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Review Article

Clinical and molecular markers guide the genetics of pheochromocytoma and paraganglioma

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ABSTRACT

Over the past two decades, research into the genetic susceptibility behind pheochromocytoma and paraganglioma (PPGL) has surged, ranking them among the most heritable tumors. Massive sequencing combined with careful patient selection has so far identified more than twenty susceptibility genes, leading to an overdetection of variants of unknown significance (VUS) that require precise molecular markers to determine their pathogenic role. Moreover, some PPGL patients remain undiagnosed, possibly due to mutations in regulatory regions of already known genes or mutations in undiscovered genes. Accurate classification of VUS and identification of new genes require well-defined clinical and molecular markers that allow effective genetic diagnosis of most PPGLs.

There are very few, if any, tumors with an inherited susceptibility similar to paragangliomas (PGL) and pheochromocytomas (PCC) (altogether PPGLs). Forty percent of patients with PPGLs carry a germline mutation that increases their risk of cancer and can be passed on from parent to child. Apart from the "classical" genes involved in the development of the disease discovered in the 1990s (i.e., *RET*, *VHL,* and *NF1*), many other genes have been identified in the last 25 years, taking advantage of the next-generation sequencing (NGS) technologies [1]. The heterogeneous prevalence of mutations in these "new genes" and their gene-specific genetic features (e.g., incomplete penetrance of mutations and sex-related transmission) are responsible for a substantial molecular complexity that makes genetic diagnosis of PPGL very challenging.

Genetic testing algorithms in which relevant clinical features of the patient are considered, such as family history of the disease, age of onset, and presence of multiple or bilateral tumors, are no longer used to decide whether to perform genetic testing to PPGL patients. Nowadays, all patients with PPGL are referred for clinical genetic testing due to the relevant implications that detection of a mutation (either germline or somatic) has for surveillance and monitoring of the patients and their families [2,3]. It is worthy to note that the study of tumor instead of germline DNA ensures the identification of both somatic and germinal mutations, as well as allows for detecting somatic mosaicisms in patients suspected of suffering from a hereditary condition because of the presence of multiple tumors.

NGS techniques now enable the simultaneous analysis of all susceptibility genes, avoiding undesirable delays caused by the sequential study of the genes. However, the use of NGS in genetic diagnostics has led to a significant increase in the discovery of variants of unknown significance (VUS) [3], which complicates the interpretation of results and requires the use of well-established strategies to definitively link a variant to disease, either in an already known or in a potential new susceptibility gene. The use of computational algorithms and functional prediction tools to assess the potential impact of genetic variants on protein structure and function do not always permit a suitable interpretation. Moreover, co-segregation analysis of the variant with the disease phenotype across affected individuals in the same family is not always possible, even less in rare diseases. Therefore, there is an urgent need of genotype-specific molecular markers for enhancing genetic diagnostic accuracy, especially for hereditary diseases, to gain enough evidence to classify a genetic variant according to the recommendations of the American College of Genetics and Genomics [4].

Though almost 80% of PPGLs are found to carry a germline or a somatic mutation in one of the known genes, there is still a significant percentage of patients with an undetermined genetic etiology. Some of these cases may be linked to already-known driver genes, but the variant

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may be 'invisible' to current diagnostic methods. Again, the existence of a known molecular marker can guide research on a particular gene by extending its analysis to alternative methods looking for less common pathogenic alterations. On the other hand, new susceptibility genes may be altered in still-undiagnosed patients. Identification of these genes requires an adequate recruitment of patients without known mutations by using discriminatory clinical and/or molecular markers to increase the number of candidates with mutations affecting the same gene.

In this review, we will describe the clinical and molecular features associated with genetic alterations in known PPGL susceptibility genes that can be applied in the interpretation of VUSs identified by NGS (Table 1), and we will discuss current approaches to find new drivers involved in the development of this multigenetic disease.

1. Markers commonly used in clinical practice to classify PPGL-related VUS

1.1. Clinical markers

The presence of clinical features of any of the syndromes associated with PPGL in a patient is an obvious marker that can be used for the classification of VUSs. Thus, the development of clear cell renal cell carcinoma may support the pathological role of variants in *VHL* or *SDHB*, and the presence of medullary thyroid carcinoma or NF1 features is routinely used for the classification of variants in *RET* and *NF1*, respectively. Similarly, the presence of gastrointestinal stromal tumors or uterine leiomyomas concomitant with PPGL supports the pathogenicity of SDH-complex genes or *FH* germline variants, and polycythemia and giant cell tumors of bone appear in some patients carrying somatic postzygotic *EPAS1* or *H3F3A* mutations. Recently, we described that idiopathic intellectual disability in a patient with H&N PGL may be a clinical marker for *DNMT3A* germline mutations [5] and can be therefore used to classify variants in this new gene. Clinical features useful for accurate classification of VUS found in PPGL patients are summarised in [1].

