Application Note

# Reporting of NGS sequencing data in clinical practice

## Introduction

Today, in the era of next-generation sequencing (NGS), the challenge is no longer how to produce large quantities of genomics data, but how to analyze, interpret, and apply this data for meaningful use. Targeted gene panels using NGS are now standard in many clinical labs; however, as the cost and processing time of NGS continues to decrease, whole-genome sequencing (WGS) and whole-exome sequencing (WES) are emerging as viable testing options for a range of diagnostic applications. But before large-panel genetic testing becomes a routine part of clinical care, diagnostic labs must first address the complexity and consistency of NGS test interpretation. To overcome these challenges, diagnostic labs need to incorporate automation tools and software solutions that can accelerate NGS analysis and provide the evidence and knowledge required to confidently interpret variants and deliver patient-specific reports.

The interpretation and reporting of NGS data should match best-practice standards already in existence for other large-scale clinical tests. Test reports should aim to be concise, but the detailed information on the overall analysis should be available on inquiry. However, when it comes to interpreting complex genetic information related to hereditary cancers, the different mutation types and their impact on the disease risks, the potential implications for family members, and its predictive power for currently healthy persons pose challenges for clinical reporting.

There are various factors that may influence the uniformity in interpreting and reporting variants detected by NGS. This can be a source of significant frustration and confusion for the treating clinicians. Therefore, the clinical testing laboratories that are preparing the diagnostic reports bear a great responsibility to deliver reports that are clear, evidence-backed, and unambiguous in order to aid the medical doctors in their clinical decision making. As NGS testing becomes routine, it is critical for diagnostic labs to standardize the interpretation and reporting of NGS data.

### Variant annotation

In recent years, bioinformatics tools have been developed to help automate certain steps of the NGS data processing workflow by extracting relevant information from different publicly available databases. This information is used to understand the significance of each genetic variant detected, such as its known pathogenicity, effect on the protein, or association with certain therapies or clinical trials.

Known as variant curation, this step in the NGS data processing workflow is almost impossible to perform manually in a timely manner. Today, online sequence repositories and journals are swelling with DNA data. On average, more than 1 million genomics peer-reviewed papers are published each year, and that number is expected to double every 2 years (1). Now, when interpreting a NGS test, a diagnostic lab must consider data and evidence from a range of diverse sources, which is both time-consuming and labor-intensive.

QIAGEN Clinical Insights (QCI®) Interpret is clinical decision support software that empowers diagnostic labs with the world's largest, manually curated knowledge base of biological and clinical findings. It manually curates, and models scientific literature and professional guidelines capturing biological, phenotypic, therapeutic, and outcomes data. For every variant in any gene and in any cancer type, the software provides computed American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) classifications, computed molecular functions, predicted effects on the protein, population frequencies, and complete bibliography encompassing clinical cases, clinical trials, functional and population studies, treatment and prognostic studies, as well as reviews and other external database reports. Yet, even with such a powerful tool, manual review, interpretation, and validation is still necessary to ensure annotation accuracy and interpretation consistency.

Accurate and detailed annotation is a challenging task, even for experienced molecular geneticists. While automation helps alleviate the data burden of variant annotation, manual review is required to determine which of the detected variants should be included in the final report.

In addition, the final report should be manually reviewed by a lab director to ensure there is a clear distinction between clinically significant variants, clinically irrelevant variants, and variants of unknown significance (VUS).

### Report summary

According to best practices, the final patient report of a clinical NGS test should include a front-page summary that clearly presents the overall test results. This summary should contain only the most relevant information, such as the patient and/or sample ID, sex, ethnicity, test indication, major findings or absence of findings, and therapeutic implications if appropriate. Figure 1 presents the first page of a sample QCI Interpret report for hereditary cancer, which includes all of the aforementioned information.

## Report clarity

The goal of a clinical NGS report is to deliver and communicate the test results in a format that is clear and understandable to the clinicians who are using this information to guide patient decisions (2-4). However, there must be a balance between efficiency and comprehensiveness; a busy clinician should be able to quickly look at the first page of a report and understand the overall test results. But then, more detailed variant annotations should be offered on subsequent pages, allowing the clinician to review all of the relevant evidence and suggestions for next steps.

