (19) as good practice if secondary findings were to be reported. Laboratories should note their secondary findings policies with greater transparency on the reports, and include a reference to the ACMG list or their own custom list of secondary findings on the report.


Clinical reporting
MUST
Easy to understand
Not too much text
SUMMARY
INDICATION
Ethnicity
DETAILED PHENOTYPE
CLASSIFICATION
ACMG #
(SOME) EVIDENCE/ BASIS FOR CLASSIFICATION
IMPLICATIONS OF THE RESULTS
STATEMENT ON GENETIC COUNSELING
TECHNICAL DESCRIPTION
GENES ANALYSED
ABERATIONS ANALYSED
DESCRIPTION OF TECHNICAL LIMITATIONS
METHOD
QUALITY REPORT
Tables
Things in order
Clear indication
Clear conclusion
Conclusion on top
Briefness in Oslo
Family studies
co-segregation

5. Interviews on clinical reporting

To develop a deeper understanding of the main challenges of reporting NGS-based diagnostics, a series of semi-structured interviews were conducted over a two-week period in June 2018. Interview subjects were drawn from across the Nordic countries, and represented both the clinical NGS laboratories that issue these reports and clinicians that use these reports in their daily work. Interviews addressed a set of pre-defined questions (Appendix 4: Interview questions), and varied in length from 20-90 minutes.

VUS

Risk of misinterpretation

VUS reporting is a complex issue, as the need to communicate potentially relevant information needs to be balanced against the risk of misinterpretation of this finding as actionable by the receiving physician, especially in the absence of a confirmed diagnosis. Several respondents reported anecdotal evidence of physicians having acted upon VUS.

Two of eight respondents deliver reports to expert users with a high level of genomic literacy, but conceded that this was not a viable future solution with increasing volumes of sequenced genomes, and potential further inclusion of VUS in electronic patient journals.

Two respondents explicitly state in their reports that VUS are not actionable, while three respondents focus on communicating the uncertainty of VUS.

Four respondents stressed the importance of increasing genomic literacy of physicians, either through direct communication, the publication of (national) guidelines or training programmes.

Reporting

All respondents (eight out of eight) report VUS that are detected in genes that are related to the requisition phenotype. Four respondents only report these VUS in situations when no available pathogenic or likely pathogenic variants related to the phenotype were found. Four respondents regularly report all VUS detected in relevant genes. Four of eight respondents expressed concern regarding policies for reporting VUS which may have clinical relevance for a patient and should not be excluded from future consideration, especially if no diagnosis was reached. One respondent suggested the idea of a 'high VUS', reflecting a gradient of evidence of pathogenicity. Two respondents noted that their policy is different for oncology samples, where they report all VUS detected in small gene panels, as the diagnostic value is greater.

Conflicting classification

All respondents (eight out of eight) deal with cases where their classification of a variant conflicts with those of other laboratories or literature, and seven respondents addressed the topic in detail. Five out of seven subjects include the conflicting classification alongside their own in the report, along with a discussion of the evidence and materials that supports the

laboratory's classification. Two respondents believed that following the ACMG guidelines for variant classification has the potential to eliminate or limit classification discrepancies. Five respondents noted the lack of tools for sharing variant classifications between laboratories as a challenge. Three respondents identified data quality issues with external classifications as a challenge.

Data management

Seven laboratories provided information on how VUS were managed at their sites: two respondents do not store VUS at all, two laboratories store pathogenic, likely pathogenic and VUS in a central database, one laboratory stores VUS from each patient via an access-controlled journal for future use within the laboratory, and two laboratories rely on Excel spreadsheets to follow VUS over time. Four respondents expressed the need for both more systematic management of variant classifications and tools that enable this.

Secondary findings

Search and reporting secondary findings

Laboratories reported various management strategies for the detection and reporting of secondary findings: five out of eight laboratories report variants in genes included in the ACMG secondary findings list if patients consent to receive these.

One laboratory does not report secondary findings, citing a lack of means to track patient consent. Two out of eight laboratories use gene panels and do not regularly analyze secondary findings. Both of these laboratories have on rare occasions in the past uncovered secondary findings, and a multidisciplinary team is put together to discuss whether these should be reported to the patient or not.

