OncoGene Dx: Paraganglioma/Pheochromocytoma (PGL/PCC) Panel Sequence Analysis and/or Exon-Level Deletion/Duplication Testing of 12 Genes

Panel Gene List: FH, MAX, MEN1, NF1, RET*, SDHA*, SDHAF2, SDHB, SDHC, SDHD, TMEM127, VHL

*Only next generation sequencing of these genes are included in this panel. Deletion/duplication analysis is not performed.

Clinical Features and Genetics:
Paragangliomas (PGL) are very rare neuroendocrine tumors that arise from neural crest tissue of the sympathetic and parasympathetic paraganglia. Pheochromocytomas (PCC) refer to paragangliomas that are confined to the adrenal medulla. These tumors are exceptionally rare in the general population and occur in approximately 1/300,000 individuals per year.6 These tumors are often benign, but may undergo malignant transformation. It has been estimated that 20-30% of those individuals diagnosed with either PGL or PCC will harbor a pathogenic variant in one of the genes included in this panel.2 The features of a personal and/or family history that are suggestive of a hereditary predisposition to PGL/PCC include: young ages at diagnosis, multiple primary tumors in a single individual, diagnosis of a tumor or cancer type that is not common in general population (such as a pheochromocytoma), and several relatives affected with cancer or tumors spanning multiple generations. Due to the rare nature of PGL and PCC, the diagnosis of one of these tumors in an individual at any age, regardless of family history, should be considered for genetic evaluation. Hereditary paraganglioma/pheochromocytoma has been described in association with several syndromes. These include hereditary paraganglioma/pheochromocytoma syndrome (SDHA, SDHAF2, SDHB, SDHC, SDHD), Neurofibromatosis type 1 (NF1) and multiple endocrine neoplasia type 2 (RET). Pathogenic variants in these seven aforementioned genes account for a significant portion of hereditary PGL/PCC predisposition and have been well described in the literature. In addition, several other well described syndromes include a risk of PGL/PCC cancer as a minor feature. These include Von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 1 (MEN1) and hereditary leiomyomatosis and renal cell carcinoma (FH). Management guidelines are available for a number of these genes and, where applicable, are listed in the attached table. Several newer genes including MAX and TMEM127, have been identified in families with PGL/PCC and may increase the risk for other cancers or tumors as well. The evidence available to date may be derived from a small number of patients with wide confidence intervals or is based upon an ethnic cohort with one specific variant. Accurate risk assessment may be complicated by the low penetrance of pathogenic variants in these genes and/or ascertainment bias.

The level of cancer risk, relative to general population risk, that is associated with pathogenic variants in each of the...
genes is outlined in the attached table. Many of these dominantly-inherited cancer predisposition syndromes are also associated with extremely rare, autosomal recessive disorders if an individual inherits two pathogenic variants in the same gene, one from each parent. For example, if both mother and father are carriers of a pathogenic FH variant, each of their children would have a 25% chance to inherit both pathogenic variants, a 50% chance to inherit one of the pathogenic variants, and a 25% chance to inherit neither pathogenic variant. If a child inherits both pathogenic FH variants, they would have a rare condition called fumarase deficiency syndrome, characterized by excessive urinary excretion of fumarate, neonatal hypotonia, growth and developmental delay, seizures, structural brain malformations, severe neurologic impairment, dysmorphic facial features, and neonatal polycythemia with death typically occurring within the first decade.2,5,9 All genes that have an associated recessive condition are noted in the attached table.

Test Methods:
Genomic DNA from the submitted specimen was enriched for the complete coding region and splice site junctions of the genes on the panel using a proprietary targeted capture system developed by GeneDx. The products were sequenced on either an Illumina MiSeq or HiSeq instrument with 2x150 or 2x100 paired-end reads, respectively. The sequence was aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Capillary sequencing was used to confirm all variants with clinical or uncertain significance and to analyze regions with inadequate coverage by Next Generation sequencing. If present, apparently homozygous variants were confirmed using alternate primer pairs to significantly reduce the possibility of allele drop-out. Concurrent deletion/duplication testing was performed for all of the genes on the panel, except RET and SDHA, using either exon-level array CGH or MLPA. Confirmation of copy number changes was performed by MLPA, qPCR, or repeat aCGH analysis. Data analysis was performed using gene-specific filtering. The array was designed to detect most single-exon deletions and duplications. For RET and SDHA, only sequencing was performed. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Benign and likely benign variants, if present, are not reported but are available upon request. The genes evaluated by this test are listed on the first page of the report.

Test Sensitivity:
DNA sequencing will detect nucleotide substitutions and small insertions and deletions, while array CGH will detect exon-level deletions and duplications. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing or array CGH. The likelihood of a false positive result is expected to be <1%. Neither sequencing nor exon-level aCGH can reliably detect mosaicism, and cannot detect chromosomal aberrations. Deletions involving more than 20bp and insertions involving more than 10bp are
not reliably detected by the sequencing methodology, and deletions or duplications of less than 250bp are not reliably detected by array CGH. Regions of certain genes have inherent sequence properties that yield suboptimal data, potentially impairing accuracy of the results. For instance, sequence analysis of NF1, among others, is complicated by the presence of pseudogenes or homologous sequences that involve multiple exons of this gene. In the absence of mRNA/cDNA studies, we cannot completely exclude the possibility of undetectable clinically significant variants in certain regions of these genes. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active leukemia or lymphoma or with known chronic myeloid or lymphoid neoplasms (such as low grade MDS, CML, ET, P. vera, PMF, CLL), there is a possibility that testing of specimens containing leukocytes may detect an acquired somatic variant, resulting in a false positive result. In this situation, please contact one of our genetic counselors to discuss the utility of submitting an alternate specimen. Additionally, rare false negatives may occur when testing for a specific variant identified at a laboratory other than GeneDx if a positive control is not provided. Based on the specific array design and technology used, the reported coordinates of duplications and deletions at the exon or gene level can slightly differ among family members tested but, in general, relatives are expected to have the same copy number variant. The ability to detect genetic variants and naming conventions can differ among laboratories.

References: