RET Gene Analysis in Multiple Endocrine Neoplasia Type 2A, Multiple Endocrine Neoplasia Type 2B, Familial Medullary Thyroid Carcinoma, and Hirschsprung Disease

Disorder Also Known As: Sipple syndrome; MEN2A; MEN2B; FMTC; Wagenmann-Froboese syndrome; Pheochromocytoma and amyloid-producing medullary thyroid carcinoma; PTC syndrome; Neuromata, mucosal, with endocrine tumors; Mucosal neuroma syndrome; HSCR; Congenital aganglionic megacolon

Clinical Features:
Multiple endocrine neoplasia type 2 (MEN2) is due to gain-of-function variants in the *RET* gene. This syndrome is characterized by medullary thyroid carcinoma (MTC), pheochromocytomas, and hyperparathyroidism. Less common features include Hirschsprung disease (HSCR) and cutaneous lichen amyloidosis (CLA).

MEN2 can be classified into three clinical subtypes: MEN Type 2A (MEN2A), MEN Type 2B (MEN2B), and Familial Medullary Thyroid Carcinoma (FMTC). MTC is the most common manifestation in all subtypes of MEN2, with a >90% risk for affected individuals to develop this cancer. In MEN2A, MTC can develop in childhood through mid-adulthood. This subtype is also associated with an increased risk for pheochromocytoma (50%) and parathyroid adenoma/hyperplasia (20-30%).[1] Individuals with MEN2B have a 100% risk of MTC, which develops in childhood, and they are also at increased risk for pheochromocytoma (50%).[1] Patients with MEN2B may exhibit other characteristic features such as a marfanoid habitus, mucosal neuromas of the lips and tongue, ganglioneuromatosis of the intestine, enlarged corneal nerves, and gastrointestinal problems. FMTC describes multiple family members with MTC in the absence of pheochromocytomas or hyperparathyroidism.

Loss-of-function *RET* variants are associated with another distinct disorder, familial and sporadic Hirschsprung disease (HSCR).[2] HSCR is associated with congenital absence of parasympathetic ganglia in the bowel. With an incidence of 1 in 5000 births, it is the main genetic cause of functional intestinal obstruction in infants and children. While HSCR presents as an isolated finding in about 70% of patients, approximately 30% of cases are considered syndromic.

Inheritance Pattern:
MEN2A, MEN2B, FMTC, and HSCR are inherited in an autosomal dominant manner. Approximately 5-10% of MEN2A and 50% of MEN2B cases are *de novo* (new).[3]

Test Methods:

Using genomic DNA from the submitted specimen, the coding regions and splice junctions of *RET* are PCR amplified and capillary sequencing is performed. Bi directional sequence is assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequencing or another appropriate method is used to confirm all variants with clinical or uncertain significance. If present, apparently homozygous variants are confirmed using alternate primer pairs to significantly reduce the possibility of allele drop-out. 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.

Test Sensitivity:

The clinical sensitivity of sequencing analysis of *RET* depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of multiple endocrine neoplasia type 2 as outlined above. Sequence analysis is expected to identify pathogenic variants in >95% of individuals with MEN2A, MEN2B, and FMTC.[3] Sequencing and deletion/duplication analysis is expected to identify a pathogenic variant in 50% of familial and 15-20% of sporadic HSCR cases.[2,3]

DNA sequencing will detect nucleotide substitutions and small insertions and deletions. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing.

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