Other clinical features, such as multiplicity or metastatic dissemination, can be used for variant interpretation. Bilateral PCC is a common clinical feature found in patients carrying germline mutations in *VHL*, *RET*, *MAX*, and *TEM127* [6–8]. Furthermore, mutations in *SDHB* and somatic *MAML3* fusions are associated with the presence of distant metastasis [9,10], and therefore finding a VUS in these genes in a metastatic PPGL may support its pathogenicity.

Table 1
PPGL driver genes and markers useful for VUS interpretation.

Cluster	Pathway	Complex	Gene
1[b,e]	TCAc[i,mt*]	SDH[i,b,me]	*SDHA*[c,i]
			SDHAF2
			SDHB[c,m]
			SDHC[c]
			SDHD[c,i]
			FH[c,i,me]
			GOT2[me]
			IDH1/IDH2[me]
			MDH2[me]
			SLC25A11[i,me]
			DLST[me,e]
			SUCLG2[me]
			EGLN1/2
			VHL[c,m,i,e]
			EPAS1[c]
2[b,e]			*NF1*[c,m]
			MAX[c,m,i]
			TMEM127[c,m]
			RET[c,m]
			HRAS
			FGFR1
			H3-3A[c]
			DNMT3A[c,mt]
3[e]			*CSDE1*
			MAML3[c,e]

TCAc: tricarboxylic acid cycle; SDH: succinate dehydrogenase complex; c: clinical marker; m: morphological marker; i: immunohistochemical marker; b: biochemical marker; me: metabolite marker; e: gene expression marker; mt: methylation marker; mt*: DLST-mutated tumors do not show hypermethylation.

1.2. Morphological markers associated with specific genotypes

There are some morphological clues that may be helpful in classifying the VUSs found in PPGL patients. For example, VHL syndrome-associated PPGLs have a thick vascular tumor capsule and a degenerative and edematous stroma. On the other hand, hyaline globules are often abundant in *RET*-mutated PPGLs, and SDH mutant cells display granular and eosinophilic cytoplasm [11,12]. In addition, PPGLs carrying germline mutations in *RET* may show spindle cell changes and diffuse enlargement of the adrenal medulla (i.e. adrenal medullary hyperplasia). This has also been reported in some tumors from patients carrying mutations in *NF1*, *SDHB*, *MAX*, or *TMEM127* [13]. Finally, the identification of composite elements, including ganglioneuroma, ganglioneuroblastoma, neuroblastoma, and peripheral nerve sheath tumors, may indicate the presence of mutations in *NF1, MAX*, or *RET* [12].

1.3. Immunohistochemical molecular markers applied to the PPGL genetic diagnosis

Since 2009, SDHB immunohistochemistry (IHC) has been widely and routinely used to guide and prioritize the genetic analysis of PPGLs towards the SDH genes [14]. Today, most genetic testing labs use NGS to analyse all susceptibility genes simultaneously, and SDHB IHC is used to interpret VUSs involving any of the SDH genes (Fig. 1). The absence of SDHB staining in SDH-mutated tumor cells is found regardless of the type of mutation, and therefore this technique is especially helpful for missense VUS classification. As a result, a large multinational study found that only 6% of SDH-mutated PPGLs showed SDHB immunopositivity [15], highlighting the usefulness of SDHB IHC in the assessment of SDH VUSs. Conversely, positive SDHD immunostaining has been observed in SDH-mutated PPGLs and has been proposed as a very useful tool to interpret SDH gene variants [16], while a negative SDHA IHC can only be used in the interpretation of VUSs affecting the *SDHA* gene [17] (Fig. 1). It is noteworthy that head and neck (HN) PPGLs are frequently embolised prior to surgery, and this procedure may result in false-positive IHC results (PPGL Working Group, ENS@T meeting 2023). This is particularly relevant when using IHC techniques, as the most common mutations found in parasympathetic PPGLs affect the SDH genes.