Figure 2 (page 4) presents a sample of subsequent information provided by a QCI Interpret report for hereditary cancer. By leveraging the QIAGEN Knowledge Base, the industry's largest, manually curated collection of biological and clinical findings, QCI Interpret is able to provide detailed, evidence-backed interpretations for each variant detected.

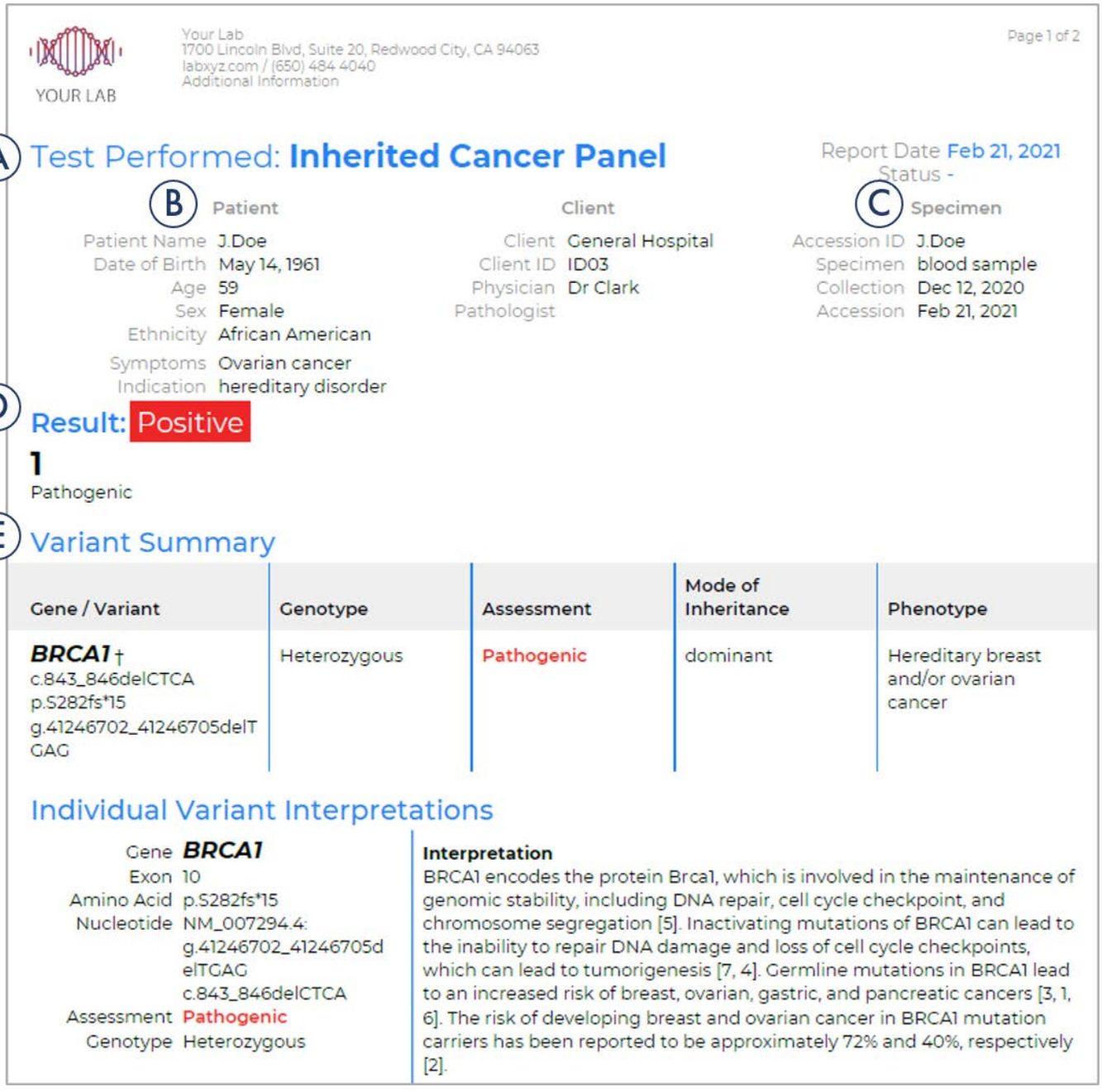



**Figure 1.** Front-page of a sample QCI Interpret report for hereditary cancer. QCI Interpret enables diagnostic labs to generate patient-specific reports with the latest, peer-reviewed evidence. A: In large, stand-out text, QCI Interpret identifies the test performed and/or panel analyzed. B: The patient information is presented at the top of the page, including name, date of birth, age, sex, ethnicity, symptoms, and indication for which he or she is being tested. C: The date of specimen collection is recorded. D: Overall test results are clearly presented. E: For the most relevant variants detected, QCI Interpret provides a front-page, high-level variant summary, including information on the gene, genotype, pathogenicity, mode of inheritance, and phenotype.

