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ORIGINAL ARTICLE

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A nomogram for predicting the presence of germline mutations in pheochromocytomas and paragangliomas

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Abstract

Purpose Up to 40% of patients with pheochromocytomas or paragangliomas (PPGLs) carry a germline mutation. This study aimed to build a nomogram using clinical information to predict the probability of germline mutation in PPGLs.

Methods The data were collected from 563 patients who were diagnosed with PPGLs between 2002 and 2015. Clinical and pathologic features were assessed with a multivariable logistic regression analysis to predict the presence of germline mutations. A nomogram to predict the probability of germline mutation was constructed with R software. Discrimination and calibration were employed to evaluate the performance of the nomogram.

Results By multivariate analysis, age at manifestation, bilateral, or multifocal tumors and family history were identified as independent predictors of the presence of any germline mutation. The nomogram was then developed using these three variables. The nomogram showed an area under the receiver operating characteristic curve (AUC) of 0. 841 (95% confidence interval [CI], 0.809–0.871). The calibration plot indicated that the nomogram-predicted probabilities compared very well with the actual probabilities (Hosmer–Lemeshow test: $P = 0.888$).

Conclusion The nomogram is a valuable predictive tool for the presence of germline mutations in patients with PPGLs.

Keywords Pheochromocytomas · Paragangliomas · Germline mutation

Introduction

Pheochromocytomas and paragangliomas (PPGLs) are catecholamine-secreting tumors that arise from chromaffin cells of the adrenal medulla (pheochromocytomas) and from extra-adrenal thoracic and abdominal paraganglia (paragangliomas). The majority of PPGLs are sporadic, but a significant number of them, currently estimated at up to 40%, are inherited as part of hereditary syndromes [1, 2]. These syndromes include multiple endocrine neoplasia type 2, von Hippel–Lindau (VHL) disease, neurofibromatosis type 1, and familial paraganglioma/pheochromocytoma syndromes (PGLs), characterized by mutations in RET, VHL, NF1, and the genes encoding the subunits (B, C, and D) of the succinate dehydrogenase (SDH) complex, respectively. Recently, novel susceptibility genes, TMEM127, MAX, and HIF-2A (only found with somatic mutations), have also been associated with tumorigenesis [3–5].

Many germline mutations of susceptibility genes occur in patients without family or medical history. Therefore, all patients with PPGLs should be engaged in shared decision-making for genetic testing [6, 7]. However, in clinical practice, universal screening of all possible genes in PPGLs is still technically or financially not feasible. The germline mutation test is unavailable in a significant proportion of familial PPGL cases because of a lack of well-established molecular diagnostic laboratories in most regions. Assessing the existence of

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heritability ("judgment in the outpatient office") is essential for the genetic diagnostic strategy. A clinical-driven strategy was recommended [8–12]. Features that indicate a high likelihood of a hereditary cause include a positive family history, syndromic features, and multifocal, bilateral, or metastatic disease. However, a single presentation can be attributed to any of several susceptibility genes, and the applicability of clinical indicators to predict the presence of germline mutations is still not clear.

Statistical prediction models are widely used for cancer outcome prediction. Among these models, nomograms create user-friendly graphical representations of complex models to generate the probability of an event based on the individual profile of each patient [13, 14]. In this study, we hypothesized that a nomogram obtained from common clinical parameters could guide us in prioritizing gene testing. We performed a retrospective study to create a risk assessment nomogram model comprising clinical risk factors to predict the greatest likelihood of having any germline mutation. The aim was not to determine whether factors related to hereditarily derived clinical parameters could be used to distinguish patients with hereditary from sporadic disease but rather to establish a practical tool to predict the existence of germline mutations in patients with PPGLs in the outpatient office.

Materials and methods

Patients

We conducted a retrospective study using data from 563 patients with PPGLs from January 2002 through December 2015. Diagnoses of PPGLs were based on plasma or urinary levels of catecholamines or catecholamine metabolites (metanephrines: MN and NMN) and confirmed by imaging and/or surgical histology. The extra-adrenal disease was defined as the initially discovered tumor at an extra-adrenal site. Recurrence was defined as the reappearance of the disease after eradication of the tumor. Malignant PPGLs were defined as the presence of metastatic lesions at non-chromaffin sites, such as bone, liver or lung. In known familial cases of PPGL, only the probands were included. The data were collected under the conditions of regular clinical care, and the ethics committee approved the retrospective use of these data for scientific purposes. Written informed consent, which also allowed for the collection of specimens and clinical data, was obtained from the patients.