DLST antibody can be used to interpret tricarboxylic acid cycle (TCAc)-mutations since PPGLs carrying such mutations show a strongly positive IHC [18]. In the same way, to identify tumors carrying somatic or germline mutations in any of the PPGL susceptibility genes related to the TCAc, the levels of 5hmC can also be assessed by IHC in FFPE tissue samples [19]. Mutations in these genes (i.e., SDH genes, *MDH2*, *IDH1/2*, or *FH*) cause accumulation of oncometabolites, such as succinate, fumarate or D-2-hydroxyglutarate (D-2HG), which competitively inhibit the activity of α-ketoglutarate-dependent dioxygenases, including both histone demethylases and DNA hydroxylases, leading to a DNA hypermethylation that can be assessed by 5hmC IHC [20] (Fig. 2). Moreover, the accumulation of fumarate caused by *FH* loss-of-function mutations

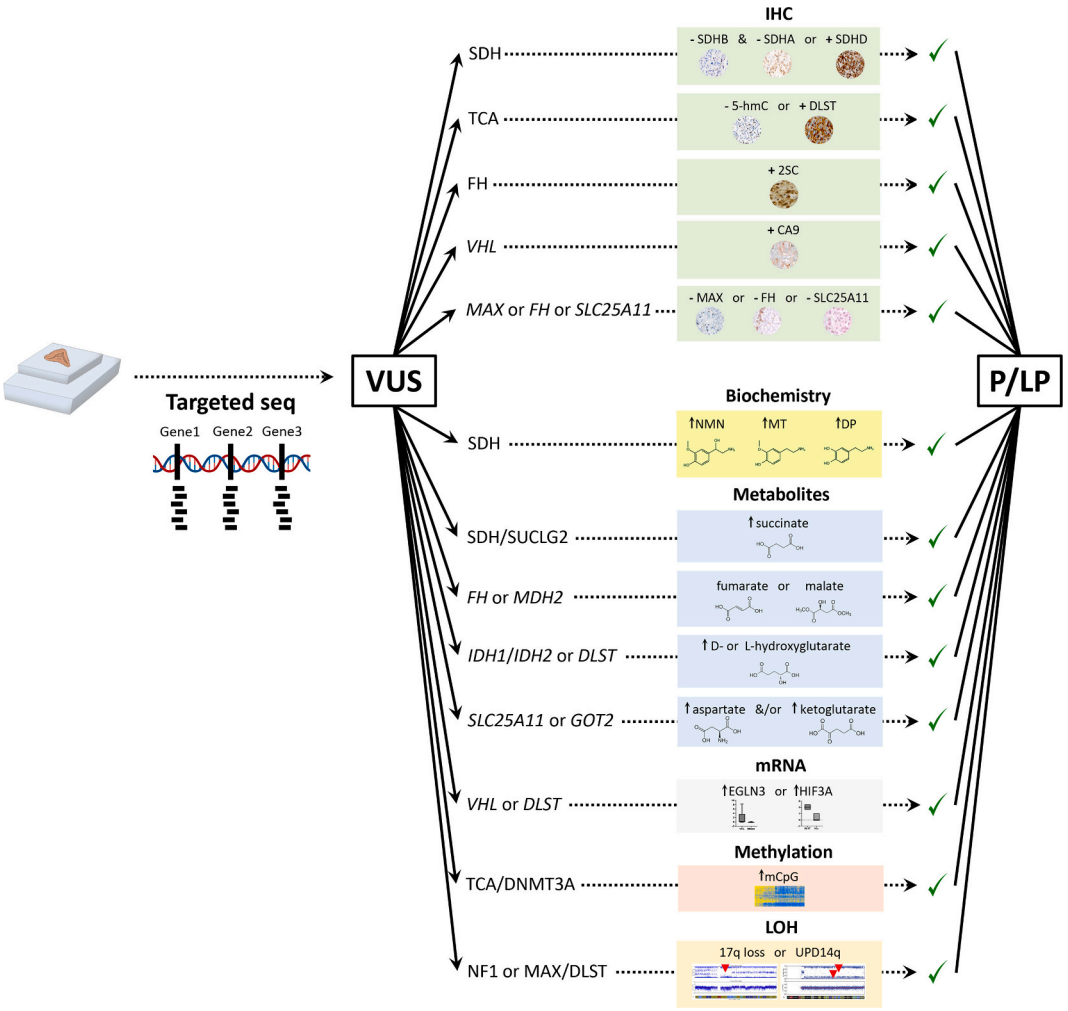

Fig. 1. Molecular markers used in the interpretation of variants of unknown significance (VUS) detected in PPGL susceptibility genes by targeted sequencing. SDH: succinate dehydrogenase genes; TCA: tricarboxylic acid cycle-related genes; − and + in the immunohistochemistry (IHC) panel indicate negative or positive IHC; P/LP: pathogenic/likely pathogenic variant; NMN: normetanephrine; MT: methoxytyramine; DP: dopamine.

