Original Article

Bilateral pheochromocytomas in MEN2A syndrome: a two-institution experience

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ABSTRACT

Background:

Bilateral pheochromocytoma (PHEO) is more frequently found in patients with multiple endocrine neoplasia (MEN) 2A carrying a *RET* germline mutation located in codon 634 (C634). However, it is unclear whether different amino acid substitutions within C634 cause differences in bilateral PHEOs expression. We aimed to answer this by pooling data from two Asian institutions.

Methods:

Sixty-seven patients had confirmed C634 germline mutation. Age-dependent penetrance of bilateral PHEO was calculated from date of birth to the date when bilateral PHEO was first diagnosed or when the contralateral gland became a PHEO (if the patient already had one adrenal gland removed). Age-dependent penetrance was estimated by the Kaplan-Meier Method and compared by log-rank test.

Results:

The 4 different amino acid substitutions included C634R (arginine) (n=19, 28.4%), C634Y (tyrosine) (n=36, 38.8%), C634G (glycine) (n=4, 6.0%) and C634W (tryptophan) (n=8, 11.9%). The age-related penetrance of PHEO was similar between C634R, C634Y, C634G and C634W (by age 40, 69.8%, 55.2%, 25.0% and 56.2%, respectively) (p=0.529). However, the age-related penetrance of bilateral PHEO in C634R was significantly higher than C634Y (by age of 40, 59.3% vs. 25.2%, p=0.046) or C634Y, C634G and C634W combined (59.3% vs. 21.5%, p=0.024). Nevertheless, the accumulative risk of bilateral PHEOs across all four C634 mutations almost approached 100% over time.

Conclusions:

The accumulative risk of bilateral PHEOs almost reached 100% but its onset was significantly earlier in C634R mutation. These findings implied that those with C634R mutation might benefit from earlier screening of contralateral PHEO than other C634 mutations after an unilateral adrenalectomy.

INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN2) is a rare autosomal dominant syndrome with a worldwide prevalence of between 1 per 30000 to 1 in 50000 population. It has traditionally been divided into three syndromes, namely MEN 2A, MEN 2B and familial medullary thyroid carcinoma (MTC). MEN2A is characterized by MTC, pheochromocytoma (PHEO) and hyperparathyroidism. Although MTC is a universal feature, it varies with age of onset and aggressiveness depending on the type of *RE*arranged during *T*ransfection (*RET*) codon mutation[1-3]. Nevertheless, once confirmed with a *RET* germline mutation, prophylactic or early total thyroidectomy is advocated[4]. Relative to MTC, the age-dependent penetrance of PHEO have been less well-described[3]. One reason might be because it is generally believed only a third of MEN2A patients would develop a clinically apparent PHEO[1-5]. However, it is now recognized that some patients would develop small subclinical tumors and / or hyperplasia of the adrenal medulla bilaterally over time[1,2]. Clinically apparent bilateral PHEOs is a particularly prominent feature in patients with mutations located on codon 634 (C634) (exon 11)[6,7] and since this codon mutation is the most frequent mutation in MEN2A families, it would be important to have a better understanding its association with bilateral PHEOs. However, to date, reports on MEN-related bilateral PHEOs particularly from the Asian population have been scarce[6,8,9]. Furthermore, since there are a number of different amino acid substitutions for the same cysteine on C634 mutation, it would be important to examine whether there are subtle but potential differences in age of onset and penetrance of bilateral PHEOs caused by various amino acid substitutions within C634[6,10]. This information will be useful in tailoring more accurate clinical care. A recent Japanese study found that patients with C634R (from cysteine to arginine) had a higher penetrance of PHEO than C634G (from cysteine to glycine) / C634F (from cysteine to phenylalanine) combined ($p<0.01$) but did not report its relationship with bilateral PHEOs[6]. Therefore, our study aimed to compare the age of onset, penetrance and outcomes of PHEO and bilateral PHEOs between different amino acid encoded by codon 634 after pooling data from two Asian institutions.