In the absence of patient consent for laboratories that do report secondary findings, policies vary. One laboratory always reports the secondary finding to the requisitioning clinician who takes on responsibility for further management. Another laboratory states in the clinical report that a secondary finding has been identified but not reported due to lack of consent, without further specifying what the finding is.

Of the laboratories that use the ACMG secondary findings list, one laboratory stated that if patients consented and secondary findings were found, these were treated as any other actionable causative variants with respect to reporting and clinical follow-up. Two laboratories report secondary findings related to variants which are informative for the risk of developing a disease, or are related to diseases with no current detectable phenotype, and an additional laboratory would report these if a patient specifically requested. These respondents all recommended a cautious approach and noted that these findings have a higher threshold for reporting and require minimally: confirmatory testing, review of family history, consultation with the clinician, awareness of the patient's genetic literacy and genetic counselling.

Importantly, seven out of eight respondents believed that patients have the right to receive secondary findings related to diseases with no clinical interventions if they request them. Of these, three out of eight respondents do not normally search for or return these results. Three laboratories had mechanisms in place for this situation: one relied on a multidisciplinary board to review and decide on action, one had a policy for mandatory genetic counselling, and at one site reporting secondary findings is the responsibility of the requisitioning physician.

Reporting secondary findings to family members

Secondary findings in genetic testing can be of significant relevance to family members, and in some cases laboratories or the requisitioning physician could have a legal or moral imperative to ensure family members are informed of genetic risks or carrier status. One laboratory reported that contacting the family of a patient was legally prohibited in their country. Two organizations rely on genetic counsellors to decide whether contacting a patient's family directly is appropriate. In two organizations, physicians rely on the patient themselves to contact family members, and one of these supplies the patient with a letter they can use for this purpose. One organization believes that carriers should be informed directly, and recommends follow-up with a genetic counsellor, oncologist or other appropriate professional.

Reanalysis

Strategies for the periodic reanalysis of data for unsolved cases to leverage changes in knowledge and technology was nominated by six out of eight respondents for discussion. None of the six respondents who nominated this topic had a strategy in place at their institution for routine reanalysis. One respondent indicated this was primarily due to limited computing capacity.

Three out of eight respondents had routines in place to inform the referring clinician and patients of the possibility of reanalysis in the case of no findings. Reanalysis thus occurs on an ad hoc basis, and can be triggered variously by the patient, the referring clinician or the laboratory itself with the emergence of more detailed phenotypes or new target genes. Only one respondent reported a systematic approach to reanalysis of all previous patients with no findings when in silico panels are updated to include new genes. There was no consensus on the optimal frequency of reanalysis.

In the case that laboratories track VUS and these are reclassified as pathogenic or likely pathogenic, management strategies varied. One laboratory relied on a board to decide whether informing patients was beneficial, and three would on an ad hoc basis notify requisitioning physicians that a VUS had been reclassified and that further diagnostic or therapeutic options may be available. One respondent issues amendments to clinical reports indicating the VUS reclassification when this is likely to be clinically relevant.

Data delivery to patient

The delivery of data to patients was identified as a challenge by five out of eight respondents. All respondents that discussed this topic believed that patients should have access to either clinical reports or their raw data. Three out of five respondents had either directly or indirectly experienced a request for data from patients, and had in all cases fulfilled these requests. Respondents indicated this happened very rarely, and most could recall only a few cases out of thousands of patients.

There was no consensus in the format in which the data should be delivered, such as a clinical report, a modified report for patient use, or raw data as .vcf, .bam, or .fastq files. One respondent raised the topic of encrypting .fastq files for secure transfer.

Three respondents also urged caution when delivering genetic data to patients, and strongly recommended that delivery is accompanied by an offer for genetic counselling to provide patients the assistance they may need to manage their genetic data. One laboratory indicated that if reports are delivered to patients, these should be readable for the patient as well as medical professionals.

VUS

- All labs correctly reported VUS with accompanying information about its potential relevance
- Evaluators were generally able to correctly identify the presence of pathogenic, likely pathogenic and VUS variants with a success rate of 85%

Secondary findings

- Secondary findings were correctly identified as being present in 97% of the cases
- No reports included information on the labs' secondary findings policy
- Information on SF policy was hard to identify in the reports by evaluators

VUS

- 8/9 repondents report only VUS detected in genes/panels relevant for phenotype
- 1/9 favoured not reporting VUS as a general rule
- 4/9 respondents report identified VUS classification conflict

Secondary findings

- 4/9 returned results in ACMG secondary findings if consented to
- 2/9 avoid issue by not looking in any other genes
- 1/9 never returns secondary findings

Figure 5. Findings from report evaluations through benchmarking and in-depth interviews relating to the four recurrent challenging topics.