produces a succination of proteins by covalent modification of cysteine residues to S-(2-succinyl) cysteine (2SC) that can be tested by an antibody against these modified residues [21]. Carbonic anhydrase 9 immunostaining at the plasma membrane of PPGL cells can be used to validate both germline and somatic *VHL* mutations [22]. Finally, MAX, FH or SLC25A11 IHC can be used for the interpretation of VUSs affecting these genes [23–25]. Obviously, the extent to which these latter immunohistochemical techniques are used depends on the prevalence of variants in the respective genes (e.g. the overall prevalence of *MAX* mutations in PPGL patients is less than 2%, as is the case for *FH* and *SLC25A11*).

1.4. Genotype-biochemical phenotype associations

PNMT is an enzyme preferentially localized in adrenal medulla that converts norepinephrine to epinephrine. The differences in PNMT expression between the two main PPGL transcriptional clusters (clusters 1 and 2), described in more detail subsequently, allow them to be distinguished by biochemical testing (Table 1), as they show different patterns of increase in plasma free metanephrine and normetanephrine [26]. In cluster 1 noradrenergic PPGLs, there is a significant increase in normetanephrine with little or no increase in metanephrine, whereas cluster 2 adrenergic PPGLs have elevated tumor-derived plasma metanephrine levels. *MAX*-mutated tumors, belonging to cluster 2, have an intermediate catecholamine biochemical profile, with detectable PNMT expression and some epinephrine production, resulting in a modest increase in tumor-derived metanephrine [6].

SDH-mutant PPGLs not only lack epinephrine production, but also show reduced norepinephrine production, often accompanied by elevated dopamine levels. In such cases, biochemical assays show elevations in both plasma normetanephrine and methoxytyramine, with certain tumors showing significantly higher increases in dopamine compared to norepinephrine metabolites (Fig. 1) [27].

2. Research-based strategies useful for variant interpretation

2.1. Metabolite-based genetic variant interpretation

As previously mentioned, mutations in several TCA-cycle related genes lead to the accumulation of oncometabolites than can be assessed by nuclear magnetic resonance spectroscopy and mass spectrometry coupled either to gas chromatography or to liquid chromatography [28]. Thus, the detection of high succinate to fumarate, or fumarate to malate ratios, can be used to support a pathogenic classification of SDH genes and *FH* VUSs, respectively [28,29] (Fig. 1). Similarly, higher levels of malate and D-2HG have been observed in PPGLs carrying *MDH2* and *IDH1/2* pathogenic mutations, respectively, and a specific L-2HG accumulation has been reported in tumors carrying *DLST* mutation [18], which can be used to interpret *DLST* VUSs. Surprisingly, a high succinate to fumarate ratio can be used to uncover *SUCLG2* mutations since it has

