**Analytical workflow**

After demultiplexing, sequences were aligned to the reference human genome hg19 using the Burrows-Wheeler Aligner. Downstream processing was carried out with the Genome Analysis ToolKit (GATK), SAMtools, and Picard, following documented best practices ([http://www.broadinstitute.org/gatk/guide/topic?name=best-practices](https://courriel.aphp.fr/owa/redir.aspx?C=SRvno_iTPopS3XsMDPWh-sqfXWszsiihD4HvmY7zsSEeWLLPGmDWCA..&URL=http%3a%2f%2fwww.broadinstitute.org%2fgatk%2fguide%2ftopic%3fname%3dbest-practices" \t "_blank)). Variant calls were made with the GATK Unified Genotyper, Freebayes and Samtools. The annotation process was based on the latest release of the Ensembl, dbSNP, 1000 Genome, gnomAD and Exome Variant Server database. Variants were annotated and filtered using the Polyweb software interface designed by the Bioinformatics platform of University Paris Descartes. The nomenclature of each variant was based on [http://sequenceontology.or](https://courriel.aphp.fr/owa/redir.aspx?C=mG-T8Ggk60SH3W43CfxoA_QE8RaDm6jfYGyV3x5hSx4eWLLPGmDWCA..&URL=http%3a%2f%2fsequenceontology.or" \t "_blank), and HGVS nomencalure ([http://varnomen.hgvs.org/](https://courriel.aphp.fr/owa/redir.aspx?C=BzX0_KyHUx_oAy1NRwVjYM3IwgdnFeIbVWXCyV6p764eWLLPGmDWCA..&URL=http%3a%2f%2fvarnomen.hgvs.org%2f" \t "_blank)).

For each MiSeq run, the quality of the run was determined by a Qscore (Q30)>80% and by the analysis of a control DNA (presence of expected variants with expected corresponding allelic ratio).

Polymorphisms present in more than 1% of the population (based on frequencies reported in public database) and repeated artefactual variants (i.e. located in homopolymeric regions) were filtered out. Low coverage was defined as <30X and all regions with less than 30X depth of coverage were analyzed using Sanger sequencing. ‘Splice’ variants corresponded to variants occurring in canonical splice sites whereas ‘intronic’ variants corresponded to variants outside the consensus splice sites regions.

Regarding gene-specific database, available PPGL-related variant database described in the NGSnPPGLs Consensus Statement1 were used, mainly the Universal Mutation Database (UMD) for *VHL* variants, the ARUP Scientific Resource for Research and Education (MEN2) RET database and the Leiden Open (source) Variation Database (LOVD) for other genes.

**Identification of rearrangements using the NGS panel**

In order to evaluate duplication and large deletions events using the NGS panel, the method was adapted from Goossens et al, Hum Mut, 2009.2 A coverage metrics (Ci, a, m) was computed per individual (i), per amplicon (a), per multiplex (m), based on the number of reads overlapping a specific amplicon in the corresponding multiplex PCR divided by the total number of mapped read pairs for this sample in the corresponding multiplex. For each individual on each amplicon, the CNV was given by the ratio of the coverage metrics (CM) of this sample on the mean of CM of a control set of samples for this amplicon. As obtaining the same CNV event in all samples of a run was not expected, the control set for a specific amplicon (A) corresponded to all other unrelated samples sequenced in the same run with the same design. Samples with less than 20 mean coverage and maximum and minimum CM samples were excluded from the amplicon control set. In case of less than 5 samples in the control set or when the coverage of the tested sample was lower than 20, the amplicon was flagged as not computable.

**Establishment of optimal multiplexing conditions**

MiSeq runs were tested with multiplexing of 48 and 36 samples but increasing the number of samples led to lower coverage and more gaps to sequence by Sanger method, which would be time consuming and expensive. Runs with less than 24 samples would increase the cost of sequencing by patient. We therefore chose 24 samples multiplexing for the NGS experiments.

**Precautions for FFPE-extracted DNA**

In FFPE samples, deamination of nucleotides can occur causing C:G>T:A changes and then can produce false positives or background noise during next-generation sequencing. Thus, in case of FFPE-extracted DNA analysis, the good NGS quality (correct mean coverage and Q30>80%) and the absence of increased background noise compared with others samples tested in the run were checked. Moreover, when a variant of interest was identified in an FFPE-extracted DNA, the absence of strand bias, the concordance of SNP between germline and tumor DNA were checked and the presence of the variant was confirmed by Sanger sequencing.

**Supplemental bibliography**

1. Group NGSiPS, Toledo RA, Burnichon N, Cascon A, Benn DE, Bayley JP, Welander J, Tops CM, Firth H, Dwight T, Ercolino T, Mannelli M, Opocher G, Clifton-Bligh R, Gimm O, Maher ER, Robledo M, Gimenez-Roqueplo AP, Dahia PL. Consensus Statement on next-generation-sequencing-based diagnostic testing of hereditary phaeochromocytomas and paragangliomas. *Nat Rev Endocrinol* 2017;13(4):233-47. doi: 10.1038/nrendo.2016.185

2. Goossens D, Moens LN, Nelis E, Lenaerts AS, Glassee W, Kalbe A, Frey B, Kopal G, De Jonghe P, De Rijk P, Del-Favero J. Simultaneous mutation and copy number variation (CNV) detection by multiplex PCR-based GS-FLX sequencing. *Hum Mutat* 2009;30(3):472-6. doi: 10.1002/humu.20873