Reanalysis

- No reports gave information on potential future reanalysis, even when reporting VUS

Data delivery to patient

- No reports adressed the return of data or the clinical report directly to the patient

Reanalysis

- Only 1/7 respondents systematically reanalyze data
- 3/7 respondents inform clinician of possibility of future reanalysis - but NOT through clinical report

Data delivery to patient

- 6/6 respondents agreed strongly that the patients had the right to receive their data if they wanted
- 3/6 respondents had experienced one or two cases of patients requesting their data throughout their careers, indicating these are rare events so far
- 2/6 respondents suggested patient genetic counseling be offered when returning data

SUGGESTED IMPROVEMENTS

- Highlight main findings using colour or tables
- Avoid specifying familial relationships unless necessary
- List the genes analysed by the assay
- List the genes the lab reports secondary findings for
- Include the policy for reporting VUS

6. Discussion

As the use of NGS-based diagnostics in regular clinical practice expands, the need for open dialogue surrounding best practices, risk management and ensuring quality is increasing. To address the shared challenges of implementing precision medicine in a Nordic perspective, stakeholders involved in the implementation of clinical NGS diagnostics across the Nordic countries founded NACG as a grass-roots organization to cooperate on improving the quality and performance in their respective health systems.

The NACG model focuses on practical implementation, benchmarking and discussion, and as such follows a workshop format rather that of a traditional scientific conference. Through a user-driven, interactive process, NACG organically identifies and addresses challenges in precision medicine. Clinical reporting was identified as a topic of interest through this process, and was addressed by a team of 23 participants drawn from across the Nordic countries.

Our review of existing recommendations on clinical reporting and in-depth discussions surrounding the contents and design of good clinical reports identified numerous topics and challenges. Published recommendations often conflict with one another and require significant interpretation by individual laboratories before they can be put into practice. However, there is a general understanding that reports should focus on clarity, brevity and the effective delivery of sufficient information to understand key findings via a report summary. Supporting information, QC metrics and in-depth technical details should be relegated to subsequent sections of the report. An interactive workshop session to identify and categorize information elements showed substantial overlap with the topics in the recommendations, though there was not always consensus on whether different elements should be included in or excluded from the report.

A systematic peer-review benchmarking of Nordic clinical NGS reports indicated that even though laboratories were well aware of the challenges surrounding clinical reporting, there is room for improvement in practice. Through this exercise, difficulties in understanding key information were identified as being due to report design but also the limitations of local IT infrastructure. One participant indicated that the findings of this exercise would be of direct value within their institution when lobbying for improvements to their reporting system.

In-depth semi-structured interviews on key topics revealed how variable reporting practices are in the clinical setting, but also gave insight into some of the differences between systems, and nuance behind issues surrounding clinical reporting. It should also be noted that the interviews conducted here were with individuals with a high level of genetic literacy. As clinical NGS becomes broadly implemented, more work is needed to develop solutions that scale and allow clinicians and patients with less genetic literacy to interact with these issues. Studying these interactions will be of great value to inform best practices.

Together, the four activities performed in this study uncovered multiple challenging areas when considering how to manage and report NGS-based test results in a clinical setting. For VUS, there was

broad recognition that the medical knowledge on a particular VUS can vary significantly, and that the category encompasses a range of uncertainties. Challenges included the development of systematic policies that capture if, when and under which circumstances VUS should be reported, reporting of VUS in a way that is comprehended by patients and physicians, and the wider consequences of these policies, for example for family members. The need for better management of classified variants was identified, as well as data sharing between laboratories to drive quality and resolution of classification discordances.

There was substantial variation in how participating laboratories managed secondary findings, both for patients and potentially affected family members. Although the use of the ACMG secondary findings list after informed consent was a generally accepted approach, implementation varied. Additionally, there was recognition that the ACMG secondary findings list contains only a small set of variants, and that other secondary findings may be important for specific populations or therapeutic areas. Some organizations were faced with technical limitations that prevent the implementation of their preferred policy.