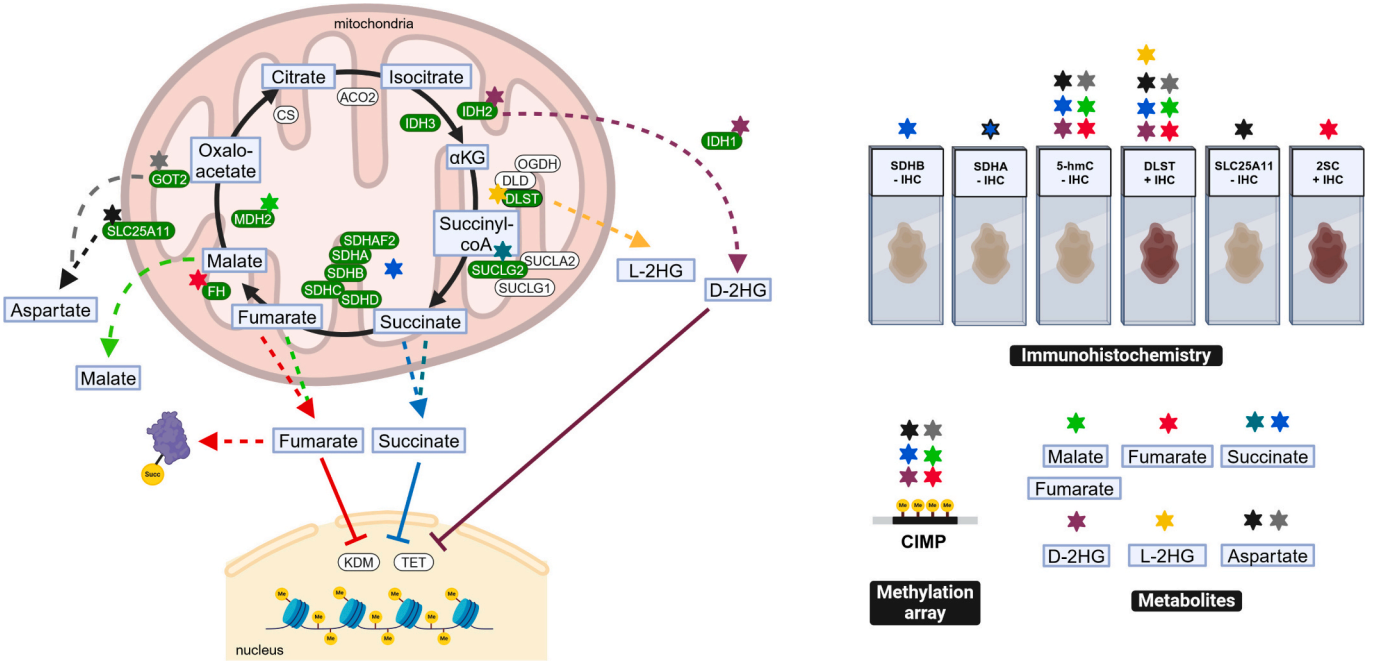

Fig. 2. Schematic representation of molecular markers (right part of the figure) associated with TCAc disruption (left part of the figure). Coloured stars represent mutations affecting the different genes involved in the TCAc. A blue star indicates a mutation in one of the five SDH genes and the blue star with a black outline indicates a specific *SDHA* mutation. D-2HG: D-2-hydroxyglutarate; L-2HG: L-2-hydroxyglutarate; CIMP: CpG island methylator phenotype. Figure created using BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been observed that PPGLs carrying *SUCLG2* mutations also exhibit a downregulation of SDHB expression [30]. Altered aspartate and ketoglutarate levels are also observed in *SLC25A11* and *GOT2* mutated PPGLs since these mutations affect genes involved in the malate-aspartate shuttle, which rewires metabolites to the TCAc [23,31]. In addition, a high succinate to fumarate ratio, as well as occurred with a negative SDHB IHC, can identify PPGLs candidate to harbor somatic methylation of the *SDHC* promotor, an uncommon and difficult to identify tumorigenic mechanism. One of the main weaknesses of these metabolite-based methods is the percentage of sustentacular cells within the tumor, since the presence of these cells in the measured sample may lower oncometabolite levels. This is something often observed in HNPPGLs, making the metabolome-guided classification of SDH variants in these particular tumors more challenging.

Interestingly, another application of metabolite's detection in the genetic diagnosis of SDH-mutated PPGLs is the measurement of succinate by in vivo magnetic resonance spectroscopy [32]. Considering the innocuousness of this non-invasive method, it can be included in the clinical guidelines for PPGL management, and also in other SDH-mutated tumors such as gastrointestinal stromal tumors and pituitary adenomas [33].

2.2. Gene expression and methylation signatures

Different studies have demonstrated that whole-genome gene expression profiling can be used to identify to which genetic cluster a given tumor belongs and ultimately which mutation could harbor it [9,34,35]. Three main transcriptional PPGL clusters are currently recognized: cluster 1 splits into *subcluster 1 A*, which includes PPGLs with loss-of-function mutations in TCAc-related genes (SDH genes, *FH*, *IDH1/2*, *MDH2, GOT2*, *SCL25A11*, and *SUCLG2*), and *subcluster 1B*, which encompasses tumors with pathogenic variants in *VHL*, *EPAS1*, and *EGLN1/2*, involved in the oxygen-sensing pathway; cluster 2 PPGLs show mutations in kinase signaling-related genes such as *NF1*, *RET, HRAS, MAX*, *FGFR1,* and *TMEM127*; cluster 3 encompasses tumors carrying alterations in *CSDE1* and *MAML3* involved in the Wnt pathway. Thus, genome-wide transcriptional data or cluster-specific transcriptional signatures may be used to interpret the pathological role of a VUS in one of the PPGL susceptibility genes. Besides expression profiling, RNA-sequencing (RNA-seq) can also be used to guide the reclassification of VUSs and detect previously unknown causative variants by revealing aberrant splicing at the RNA level.