Detection of germline mutations

The presence of germline mutations and large deletions of susceptible genes (RET, VHL, and SDHx) in patients with a positive family history and syndromal features was investigated according to standard procedures [15, 16]. Biochemical classification was used to further detect some rare mutations, such as in the TMEM127, SDHAF2, and MAX genes, as recommended by Karel Pacak et al. [17]. For mutation-negative patients according to the above flowchart, we further tested the main genes (including VHL, RET, SDHx) in patients with available samples, and regular follow-up included 25 patients with bilateral or multifocal tumors and patients with family history, due to their clinical importance. In total, 111 patients had hereditary PPGLs, including 25 with VHL syndrome, 50 with RET, 1 with NF1, 19 with mutations in the SDHB gene, 2 with mutations in the SDHD gene, 4 with mutations in the SDHA gene, 1 with mutations in the SDHC gene, 5 with mutations in the SDHAF2 gene, 2 with mutations in the MAX gene and 2 with mutations in the TMEM127 gene (Supplementary Table 1). The diagnosis of NF1 was based on clinical criteria [18].

Statistical analysis

To determine which clinical parameters predicted the presence of a germline mutation in any gene, we compared the group of patients carrying mutations in any of the tested genes with the mutation-negative group using the chi-squared test and two-sample paired *t*-test for categorical and continuous variables, respectively. The predictors or variables identified were tested in univariate and multivariate logistic regression analyses for association with the mutation. All variables that were univariately associated with mutation status at a level of $p < 0.05$ were candidates for inclusion in the multivariate model and were retained in the model if they remained significantly associated with the outcome.

The nomogram performance was quantified with respect to discrimination and calibration. Moreover, the nomogram was subjected to 1000 bootstrap resamples for internal validation to obtain relatively unbiased estimates of the performance of the model. The discriminatory ability of the model was quantified using the area under the receiver operating characteristic curve (AUC). A 95% CI was calculated for each AUC. In general, an AUC > 0.75 was considered to be relatively good discrimination. Calibration of the nomogram was assessed by plotting the observed outcome probabilities and the probabilities predicted by the logistic model. A plot along the 45° line corresponds to a model in which the predicted probabilities are identical to the actual probabilities, therefore indicating perfect calibration. The Hosmer–Lemeshow (H–L) test was used to examine how well the percentage of the observed probability matched the percentage of predicted probability over deciles of predicted risk. A *P*-value > 0.05 was considered well calibrated, meaning that there was no significant difference between observed and predicted probability.

Statistical significance was set as $P < 0.05$ in a two-tailed test. All statistical analyses were performed using SPSS version 24 (SPSS, Chicago, IL), and R software version 3.6.0 (http://www.r-project.org) with the rms package.

Results

Population characteristics

The demographic, clinical, and pathological characteristics of the patients are presented in Table 1. Overall, 111 (19.7%) patients harbored germline mutations, Sex, primary tumor size, primary tumor site, plasma-free metanephrine, and normetanephrine did not differ significantly between patients with and without germline mutations (Table 1). However, patients with germline mutations had a lower age at manifestation compared with patients without germline mutations ($P < 0.001$). Compared with the patients without germline mutations, the patients with germline mutations were more likely to have recurrence (18.0% versus 3.8% of patients; $P < 0.001$) and have a family history (31.5% versus 1.1%; $P < 0.001$). A total of 54.1% of the patients with germline mutations had bilateral or multifocal tumors, compared with 8.4% of those with mutation-negative tumors ($P < 0.001$). There was no significant difference between the two groups with respect to malignant tumors (11.7% versus 10.0% of patients; $P = 0.586$).

Clinical risk factors for germline mutations

Table 2 summarizes the univariate and multivariate logistic regression analyses. Univariate analysis showed that age at manifestation, bilateral or multifocal tumors, recurrence, and family history were significantly associated with germline mutation. The final multivariate logistic regression analysis yielded three statistically significant independent predictors: age at manifestation (OR 0.96, 95% CI 0.94–0.98), bilateral or multifocal tumors (OR 6.63, 95% CI 3.70–11.86) and family history (OR 18.62, 95% CI 6.52–53.20).

