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Paraganglioma/Pheochromocytoma (PGL/PCC) Predisposition Next Generation Sequencing (NGS) Panel: Information for Ordering Providers

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What is Next Generation Sequencing (NGS)?
NGS is a high-throughput DNA sequencing technology that allows sequencing of multiple regions of the human genome at one time. This enables the simultaneous analysis of many genes known to be associated with a particular phenotype (i.e. gene panels). For some genes, additional analysis for copy number variants may be performed in conjunction with NGS.

Since not all genes associated with a given phenotype/presentation are known or included in the panel, a pathogenic variant will not be identified for every patient. **The absence of a pathogenic variant does not exclude a clinical diagnosis.**

Testing may identify a genetic variant for which there is currently insufficient evidence to conclude that it is either disease-causing or benign (called a variant of uncertain significance). Such variants cannot be used to alter the clinically established risk of disease.

Why order a NGS panel for my patient?
Phenotypes are often genetically heterogeneous, meaning that the condition is caused by a pathogenic variant(s) in any one of a number of genes. Instead of sequentially testing each of those genes, patients with a particular phenotype should be offered a targeted NGS panel. In some circumstances, it may be more appropriate to test only one gene instead of a panel of genes. In such situations, please contact the laboratory to discuss your patient.

Individuals who carry a pathogenic variant in a hereditary cancer gene have an increased risk of certain cancers compared to the general population. Cancer risks depend on the gene(s) in which the variant(s) is identified.
These individuals are eligible for increased cancer screening and/or risk reducing surgeries and therapeutic interventions. In addition, results may influence treatment plans for individuals with cancer.

Background
Paragangliomas and pheochromocytomas may occur in patients with several different inherited cancer susceptibility syndromes, including PGL/PCC syndrome, multiple endocrine neoplasia type 2, von Hippel–Lindau disease (*VHL*), and neurofibromatosis type 1 (*NF1*).

Hereditary cancers syndromes involving PGL/PCC are typically inherited in an autosomal dominant manner. The clinical findings associated with PGL/PCC can vary greatly within a family, despite the fact that affected individuals within a given family carry the same pathogenic variant.

Associated Disorders:
Some of the genes on this panel are associated with other rare disorders including:

> **Fumarate Hydratase Deficiency** is an autosomal recessive disorder caused by pathogenic variants in *FH*. Individuals with fumarate hydratase deficiency (two pathogenic *FH* variants) have brain anomalies, epilepsy, dysmorphic features, and global developmental delay. Most affected individuals die in early childhood.
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> **Mitochondrial complex deficiencies** are rare autosomal recessive conditions with highly variable phenotypes. Pathogenic variants have been reported in *SDHA, SDHB,* and *SDHD.*

If a pathogenic variant is identified in one of these genes, the patient and/or their family members may be at increased risk for specific cancers or other conditions. Genetic counselling is recommended for these families.

Indications for testing

Any patient with a pheochromocytoma or paraganglioma is eligible for testing. Patients with a personal and/or family history consistent with a pheochromocytoma/paraganglioma syndrome should be referred to a clinical geneticist for assessment.

Ordering privileges

This panel may be ordered by Clinical Geneticists or Endocrinologists. Presymptomatic testing for a known pathogenic variant in the family is restricted to Clinical Geneticists.

Paraganglioma/Pheochromocytoma NGS panel

The genes included in this panel may be associated with a spectrum of cancer types or a well-described hereditary cancer condition. The associated cancer risks depend on the gene in which the variant is identified.

Gene(s)	Associated cancers and/or clinical features	Associated Hereditary Syndrome
SDHA, SDHB, SDHC, SDHD SDHAF2	Head and neck paragangliomas, extra-adrenal paragangliomas and/or pheochromocytomas, GISTs and renal clear cell carcinoma (rare)	Hereditary paraganglioma/ pheochromocytoma syndromes
MAX, TMEM127	Susceptibility to paraganglioma/pheochromocytoma	
RET	Medullary thyroid carcinoma, pheochromocytoma, parathyroid adenoma or hyperplasia, mucosal neuromas of the lips and tongue, ganglioneuromatosis of the gastrointestinal tract	Multiple endocrine neoplasia, type 2 (includes MEN2A and MEN2B)
	Medullary thyroid carcinoma	Familial medullary thyroid cancer
VHL	Renal tumors, adrenal pheochromocytoma, neuroendocrine tumors and hemangioblastomas of the brain, spinal cord, and retina. The lifetime risk of renal cell carcinoma is approximately 25%-70%.	Von Hippel Lindau syndrome
FH	Renal cancer (20% lifetime risk), cutaneous and uterine leiomyomas/fibroids (~98% lifetime risk).	Hereditary leiomyomatosis and renal cell carcinoma
NF1	Multiple café au lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and Lisch nodules. Learning disabilities may be present. Pheochromocytomas are rare in NF1 overall; however, they occur in 20%-50% of individuals with NF1 and hypertension.	Neurofibromatosis, type 1