Sometimes, the expression of a particular gene is itself a molecular marker to genetically classify a PPGL. Most of these expression markers have to do with genes belonging to the oxygen-sensing pathway. For instance, *EGLN3* mRNA overexpression is a hallmark of *VHL*-mutated tumors, including PPGLs [34,36], and *HIF3A* overexpression is an exclusive molecular feature observed in *DLST*-mutated PPGLs [18] (Fig. 1). However, *EPAS1* mRNA expression can only be used to distinguish between cluster 1 (high expression) and cluster 2 (low expression) PPGLs. On the other hand, a high expression of *MAML3* has been observed and is a molecular marker for *MAML3*-related PPGLs [9].

As the accumulation of oncometabolites cause a global hypermethylation or CIMP (CpG island methylator phenotype) [20], this feature can be used to classify variants or to select PPGLs for the study of additional mechanisms affecting TCAc-related genes. An exception to this molecular characteristic can be found in *DLST*-mutated tumors, which behave as cluster 2 PPGLs regarding methylation profiling. A schematic representation of how TCA dysfunction can be detected using multiple molecular markers is shown in Fig. 2. Similarly, *DNMT3A*-mutated HNPPGLs show a specific methylation signature enriched with homeobox-containing genes involved in neural crest differentiation and pattern specification and embryonic morphogenesis processes [37]. As occurs with the expression data, the methylation of a specific gene (i.e., *RBP1*) can be used as a marker of a hypermethylation phenotype and be helpful in the interpretation of VUSs [31].

2.3. Loss of heterozygosity (LOH) mechanisms

Following Knudson's two-hit model, mutations in TSGs should be coupled with inactivation of the remaining allele usually by mutation, methylation or loss of the wild-type allele (i.e., LOH). As a result, finding

LOH in a region where a potential pathogenic germ line mutation of a TSG is identified supports and enhances the likelihood of pathogenicity and may be therefore a molecular marker useful for variant interpretation (Fig. 1). That was the case of the study that first revealed the high frequency of somatic mutations in *NF1* in PPGLs. Welander et al. found that copy number losses in chromosome 17 were molecular markers of the presence of *NF1* mutations in PPGLs [38], and therefore this marker can also be used to interpret somatic *NF1* VUSs. Another example is the uniparental disomy of chromosome 14q, which is the most frequent second hit observed in tumors carrying mutations in *MAX* and *DLST* and that could support a pathogenic role of variants under study in these TSGs [18,24]. However, other chromosomal losses (e.g. affecting chromosomes 1, 3 and 11) are too frequent in PPGLs, regardless of the mutation, to be used as markers of specific gene mutations.

Reversely, many oncogenic activating mutations are followed by the gain or amplification of the specific *locus*. This is the case of the chromosomal gain of 2q observed in *EPAS1*-mutated PPGLs that may guide the genetic diagnosis of VUSs in the gene [39].

2.4. In vitro functional assays and in silico tools

Computational predictions combined with functional models can be used to correctly classify VUS involving a susceptibility gene, in the absence of informative pedigrees for assessing the cosegregation of a novel variant with a hereditary condition, clinical information or molecular data. A decade ago, we proposed a combination of a consensus computational prediction, clinical and molecular data, and a functional assay designed to assess the effect that a *MAX* VUS may have on MYC's *E*-box transcriptional activation to classify *MAX* variants [40]. More recently, Flores et al. [41] functionally classified 15 *TMEM127* variants as pathogenic or likely pathogenic mutations by evaluating their effect on loss of membrane-binding ability, stability, or internalization capability. Another example of this kind of approaches is the interpretation of *MDH2* VUSs, which was carried out using an algorithm based on 20 computational predictions, by implementing cell-based enzymatic and immunofluorescence assays, and/or by using a molecular dynamics simulation approach [42]. After the finding of the first *DLST* variants in patients with PPGL, we conducted $^{13}C_5$-glutamate labelling studies to trace the carbon flow in the TCAc in a heterologous cell-based assay designed to assess the impact of several missense VUSs on DLST function [18]. There are other examples that use knockout cell lines to study the impact of VUSs found in several susceptibility genes by assessing the impact of the variants in some of the previously mentioned molecular markers related to the presence of pathogenic mutations [23,30].

Functional assays are challenging as they require experience in designing appropriate experiments to assess the effect of the variant, which will vary depending on the function of the gene and location of the substitution in the protein. Disease diagnostic labs should be prepared to classify variants using functional assays, but it should be noted that these assays are time-consuming and require significant resources.