Prediction nomogram

A nomogram was constructed based on the results of the multivariate logistic regression (Fig. 1). The discrimination accuracy of the model was 0.841 (95% CI, 0.809–0.871; (Fig. 2a), indicating excellent accuracy in discriminating germline mutation-positive versus germline mutation-negative cases in this group of patients. At an optimal cutoff value of 22%, the sensitivity and specificity were 64.0% and 90.9%, respectively. To assess the accuracy of the nomogram for our collective data, the actual probabilities were plotted against the calculated probabilities for each decile of patients. The calibration plot indicated that the nomogram-predicted probabilities compared very well with the actual probabilities ($P = 0.888$, Hosmer–Lemeshow test, Fig. 2b).

Discussion

Routine testing of tumor susceptibility genes is now often carried out in patients with PPGLs; however, in many regions, mutation testing is not available. Here, we integrated clinical factors to develop a logistic regression-based model to predict the presence of germline mutations in PPGLs. The nomogram had excellent discrimination properties in patients, with an AUC of 84.1%. This study was the first, to our knowledge, to incorporate multiple clinical variables into a user-friendly nomogram for predicting germline mutations in PPGLs.

Considerable effort is being expended to establish guidelines and algorithms for the cost-effective genotyping

Table 1 Clinicopathological features of the patients with and without germline mutation

Clinicopathological features	All patients ($N = 563$)	Mutation positive ($N = 111$)	Mutation negative ($N = 452$)	P
Gender (male/female)	248/315	51/60	197/255	0.653
Age at manifestation (year)	40.1 ± 14.3	31.3 ± 11.0	41.9 ± 13.9	<0.001
Primary tumor size (cm)	5.9 ± 2.8	6.1 ± 3.1	5.5 ± 2.9	0.195
Primary tumor site (adrenal/extra-adrenal)	428/135	87/24	352/100	0.909
Plasma-free metanephrine[a] (pg/ml)	86.3 (60.2, 375.1)	89.7 (59.6, 700.9)	85.9 (61.1, 277.3)	0.526
Plasma-free normetanephrine[a] (pg/ml)	1078.7 (375.1, 2646.7)	1178.0 (310.1, 3107.3)	1074.0 (374.7, 2581.5)	0.862
Bilateral or mutifocal tumors	98	60 (54.1%)	38 (8.4%)	<0.001
Malignant tumors	58	13 (11.7%)	45 (10.0%)	0.586
Recurrence	37	20 (18.0%)	17 (3.8%)	<0.001
Family history	40	35 (31.5%)	5 (1.1%)	<0.001

[a]Plasma-free metanephrines are expressed as medians (and interquartile range)

Table 2 Univariable and multivariable logistic regression analysis results for presence of any germline mutation

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Age at manifestation (per year increase)	0.94	0.92–0.96	<0.001	0.96	0.94–0.98	<0.001
Bilateral or mutifocal tumors						
Negative						
Positive	13.2	7.98–21.8	<0.001	6.63	3.70–11.86	<0.001
Recurrence						
Negative						
Positive	5.62	2.84–11.16	<0.001	1.33	0.52–3.45	0.555
Family history						
Negative						
Positive	41.17	15.64–108.40	<0.001	18.62	6.52–53.20	<0.001