PATIENTS AND METHODS

Patients

The two participating institutions were Queen Mary Hospital (QMH), Hong Kong and Seoul National University Hospital (SNUH), South Korea. All consecutive patients with a diagnosis of MEN2A managed at these two institutions from 2005 were included. Relevant data were pooled together. In total, there were 12 unrelated families at QMH and 16 unrelated families at SNUH identified. All at-risk or affected individuals from these 27 families agreed and underwent genetic testing at their own respective institution and among them, 93 individuals turned out to have a positive germline *RET* mutation. There were no significant differences in the way *RET* was sequenced in the two institutions. Blood samples were obtained from at-risk family members by peripheral veni-puncture. Genomic DNA was prepared from peripheral blood leukocytes by standard procedures, and *RET* mutations were screened by restriction enzyme digestion and/or direct sequencing as described previously[8]. These mutations included C634 mutations (n=67), C611Y (n=1), C618S (n=6), C620A (n=2), C631Y (n=8), D898Y (n=3), E768D (n=1), P841L (n=1) and V804M (n=4). For the purpose of the present study, only those (n=67) carrying a C634 mutation were included in the analysis.

Clinical data such as age at which each component of MEN2A (i.e. MTC, PHEO, primary hyperparathyroidism) was diagnosed, sex, date of birth, family history, proband or not, the date at which the PHEO was detected, whether the PHEO was unilateral or bilateral, surgical management and its outcome, histopathology, genetic results and latest follow-up status were recorded from these patients. In order to obtain the latest disease and health status, at the time of analysis, an electronic medical record was performed at both institutions. The latest date of follow-up or date of death and the cause of death were retrieved from the search. All causes of death were confirmed by examination of the medical record, autopsy report and / or death certificate.

Screening of PHEO in asymptomatic *RET* carriers

Both institutions adopted a similar screening protocol for PHEO. Briefly, all patients carrying a known *RET* mutation underwent annual biochemical screening for PHEO by determination of plasma and/or 24-hour urinary fractionated catecholamines and metanephrines. If the level of either the plasma or 24-urinary fractionated catecholamines and metanephrines was elevated, a

computerized tomography (CT) / magnetic resonance imaging and 131I-metaiodobenzylguanidine (MIBG) or 18F-fluoro-dihyroxyphenylalanine (DOPA) positron emission tomography or any combination of these imaging modalities were done to localize the PHEO. A diagnosis of PHEO was made based on histology (if adrenalectomy had been done) or biochemical evidence of elevated levels of serum or 24-hour urinary fractionated catecholamines or metanephrines together with a positive adrenal imaging (if adrenalectomy had not been done). Similarly, bilateral PHEO was defined either by histology and / or by elevated 24-hour urinary catecholamines and metanephrines together with positive bilateral adrenal imaging (if adrenalectomy had not been done).

Surveillance of PHEO recurrence

Similar to asymptomatic carriers, all patients with a history of PHEO were followed up annually with either two sets of plasma or 24-hour urinary fractionated catecholamines and metanephrines. Localizing imaging was done when plasma or urinary catecholamines or metanephrines were elevated by more than 2-fold above the normal limit. Local recurrence was defined as increased urinary catecholamines / metanephrines concentration associated with a positive adrenal lesion at the previous adrenalectomy site.

Other definitions

Malignant PHEO was defined as a lesion which had invaded beyond the confine of the adrenal gland or had evidence of metastasis present in either lymph nodes or in sites where paraganglial tissue was not typically present (i.e. lungs or bone). Age-dependent penetrance of PHEO was calculated based on the duration from the date of birth to the date when a PHEO was first diagnosed. Penetrance of bilateral PHEO was calculated based on the duration from the date of birth to the date when either bilateral PHEO was diagnosed or when the remaining or contralateral adrenal gland became a PHEO (if one adrenal gland had been removed completely).

Statistical analysis

Age-dependent penetrance for a PHEO and bilateral PHEO were estimated by the Kaplan-Meier Method. Penetrance between mutations was compared by log-rank test. All statistical analyses were performed using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Among the 67 patients with C634 mutations, 17 (25.4%) patients were index while the other 50 (74.6%) were non-index *RET* gene carriers. Table 1 shows the patient characteristics of these 67 patients from the two institutions. The male to female ratio was 27:40 (or 1:1.5). At the time of analysis, the mean age was 43.4 ± 19.5 years old and 2 (3.5%) patients died of medical (i.e. non MEN-related) diseases. There were no MEN-related deaths. Of the 65 patients still alive, 4 patients suffered from metastatic MTC and 1 patient suffered from metastatic PHEO. Despite their metastases, all 5 patients had stable disease.

Table 2 compares clinical characteristics of PHEO by mutations. A total of 46 (68.7%) patients were diagnosed with PHEO and among them, 20 (43.5%) patients were asymptomatic on first presentation. PHEOs with C634Y mutation were significantly more likely to be symptomatic than other mutations (like C634R, C634G and C634W) (p=0.014) but other characteristics like bilateral involvement on presentation and tumor size were similar across all 4 mutations. Although it was not statistically significant, those with C634W had 3-15 times higher level of nor-metanephrines compared to other mutations.