2.5. Additional analysis of mutation mechanisms related to known susceptibility PPGL genes

Whole-genome sequencing (WGS) is now a further tool in the identification of pathogenic alterations associated with inherited diseases. The main application of WGS in clinical genetics is the detection of non-coding alterations in susceptibility genes, such as mutations in regulatory regions like introns and promotor regions. In addition, due to technical reasons, WGS can detect alterations that amplicon amplification-based methods such as targeted-sequencing cannot, like deletions encompassing primers recognition sites. Finally, WGS, as occurs with RNA-seq, may be used to identify fusion genes and translocations. Until the publication in 2017 of the first fusions affecting *MAML3* as a new genetic driver in PPGL [9], it was thought that this kind of rearrangement was a rare event in these tumors. However, as it is now clear that many sporadic PPGLs may harbor fusions affecting *MAML3*, there is an urgent need for easy-to-apply methods to identify such alterations. PD-L1 (CD274) has been reported as a proliferation biomarker for PPGL, and more recently, it has been reported that PPGLs carrying oncogenic fusions involving MAML3, which are more prone to metastasis, are highly positive for PD-L1 IHC [43]. Moreover, two *RET* rearrangements have been recently reported in PPGLs with *RET* as the upstream partner of the fusion instead of the conventional 3′ position, suggesting that this oncogenic mechanism could be more frequent than previously expected [44]. Due to technical and cost-effective reasons, the detection of these chromosomal rearrangements is not widespread and, therefore, is not routinely carried out. Hence, the selection by PD-L1 IHC of cases to be screened for prognostically relevant translocations, such as those affecting *MAML3*, would reduce costs and increase the likelihood of finding a fusion-positive tumor, improving the clinical management of mutation carriers.

Epimutation of *SDHC* could explain a subset of SDHB IHC negative PPGLs without gross deletions and point mutations in the SDH genes. Detection of this alteration requires either whole methylation analysis of tumor DNA or a more gene-specific method such as pyrosequencing. It is worthy to note that this somatic mechanism may be postzygotic, occurring during embryogenesis and leading to mosaic individuals who develop multiple tumors. This latter has been described in individuals with Carney triad (co-occurrence of PGLs, gastric stromal tumors, and pulmonary chondromas) [45], in a patient with Carney-Stratakis syndrome (co-occurrence of PGLs and gastric stromal tumors) [31], and in PPGL patients with multiple tumors [46].

RNA-seq, apart for the identification of rearrangements, plays a crucial role as "functional assay" for reclassifying previously identified VUSs. This RNA-based approach complements inconclusive results obtained by current NGS methods and allows unveiling aberrant splicing as a disease-causing mechanism.

2.6. Molecular markers in the identification of new susceptibility genes

Currently, the main approach for the identification of a new susceptibility gene associated with a hereditary condition involves the application of NGS to DNA samples obtained from genetically unsolved patients. In such analyses, the first and key step is an adequate selection of candidates to share an alteration affecting the same gene, and this may be possible if we identify a common and distinctive molecular feature for all samples (Fig. 3), as mentioned above. For instance, the application of different omics approaches may facilitate the detection of a molecular marker that not only homogenizes the set of candidate samples but could also be used later to validate VUSs identified in the new gene. Of course, the presence of a family history of PPGL or multiple tumors in unsolved patients is also a clinically distinctive feature useful for the identification of a new susceptibility gene. However, the number of families without a genetic explanation is currently scarce, and they are probably candidates to carry an alteration in one of the already-known genes. Therefore, the collection of enough patients with a family history of PPGL and without known mutations will require the cooperation of reference research groups. Collaborative consortia are now more necessary than ever to accomplish the goal of genetically diagnosing most patients with PPGL.

Regarding the material to work with, as occurs with genetic diagnosis, the best option is to use tumor DNA for the analyses to simultaneously consider the possibility of germline and somatic mutations, even in the case of patients with family history of the disease. As previously demonstrated, the presence of multiple PPGLs in a patient does not preclude the presence of a somatic postzygotic mutation that would only be detected in tumor DNA [47,48]. Similarly, although extremely rare, there are also examples of phenocopies in PPGL families [49].