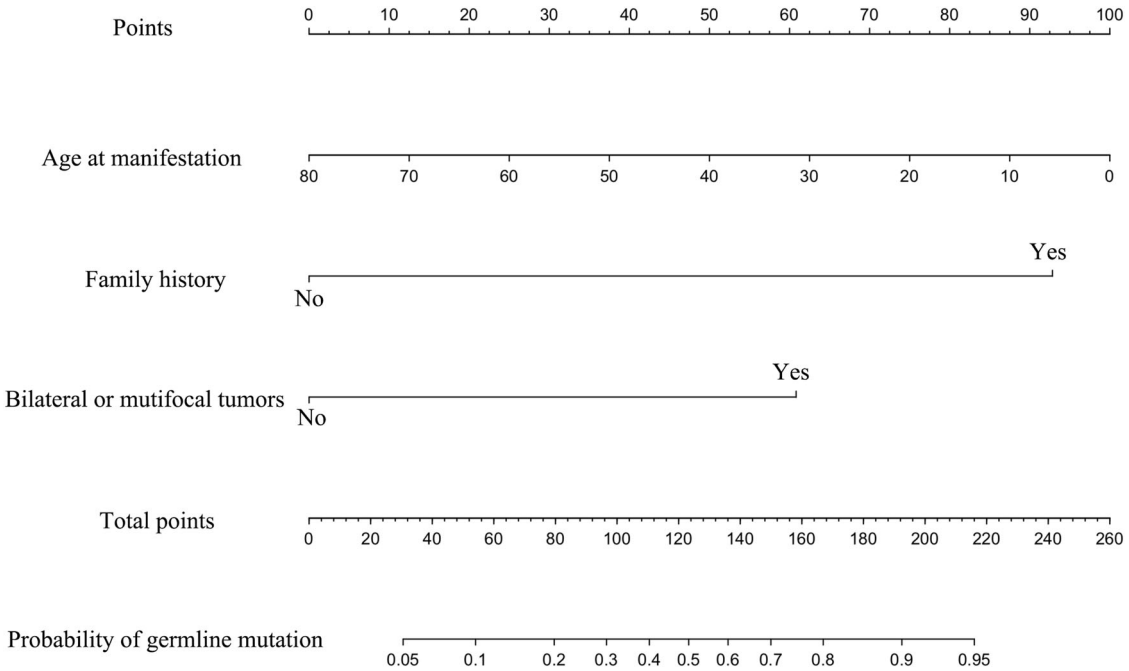

Fig. 1 Nomogram to predict the presence of germline mutations based on age at manifestation, bilateral or multifocal tumors and family history. **Instructions for physicians:** Locate the age on the age at manifestation axis. Draw a line straight upward to the points axis to determine the number of points for the probability of the presence of germline mutation for the age at manifestation. Repeat the process for each of the remaining axes. Sum the points for each of the predictors. Locate the final sum on the total points axis. Draw a line straight down to find the probability of germline mutation

of patients with PPGLs. Next-generation sequencing (NGS) is a time-efficient and cost-effective alternative for doctors [19]. However, for patients with classical syndromes, a clinical feature-driven diagnostic algorithm to establish the priorities for specific genetic testing in PPGL patients with suspected germline mutations is still valuable. Risk factors such as family history, age < 35 years, and extra-adrenal, bilateral, or malignant tumors have been reported [17, 20]. Consistent with previous results [8–10], we found, through multivariate analysis, that younger age at manifestation, bilateral or multifocal tumors, and family history were associated with the presence of germline mutation.

In our results, family history was the strongest factor for germline mutation (OR 18.62, 95% CI 6.52–53.20). Overall, 35 of 40 patients (87.5%) in this subgroup were mutation carriers. There is no doubt that family pedigree or identification of a PPGL-susceptibility gene mutation in a relative may benefit earlier diagnosis and treatment. Detailed consultation at the clinic is necessary. In addition, there is a broad consensus that patients with hereditary