Reanalysis of data in the case of no findings was also a challenging area. While most institutions have policies and procedures for reanalyzing data on a sporadic basis, many expressed that the systematic and automated reanalysis of unsolved cases would have clinical benefit. Additionally, as patients' genetic literacy rises, an informed discussion is needed on how best to deliver genetic data to patients and with what level of support.

7. Conclusions

Effective and accurate information flow in NGS in the clinical, encompassing both delivery and comprehension, is strongly influenced by clinical report structure and design. The results of this study indicate that the critical clinical genetics report is ripe for a redesign process from the ground up, prioritizing fitness for purpose and user needs.

Other topics in various stages of the NACG method include a critical evaluation of the requisitioning process, a focus on validation and verification techniques, and the development of methods for comparing and benchmarking variant prioritization processes and algorithms. By focusing on practical issues relevant to clinical laboratories and employing a ground-up and agile workshop format, NACG will continue developing as a Nordic forum representing diverse parties involved with the implementation of precision medicine in the Nordic health sphere.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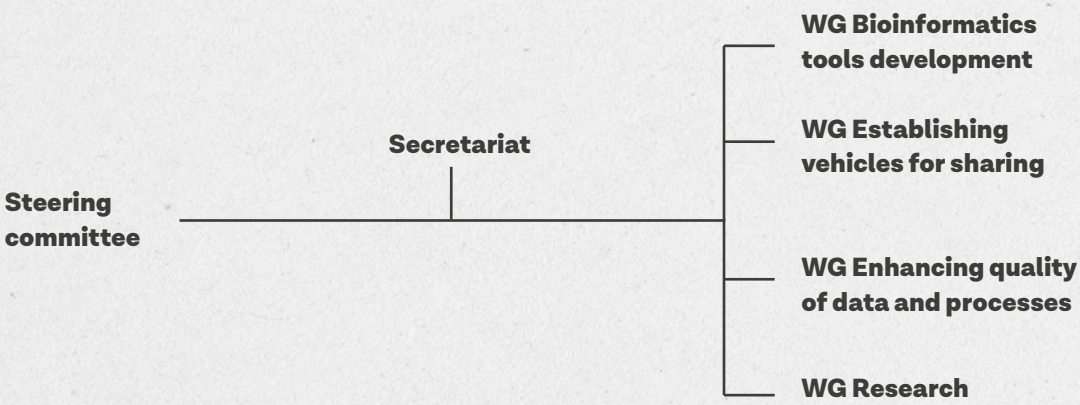
Appendix 1: About NACG

The Nordic Alliance for Clinical Genomics (NACG) is an independent, non-governmental, not-for-profit Nordic association. Its mission is to share technology competence and trusted genomics data, and to serve as a resource for research. NACG has defined the following goals:

- To facilitate the responsible sharing of genomic data, bioinformatics tools, sequencing methods and best practices for interpretation of genomic data.
- To enhance quality of genomic data and processes, and explore methodologies to provide assurance.
- To understand legal barriers to the implementation of personalised medicine and to engage with key stakeholders that influence these barriers.
- To develop demonstration projects that challenge perceived legal barriers that limit responsible and ethical sharing of genomic and health data.
- To build bridges between research and clinical communities, technologies and practices to foster innovation

NACG is coordinated by a Steering Committee and Secretariat. To achieve these goals, work in the alliance is organized through four Working Groups. For more information please see nordicclinicalgenomics.org or email post@nordicclinicalgenomics.org



STEERING COMMITTEE MEMBERS			
Role	**Name**	**Affiliation**	**Country**
SC Chair	Dag Undlien	Oslo University Hospital	Norway
SC Vice Chair	Valtteri Wirta	SciLifeLab	Sweden
SC Vice Chair	Karin Wadt / Morten Dunø	Department of Clinical Genetics, Rigshospitalet	Denmark
SC Member	Joakim Lundeberg	SciLifeLab	Sweden
SC Member	Jón Jóhannes Jónsson	Dept. of Genetics and Molecular Medicine, Landspitali - National University Hospital / Dept. of Biochemistry and Molecular Biology, Faculty of Medicine, University of Iceland	Iceland
SC Member	Maria Rossing	Center for Genomic Medicine, Rigshospitalet	Denmark
SC Member	Stephen McAdam	DNV GL	Norway