How do I order an NGS panel?
Discuss the advantages and limitations of testing with your patient (see above). If your patient consents to the testing:

1. Complete the Molecular Genetics Laboratory Cancer and Endocrine Next Generation Sequencing Requisition (available at Genetics and Genomics) providing all relevant clinical and family history information. **Incomplete requisitions will not be accepted and will result in test delays.**
2. Provide the fully completed requisition to your patient and **direct them to their local collection lab for a blood draw.**
3. For patients without a valid Alberta PHN, please contact the laboratory genetic counsellor to discuss test availability, billing and sample requirements.

My patient has a family history of a known pathogenic variant. Is an NGS panel the appropriate test for my patient?
No. Once a pathogenic variant has been identified in the family it is best to begin testing by looking for the variant that has already been identified in the family.

Methods
Genomic DNA is sequenced on an NGS instrument. Analysis includes the coding region of the gene, including 15bp of intronic/coding boundaries. If a clinically relevant variant does not meet the validation requirements it is confirmed by Sanger sequencing. Additional deletion/duplication testing may be performed by a variety of methods, including, but not limited to: comparative genomic hybridization, NGS-based dosage analysis, multiplex ligation-dependent probe amplification, and quantitative PCR. Confirmation by a secondary method is carried out when necessary. The methods used to generate results are identified on each patient report.

Test Performance
NGS detects nucleotide substitutions, small insertions and deletions and copy number variants. This test is expected to detect >95% of variants in the coding regions of the tested genes.

When can I expect results?
Results may take up to 4 months.

Can testing be expedited to facilitate medical management of a patient?
Expedited testing (~1 month from the time the sample is received) is available if required for immediate surgical or therapeutic management. Please provide details on the requisition form regarding the reason for expedited testing as well as a target date for results.

How are results reported?
Results are sent to the ordering provider. For some tests, results will not be sent to 'copy to' physicians by the laboratory, but may be obtained by contacting the ordering health care provider.

What Types of Results Can I Expect?

Type of NGS result	Interpretation
Pathogenic Variant	A variant has been identified that is disease causing.
Likely Pathogenic Variant	A variant has been identified and there is significant but not conclusive evidence that the variant is disease causing.
Variant of Uncertain Significance	A variant has been identified and there is not sufficient evidence to classify the variant as pathogenic/likely pathogenic or benign
No Pathogenic Variant (Uninformative)	No variants of clinical or uncertain significance were detected. This is an uninformative result and no explanation has been identified for the patient's phenotype. There may be other genes or variants not assessed by the current NGS panel associated with the patient's phenotype. A genetic condition or genetic component to the phenotype has not been excluded.

NOTE: Benign, or likely benign variants (variants known not to be disease causing) are not reported.

My patient has a variant. What are the next steps?

Your patient should be managed based on their diagnosis and clinical presentation. If your patient has a pathogenic variant or a likely pathogenic variant, genetic counselling may be indicated to discuss the implications for other family members. If your patient has a variant of uncertain significance, a referral to Clinical Genetics may aid in the assessment of the variant.

My patient's results are uninformative. What are the next steps?

A referral to Clinical Genetics may still be appropriate for your patient if they have a significant family history suggestive of hereditary cancer syndrome and/or desire additional counselling regarding their results.

Contact Information

Health care providers can contact the Calgary Laboratory Genetic Counsellors at 403-955-3097.

Requisition forms, contact information and other resources can be found at:
Genetics and Genomics

References

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National Library of Medicine (US). Genetics Home Reference [Internet]. Bethesda (MD): The Library; 1993-2018 [cited 2017 Dec]. Available from: https://ghr.nlm.nih.gov/