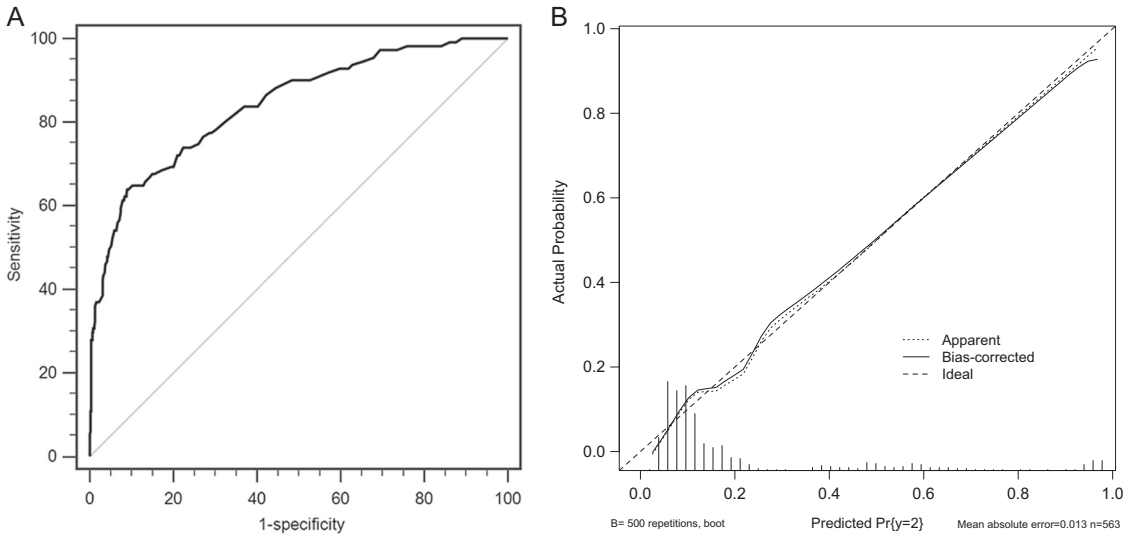

Fig. 2 Validation of the nomogram to predict probability of the presence of germline mutation. **a** Discrimination. Area under the receiver operating characteristic (ROC) curve (AUC) is 84.1% (95% CI, 0.809–0.871). **b** Calibration of the nomogram to predict germline mutation in patients with PPGLs. The horizontal axis represents the predicted probability of the presence of germline mutation, and the vertical axis represents the actual probability of the mutation. Perfect prediction would correspond to the 45° broken line. The dotted and solid lines indicate the observed (apparent) nomogram performance before and after bootstrapping

PPGLs typically present with multifocal disease and at a younger age [12, 21]. Multifocal or bilateral PPGLs were strongly associated with mutations in VHL or RET in our clinical practice, which makes them the second strong risk factor for mutation. A total of 53 of 54 patients (98.1%) in the bilateral group and 11 of 16 (68.8%) in the multifocal group were VHL or RET mutation carriers. The remaining patients had SDHx mutations. An age cutoff of 45 years has been recommended to better identify mutation-positive cases [8]. We also recommend 45 years for Chinese patients (90.1% of patients younger than 45 were mutation carriers).

We built a nomogram model to assess the probability of germline mutation combined with these risk factors. Doctors can make a preliminary judgment on the existence of germline mutations in the outpatient clinic. As nomograms can generate individualized predictions, these models can be used to provide better information to the patient about their probability of mutation. This information can help clinicians to involve patients in the genetic screening decision-making process and may consequently improve their compliance. The American Society of Clinical Oncology suggests that all patients with a risk of heritability higher than 10% should undergo testing [22]. For our patients, at an optimal cutoff value of 22%, the sensitivity and specificity were 64.0% and 90.9%, respectively. For patients with mutation probability above 22%, patterns of increases in plasma normetanephrine, metanephrine, and methoxytyramine should provide further information to determine the most appropriate genes to test [23, 24].

Genetic testing of inherited mutations allows the identification of cooccurring cancers in hereditary syndromes and screening of at-risk relatives, with an impact on health care. Combined with the consideration of clinical factors in the nomogram, the data derived from diagnostic testing (patterns of increases in plasma metanephrines) and further clinical information (coexistence of tumors included in PPGL syndrome) should offer a useful approach to select genes for further gene testing [11, 24]. Although our nomogram used fewer variables, the clinical utility of the nomogram was still considerable. Patients could be informed of their individual likelihood of germline mutation and, based on this information, make a decision regarding the gene test.

Our study has some limitations. First, our analyses focused on the germline mutation status rather than specific genes, and selected genes according to the clinical information for the individual were analyzed in the next step. Second, the sensitivity and specificity of the genetic screening approach may need further investigation. In patients with a negative family history and lack of syndromal features, SDHB immunostaining was used as a substitute method. Thus, formal genetic testing was omitted, which may have led to the presence of a significant number of young patients, patients with bilateral or multifocal tumors and patients with family history in the mutation-negative cases. Third, although our cohort represents the largest group of Chinese patients from a single center, this might not be relevant to referral patterns outside major cancer centers. More robust validation is necessary on an external dataset. The mutation rate in the cohort was lower than reported, and NGS was not used, which may have led to inaccurate results. Fourth, although we constructed an

improved prognostic instrument based on a contemporary dataset, the nomogram does not specifically make treatment recommendations but simply provides an assessment of individual risk for germline mutation. This information enables a more informed clinical decision-making process but does not substitute for it.

In conclusion, our nomogram provides an easy-to-use tool with which to simultaneously incorporate several important clinical variables into the estimate of the risk of germline mutations in patients with PPGLs. This information could help inform decision-making by physicians and patients.

Data availability

Materials and data are available from the corresponding author upon reasonable request.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethics Committee of the Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

Informed consent All patients gave their written informed consent.

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