SECRETARIAT			
Role	**Name**	**Affiliation**	**Country**
Secretariat	Guro Meldre Pedersen post@nordicclinicalgenomics.org	DNV GL	Norway

WORKING GROUP LEADERS			
Working group	**Working group leaders**	**Affiliation**	**Country**
Bioinformatics tools development	Kjell Petersen / Tony Håndstad	University of Bergen Oslo University Hospital AMG	Norway
Establishing vehicles for sharing	Henrik Stranneheim / Chiara Rasi	SciLifeLab	Sweden
Enhancing quality of data and processes	Sharmini Alagaratnam / Courtney Nadeau	DNV GL	Norway
Research	TBD		

Appendix 2: Clinical cases

Clinical cases were based on real patients but modified and anonymized - names and birth dates are not real, and some of the findings were modified. The described methods do not represent best practice and are for illustrative purposes only. NACG does not endorse any of the mentioned reagents, software or sequencing platforms.

Patient ID: CASE1_MN
Name: Mattias Nordahl
Gender: Male
DOB: 22.02.2018
Physician: Jon Johansen
Diagnosis Arthrogryposis multiplex congenita (AMC)

Phenotype
Contractures in fingers, feet and knees. Big skull with prominent forehead with hemangioma that spreads over thoracic spine and sacral pit. Short nose, long filtrum, downward mungipor, long eyelashes.

Test results

Gene	MYH3 (NM_002470.3)
Variant coordinates	c.700G>A (p.Ala234Thr)
Zygosity	homozygous, parents are carriers
Classification	likely pathogenic variant

Patient ID: CASE2_AH
Name: Anna Høvik
Gender: Female
DOB: 18.05.2013
Physician: Jon Johansen
Diagnosis Arthrogryposis multiplex congenita (AMC), muscle hypotonia, psychomotor development retardation

Phenotype
Brain MR shows suspected dysgenesis and delayed myelination,

microcefalia – 3 SD. Parents are healthy

Gene	RARS (NM_002887.3)	RARS (NM_002887.3)	BRCA2 (NM_000059.3)
Variant coordinates	c.668G>A (p.Arg223His)	c.1568T>A (p.Met523Lys)	c.8648delC (p.Pro2883Hisfs)
Zygosity	heterozygous, inherited from father	heterozygous, inherited from mother	heterozygous, inherited from mother pathogenic variant
Classification	variant of uncertain significance	variant of uncertain significance	Variant was not verified due to the lack of association with requisition phenotype.
Notes			Variant was not verified due to the lack of association with requisition phenotype.

Patient ID: CASE3_IC
Name: Ivar Carlson
Gender: Male
DOB: 28.01.1989
Physician: Jon Johansen
Diagnosis Dyskinetic Cerebral Palsy

Phenotype
Epilepsy, scoliosis, speech disorder.

Test results

Gene	GRIN2A (NM_000833.4)
Variant coordinates	c.3228C>A (p.Asn1076Lys)
Zygosity	homozygous, parents are carriers
Classification	heterozygous, inherited from motherlikely benign variant

Methods
Blood samples from the family trios were collected and received for sequencing on the same day. RNA capture baits against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCDS, RefSeq and Gencode databases) was used to enrich regions of interest from fragmented genomic DNA with Agilent's SureSelect Human All Exon V6 kit. The generated library was sequenced on an Illumina NextSeq 500 System to obtain an average coverage depth of ≥ 80x. An end-to-end in-house bioinformatics pipeline was applied including conversion of base calls to fastq files (bcl2fastq2 Conversion Software v2.20), alignment of reads (bwa v. 0.7.17) to GRCh37/hg19 genome assembly, preprocessing of alignment files (GATK v.3.8.1), variant calling (GATK Haplotype caller v.3.8.1), variant filtering and annotation with (Illumina Variant Studio 2.2). Identification of complex structural variants was not part of the pipeline. Evaluation was focused on coding exons along with flanking +/-20 intronic bases. All pertinent inheritance patterns were considered. In addition, provided family history and clinical information were used to evaluate identified variants. All identified variants were evaluated with respect to their pathogenicity and causality, and were categorized into five classes. Only rare variants with AF < 0.01 currently associated with patient phenotype were verified and reported. Verification was done by Sanger sequencing. Rare variants not associated with the requisition phenotype and classified as pathogenic or likely pathogenic were also reported if patients consented to receiving secondary findings.