The use of WGS instead of exome sequencing enables almost all possible mutational mechanisms to be covered, identifying regulatory mutations (e.g., deep intronic splice variants) and fusions in addition to

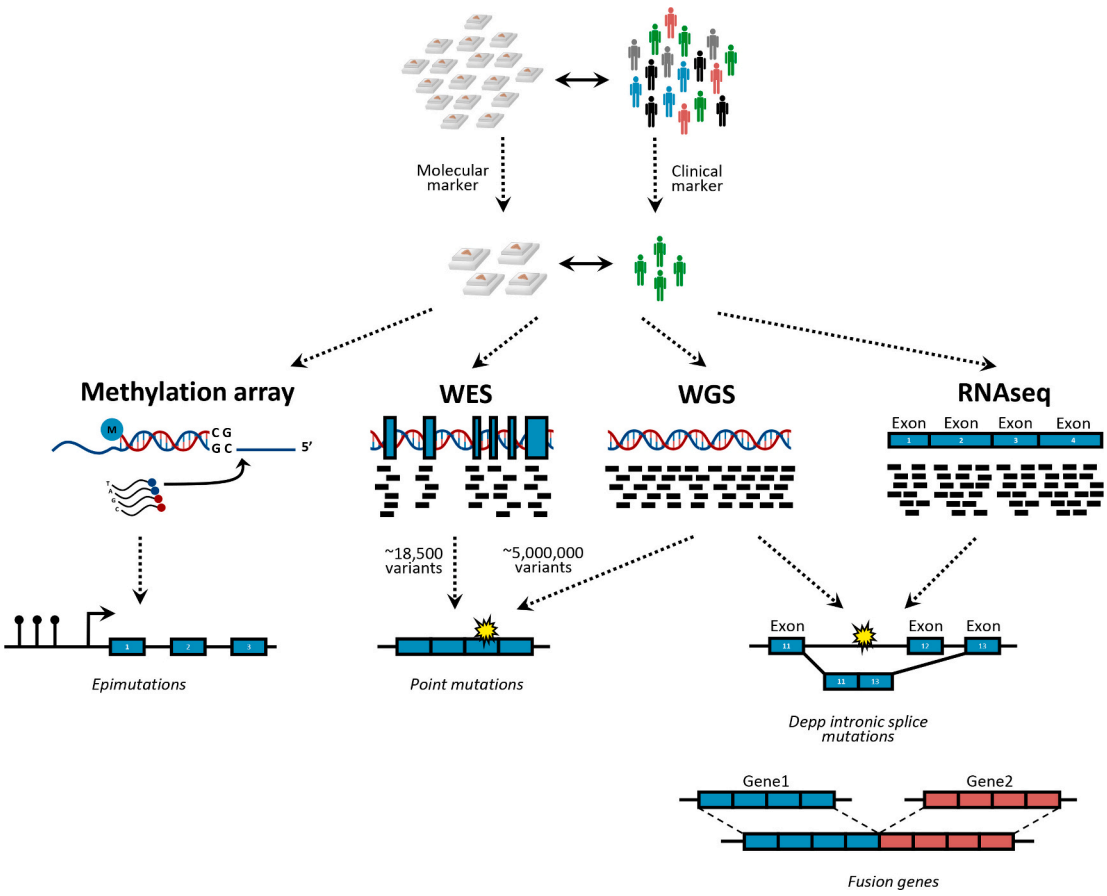

Fig. 3. Omic strategies used for new susceptibility gene identification. WES: whole exome sequencing; WGS: whole genome sequencing; RNAseq: RNA sequencing.

coding mutations. While the current reduced cost of WGS does not appear to be an obstacle for this type of research, it is important to remember that this technology involves a massive amount of data and variants, with an average number of variants detected per whole genome of ~5,000,000 compared to 18,400 variants per exome [50]. Finally, somatic epimutations and rearrangements of still unknown susceptibility genes can be detected using alternative methods such as methylation arrays and RNAseq ((Fig. 3) focused on tumor DNA to cover more mutational mechanisms.

3. Conclusions

VUSs represent the vast majority of variants identified by NGS technologies. Assessing the pathogenicity of VUS is a long-term, intricate, and expensive procedure that requires the collaboration of several experts, including bioinformaticians, biologists, and clinicians [51]. As scientific knowledge evolves and additional data becomes available, the interpretation of VUS may change over time. Periodic re-evaluation of VUS, considering new evidence, is necessary to ensure accurate and up-to-date clinical management. The use of well-established molecular markers can enhance genetic diagnostic accuracy by allowing the classification of VUSs and enabling timely follow-up with patients and their relatives. This is particularly relevant for mutations that contribute to the genetic background of rare diseases, where some genes are mutated in only 1 or 2 families in the world. It is therefore very difficult to estimate their pathogenicity and exact impact on patients, which also makes genetic counselling very challenging. Finally, the application of high-throughput NGS technologies to DNA tumor samples from well-selected candidates that share a distinctive molecular signature may allow the identification of novel PPGL susceptibility genes.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

No data was used for the research described in the article.

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