

Supplemental Data

DNA analysis

Genomic DNA was extracted from buffy coat leukocytes using the standard phenol-chloroform method. PCR primers were designed to amplify the entire coding sequence plus flanking splice sites using the Primer-3-web-based tool.

1. von Hippel–Lindau (VHL) disease: *VHL* gene

A 25- μ L PCR mixture contained 100 ng of genomic DNA, 0.4 μ M of each primer, 0.1 mM of each dNTP, 0.5 unit of Taq DNA polymerase (Invitrogen[®], Carlsbad, CA, United States), 2.5 mM MgCl₂ in 10X PCR buffer. PCR reaction was started with an initial denaturation at 94°C for 3 min, followed by 35 cycles of amplification in a thermocycler (PCR Sprint, Thermofisher, Waltham, MA, United States) with denaturation at 94°C for 1 min, annealing at 63°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min.

Exon	Forward primer	Reverse primer
1	TACAACGGCCTACGGTGCT	GGCTTCAGACCGTGCTATCG
2	ACCGGTGTGGCTCTTTAACAACC	TCAAGTGGTCTATCCTGTACTTAC
3	GAGATCCATCAGTAGTACAGG	AAAGCTGAGATGAAACAGTGTAAG

2. Multiple endocrine neoplasia type 2 (MEN2): *RET* gene

A 25- μ L PCR mixture contained 100 ng of genomic DNA, 0.4 μ M of each primer, 0.1 mM of each dNTP, 0.5 unit of Taq DNA polymerase (Invitrogen[®], Carlsbad, CA, United States), 2.5 mM MgCl₂ in 10X PCR buffer. PCR reaction was started with an initial denaturation at 94°C for 3 min, followed by 35 cycles of amplification in a thermocycler (PCR Sprint, Thermofisher, Waltham, MA, United States) with denaturation at 94°C for 1 min, annealing at various temperatures (below) for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min.

Exon	Forward primer	Reverse primer	Annealing temperature (°C)
10	AGGCTGAGTGGGCTACGTCT	TGCTGTTGAGACCTCTGTGG	63
11	CAGAGCATAACGCAGCCTGTA	CTATGGAAATGGGGGCAGA	63
12	GTCATGTAGCAGCTTTCAGG	AAAGTCCTCGCTCTGCTTCT	63
13	TCAAGCAGCATCGTCTTTGC	ATGGAAAGTGACCACTCAGC	63
14	CAAGAGAAAGCTGAGGCTTC	AAATAGCACGAGTCGTCAGG	63
15	CCTGACGACTCGTGCTATTT	CTTCGGTATCTTTCCTAGGC	63
16	TGTCTACAGCACTCCTCTG	CCCCACTACATGTATAAGGG	63

3. Familial Paraganglioma: Succinate dehydrogenase complex, subunit B (*SDHB*) gene

A 25- μ L PCR mixture contained 100 ng of genomic DNA, 0.4 μ M of each primer, 0.1 mM of each dNTP, 0.5 unit of Taq DNA polymerase (Invitrogen[®], Carlsbad, CA, United States), 2.5 mM MgCl₂ in 10X PCR buffer. PCR reaction was started with an initial denaturation at 94°C for 3 min, followed by 35 cycles of amplification in a thermocycler (PCR Sprint, Thermofisher, Waltham, MA, United States) with denaturation at 94°C for 25 seconds, annealing at various temperatures (below) for 25 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 5 min.

Exon	Forward primer	Reverse primer	Annealing temperature (°C)
1	CCTGTAATCCCAGCTACTCG	TTGCCCTATGCTTCCTCAGT	62
2	GTGTGAGTTTATATCCAGCG	CTTCCAAGGATGTGAAAAGC	64
3	ACCTGAGAAGACCAAATGGA	GTCTCTATCAGCTTTGGCCA	64
4	TCCAGAAGAAAGTATTTGGG	ATAGCGTAACACACATAGCAC	64
5	AGTCAGTGTCCAAGAAATGG	TGGCTTGCATCAGCTTATGT	64

6	AGGCACTTTGTTTCATGCACT	CTATTGTCCTCTTGGACTTC	64
7	CTCCCAGAGCTTTGAGTTGA	CTCTCTGCCAATCACCTCTT	64
8	ACCAGCTGAGGAAGGAGTTT	CCAAGATCTTTAAAGGAACTC	62

4. Familial Paraganglioma: Succinate dehydrogenase complex, subunit D (SDHD) gene

A 25- μ L PCR mixture contained 100 ng of genomic DNA, 0.4 μ M of each primer, 0.1 mM of each dNTP, 0.5 unit of Taq DNA polymerase (Invitrogen®, Carlsbad, CA, United States), 2.5 mM MgCl₂ in 10X PCR buffer. PCR reaction was started with an initial denaturation at 94°C for 3 min, followed by 35 cycles of amplification in a thermocycler (PCR Sprint, Thermofisher, Waltham, MA, United States) with denaturation at 94°C for 25 seconds, annealing at various temperatures (below) for 25 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 5 min.

Exon	Forward primer	Reverse primer	Annealing temperature (°C)
1	GTATCTTTTCTACGGGCACG	GACTACAGTGGTCATTGCTGT	62
2	ACTTCACAGTAACCCCAGTG	AAGTTGGACACAAGAGTCCC	64
3	GGGTTACTGTGTGGCATATG	TACATAAGACAAGCTCACAGC	64
4	GAAGCAAACAGTGACAGTGG	TTAAGAGAAGAAGGCTGTCC	64