Appendix 3: Survey questions

1. Please score the clarity of the main findings in the reports. (scale 1-4).
2. Are all pages of the reports marked with a patient ID and page number? (Yes/No)
3. Do the first pages of the reports have (check boxes):
   - Unique patient ID (e.g social security number)
   - Patient gender
   - Sample ID
   - Ethnicity
   - Availability of a family tree
   - The purpose of testing
   - Short description of used methodology
   - Short description of test limitations
   - Major findings or clearly indicate absence of findings
   - Conclusion
4. Are variants listed according to HGVS nomenclature? (Yes/No)
5. Which classes of variants are reported? (check boxes):
   - Pathogenic
   - Likely pathogenic
   - Unclassified (VUS)
   - Likely benign
   - Benign
   - It is not clear which classes of variants are reported
6. Are secondary findings reported? (Yes/No)
7. Is information provided on whether secondary findings were systematically searched for? (Yes/No)
8. Is a list of genes used to search for secondary finding provided or mentioned? (Yes/No)
9. Is information about the confirmation method of secondary findings provided? (Yes/No)
10. Is there a methodology description in the reports? (Yes/No)
11. Which steps does the methodology description cover? (Yes/No)
12. Sample collection
    - Nucleic acid extraction
    - Library preparation
    - Sequencing details
    - Bioinformatic analysis
13. Do the reports contain variants confirmation method? (Yes/No)
14. Do the reports disclose limitations of the test? (Yes/No)
15. Which limitations are disclosed in the reports?
    - Coverage gaps
    - Limit of detection
    - Inability to address specific variant types
    - Expected diagnostics yield
    - Other (specify)
16. Do the reports contain information about the possibility of future data reanalysis? (Yes/No)
17. Is the language used in the reports clear? (scale 1-4)
18. In your opinion, what was done well in the reports from this laboratory? (free text)
19. How would you improve the reports from this laboratory? (free text)

Appendix 4: Interview questions

1. VUS

a) Report it or not?
b) If a laboratory chooses to report VUS, how to make sure that a physician does not misinterpret it?
c) How to deal with VUS that are classified by other laboratories as non-VUS?
d) Data management for VUS: Database? Separate database?
e) How to mitigate unnecessary clinical follow-up due to VUS?

2. Secondary findings

a) Should secondary findings ever be reported? If so, which?
b) How to gather consent for secondary findings reporting? What happens if specific secondary findings were not addressed by consent?
c) How to handle secondary findings related to disease risk/conditions without visible phenotypes?
d) How to handle information about risk of disease for which there is no effective intervention?
e) Should secondary findings be shared with family? All secondary findings, only actionable, risk factors?
f) Should secondary findings be shared with family after death of patient?

3. Phenotype uncertainty

a) What genes should be analysed or prioritized if phenotypic data is not available or useful?
b) Which results should be reported in this case? How to indicate this is based on only genetic information?

4. Method description

a) How detailed should the description of a method be?
b) Which quality metrics should a report contain?

5. Reanalysis and reclassification

a) Should a laboratory perform regular reanalysis of data? How often?
b) How should patient/physicians be informed about possibility of future reanalysis? Who triggers reanalysis?
c) What happens when a variant is reclassified to a definitive category?
d) What about secondary findings? If a patient has opted-in and policy changes to include a new secondary finding later, is this reanalyzed?

6. Data delivery to patient

a) Should reports written for physicians be delivered to patients?
b) If patient requests to receive raw data, what should be delivered?

7. Actionable results

a) How to distinguish causative actionable and non-actionable variants (or clinical trial available)?
b) What happens when a new therapy is adopted and previously non-actionable variants become actionable?

8. No findings

How to make patients and physicians aware about uninformative negative (e.g. due to limitations of analysis pipeline) vs. true negative (e.g. when a mutation in a gene known to cause phenotype in a family is absent in tested individual)?


P

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SciLifeLab

REGION Rigshospitalet

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