As shown in Figure 2, QCI Interpret cites each line of evidence, which can be further reviewed in the report's bibliography. The detailed variant interpretations found on the subsequent pages of QCI Interpret reports are becoming increasingly important with the proliferation of NGS testing. Novel variants are uncovered on a weekly basis; hundreds of new papers are published each month; and thousands of targeted therapies and clinical trials are now available throughout the world. Clinicians and treating oncologists cannot spend hours researching the results of each patient test. They need all of this information summarized for them in easy-to-understand terminology with the option to conduct more thorough investigations via the citations given.

| | |
|---|---|
| Gene ***EGFR***<br>Exon 13<br>Amino Acid p.R521K<br>Nucleotide NM_005228.5:<br>g.55229255G>A<br>c.1562G>A<br>Assessment **Uncertain Significance**<br>Genotype Heterozygous | **Interpretation**<br>EGFR encodes the Epidermal growth factor receptor (Egfr), a receptor tyrosine kinase that functions as an oncogene. Egfr activates signaling pathways, such as the Ras/Raf/MAPK and PI3K pathways, and stimulates the cell to grow and divide [37]. Amplification, mutation, and overexpression of EGFR may cause excessive proliferation and tumor formation [7, 3]. EGFR activating mutations or amplification may predict sensitivity to Egfr-targeted therapies, including inhibitors of multiple ErbB family members, and several have received FDA approval in some tumor types [27, 33, 41]. Small in-frame deletions in exon 19 and the exon 21 missense mutation L858R account for approximately 40% of all EGFR alterations in non-small cell lung cancer [25, 8]. In-frame insertions within exon 20 of EGFR are found in over 4% of non-small cell lung cancer and associated with resistance to tyrosine kinase inhibitors [1, 26, 30, 43, 44]. |
| Gene ***STK11***<br>Exon 1<br>Amino Acid p.Y36Y<br>Nucleotide NM_000455.5:<br>g.1207020C>T<br>c.108C>T<br>Assessment **Uncertain Significance**<br>Genotype Heterozygous | **Interpretation**<br>STK11 encodes Serine/threonine-protein kinase 11, also known as Lkb1, which plays a prominent role in the regulation of cellular metabolism [14]. Lkb1 activates AMPK and negatively regulates the mTOR pathway in response to cellular energy levels [38]. Lkb1 functions as a tumor suppressor, and deletion or inactivation of the STK11 gene results in reduced cell cycle control [6, 38]. Inactivating germline mutations in STK11 are associated with Peutz-Jeghers syndrome, an autosomal dominant disorder associated with increased susceptibility to various cancers [40, 45, 32, 17]. |
| Gene ***TP53***<br>Exon 4<br>Amino Acid p.P72R<br>Nucleotide NM_000546.6:<br>g.7579472G>C<br>c.215C>G<br>Assessment **Uncertain Significance**<br>Genotype Homozygous | **Interpretation**<br>The TP53 gene encodes the tumor suppressor p53, a protein that is involved in the DNA damage cell cycle checkpoint and causes cell cycle arrest when it senses DNA damage. p53 can also activate DNA repair genes, or induce apoptosis in the presence of DNA damage. It has been called the cellular gatekeeper [22]. Loss of p53 is common in aggressive advanced cancers [5]. Carriers of a germline mutation in TP53 have Li-Fraumeni Syndrome, an inherited cancer syndrome resulting in multiple tumors in early adulthood, including breast cancer, brain tumors, and leukemias [35, 24, 39]. Expression of p53 in normal cells is low; however, TP53 alterations, including those that result in loss of p53 tumor suppressor function, may lead to stabilization and increased expression of p53, particularly in the nucleus, and several studies have shown that it may have oncogenic gain-of-function effects [20, 19, 42, 29, 16]. |

**Figure 2.** Detailed variant interpretations in sample QCI Interpret Report for hereditary cancer. For each relevant variant, QCI Interpret provides detailed interpretations using evidence from the QIAGEN Knowledge Base. In a simple-to-read format, QCI Interpret presents a two-column table with the high-level gene and variant information on the left and biological, clinical, and therapeutic implications on the right. All the information is cited by a peer-reviewed source that can be easily reviewed through the report's bibliography.

## Variants of unknown significance (VUS)

As NGS panels increase in size, so too does the number of variants of unknown significance (VUS), which are variants that cannot be classified as pathogenic/likely pathogenic or benign/likely benign because there is not enough evidence to make that distinction. While VUS tend to offer little guidance into patient management and are sometimes left out of final reports, they can be important in certain cases. For example, if no clear causative pathogenic variants are identified, but a VUS is detected in a high-risk gene in a patient with a known family history of hereditary cancer linked to that gene, including this VUS in the final report is critical.

Figure 3 illustrates how QCI Interpret handles VUS. Here, a VUS is detected in the APC gene, and QCI Interpret lists the different lines of evidence from the ACMG criteria that were triggered for the variant using content from the QIAGEN Knowledge Base.

Users can click on each piece of evidence, read and review the articles, and either agree or disagree with QCI Interpret's automatic assessment. In addition, as our understanding of genomics continues to advance, what is classified as a VUS today may be re-classified as pathogenic or benign in the future. Therefore, including VUS in final reports allows diagnostic labs to re-issue a new classification if a VUS changes, which can impact patient care. Yet, the label of VUS in final reports can also cause emotional distress to patients who may misunderstand the classification.

The handling, managing, and reporting of VUS is a nuanced topic, and the development of an appropriate VUS policy is needed. For smaller panels of high-risk genes in hereditary cancers, VUS may have clinical utility and laboratories may choose to report them. Figure 5 shows a sample QCI Interpret report for hereditary cancer that includes VUS. The handling, managing, and reporting of VUS is a nuanced topic, and the development of an appropriate VUS policy is needed.

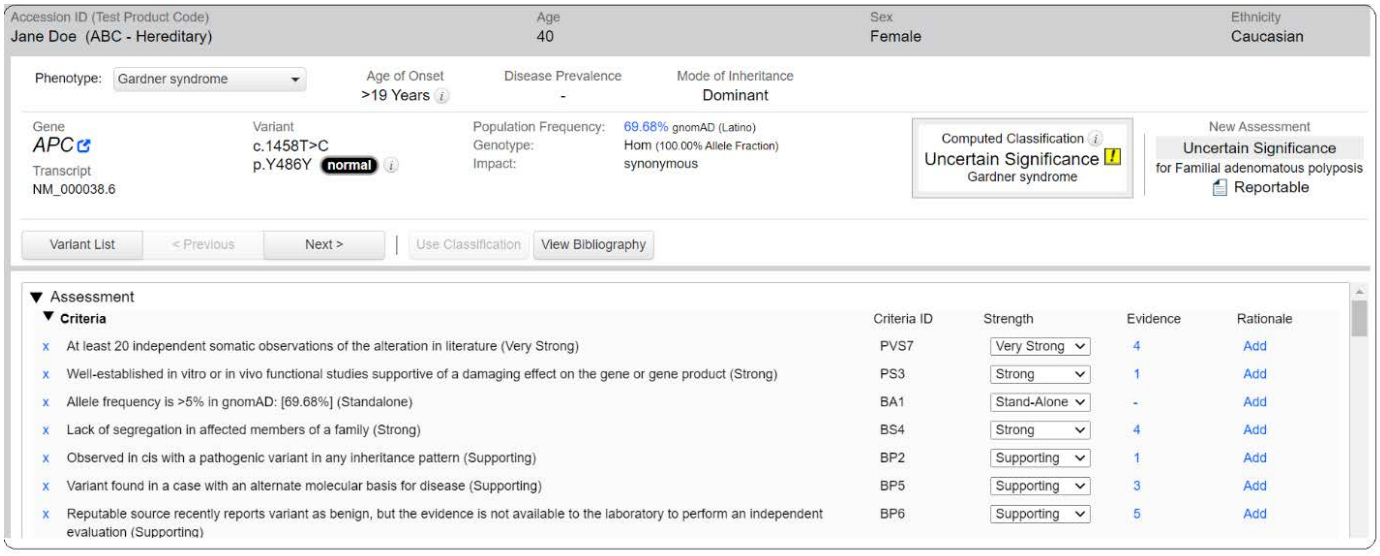
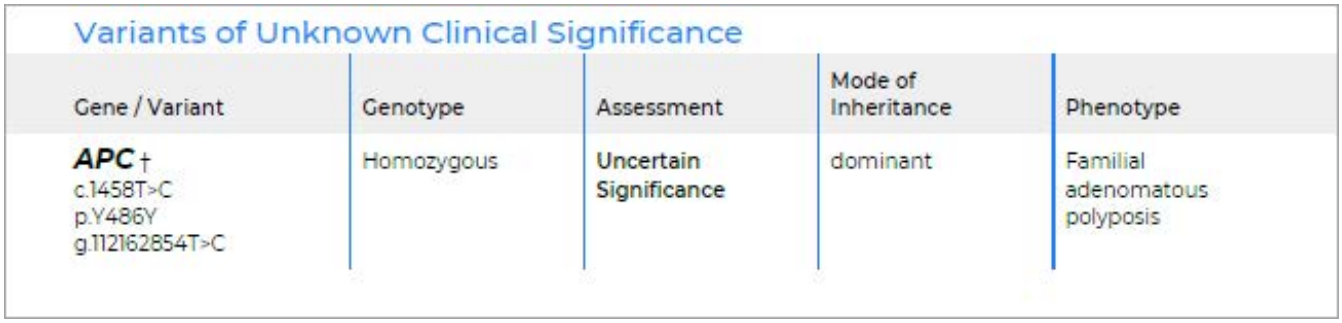



**Figure 3.** Example of an *APC* VUS in QCI Interpret with the different lines of evidence from the ACMG criteria that were triggered for the variant based on evidence from the QIAGEN Knowledge Base.



Variants of Unknown Clinical Significance

| Gene / Variant | Genotype | Assessment | Mode of Inheritance | Phenotype |
|---|---|---|---|---|
| ***APC*** †<br>c.1458T>C<br>p.Y486Y<br>g.112162854T>C | Homozygous | **Uncertain Significance** | dominant | Familial adenomatous polyposis |

**Figure 4.** Example of an *APC* VUS in a QCI Interpret report.

While there is no definitive information about pathogenicity, QCI Interpret identifies the gene and variant, the genotype, mode of inheritance, and phenotype. Users can also choose to include biological information about VUS taken from peer-reviewed literature, which may help the clinicians and treating oncologists make more informed treatment decisions.

## Report customization

The level of report customization that clinical decision support tools provide is also important. Diagnostic labs need to be able to customize reports according to lab branding, test type, client needs, as well as industry-wide guidelines and best practices. QCI Interpret offers diagnostic labs total customization of final reports. Users have the option of modifying an "out-of-the-box" report template or can work with QIAGEN's bioinformatics team to create a bespoke report that meets all of their criteria.

## Conclusion

The rapidly changing nature of science poses formidable challenges in the field of clinical genomics especially when it comes to data analysis, variant annotation, and clinical reporting. The clinical genomics reports should detail key findings from the interpretation of NGS data and should represent the link between specialized clinical genomics laboratories and the clinicians who implement patient therapy and management. As such, the reports should help in improving effective and accurate flow of information from the NGS laboratory to the report end-user.

**References**

1. Landhuis, E. Scientific Literature: Information Overload. Nature. 2016 July 21;7612:457-58.
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Learn more about QCI for hereditary cancer at

**www.digitalinsights.qiagen.com/hereditary-cancer**

QCI Interpret is evidence-based decision support software intended as an aid in the interpretation of variants observed in genomic next-generation sequencing data. The software evaluates genomic variants in the context of published biomedical literature, professional association guidelines, publicly available databases, annotations, drug labels, and clinical trials. Based on this evaluation, the software proposes a classification and bibliographic references to aid in the interpretation of observed variants. The software is NOT intended as a primary diagnostic tool by physicians or to be used as a substitute for professional healthcare advice. Each laboratory is responsible for ensuring compliance with applicable international, national, and local clinical laboratory regulations and other specific accreditations requirements.