PHEO diagnosis was made >6 months after diagnosis of MTC in 18 (39.1%), within 6 months in 23 (50.0%) and >6 months before MTC in 5 (10.9%). On first presentation of PHEO, 16 (34.8%) already had bilateral involvement while 30 (65.2%) were unilateral only. Of these 30 unilateral PHEOs, 10 (33.3%) subsequently developed contralateral PHEO after a mean (±SD) follow-up of 8.5 (±4.5) years. Therefore, at the time of analysis, 26 (56.5%) patients had bilateral PHEOs and 20 (43.5%) had unilateral PHEO. All patients with PHEO underwent unilateral or bilateral total adrenalectomy. Cortical-sparing adrenalectomy (CSA) was not performed in any of the patients. The laparoscopic approach was successful in 32 (47.8%) patients. Pathological examination confirmed PHEO in all patients. No patients suffered from extra-adrenal PHEO. To date, two (5.3%) patients were believed to have suffered from malignant PHEO. The first was a 21 years old female index patient with C634Y (from cysteine to tyrosine) mutation who presented with a huge PHEO and tumor thrombus invading into the inferior vena cava and in the right atrium. The patient is alive and has remained free of disease after successful resection. Details of this case had been described previously[11]. The second patient was also an index patient with C634R mutation who underwent an open right adrenalectomy outside our institution for a

10cm PHEO at age 32. She suffered local recurrence of PHEO 14.7 years later. Subsequent ^{131}I MIBG and DOPA scans confirmed right PHEO local recurrence with another separate uptake at T3 vertebra (see Figure 1). She subsequently underwent a right laparoscopic removal of a 3cm adrenal recurrence. Histology of the mass confirmed to contain PHEO tissue while the uptake in T3 vertebra was also confirmed to be metastatic PHEO (i.e. bone metastasis). Given these findings, the overall malignancy rate of PHEO in our cohort was 2/67 (3.0%).

Penetrance of PHEO

The overall PHEO penetrance was 20.8% by age 30 years, 53.8% by age 40 years, 76.5% by age 50 years and 95.2% by 80 years. The mean age of PHEO was 37.0 ± 12.6 years old. Within the cohort, 4 different amino acid substitution mutations were identified and they were C634R (n=19, 28.4%), C634Y (n=36, 38.8%), C634G (n=4, 6.0%) and C634W (from cysteine to tryptophan) (n=8, 11.9%). Figure 2 compares overall estimated penetrance between the 4 different mutations. At the age of 40 years old, using Kaplan-Meier analysis, the estimated penetrance rate of PHEO tended to be higher in C634R than C634Y, C634G and C634W (69.8% vs 55.2%, 25.0% and 56.2%, respectively) but this was not statistically significant (p=0.529 using log-rank). The mean age of developing first PHEO in C634R and C634Y were slightly younger than C634G and C634W (36.1 ± 11.4 years and 36.0 ± 11.3 years vs. 45.4 ± 9.6 years and 40.2 ± 21.1 years, respectively, p=0.334). When only the two most common substitutions were considered (C634R and C634Y), the estimated penetrance of PHEO were also not significantly different (p=0.518). Also, the estimated penetrance of PHEO between C634R and other non-C634R codons was not significantly different (p=0.299). Interestingly though, the youngest patient with a confirmed PHEO was a 11.8 years old male carrying a C634W mutation while the oldest patient with a PHEO was a 73.8 years old female patient also carrying a C634W mutation.

Penetrance of bilateral PHEOs

By age 40, the estimated penetrance of bilateral PHEOs in C634R, C634Y, C634G and C634W were 59.3%, 25.2%, 0.0% and 20.0%, respectively. They were not statistically different (p=0.155). However, the onset of developing bilateral PHEOs in C634R was significantly earlier than C634Y (p=0.046). Figure 3 compares the penetrance of bilateral PHEOs between C634R and C634Y mutations. By the age of 40 years old, the estimated penetrance of bilateral PHEOs in C634R was 59.3% and the risk in C634Y was 25.2%. However, by the age of 80 years old, the

estimated risk of bilateral PHEOs in C634R and C634Y approached each other (78.6% vs. 83.2%). Similarly, onset of bilateral PHEOs in C634R was significantly earlier than other C634 mutations combined (by age 40, 59.3% vs. 21.5%, *p*=0.024) but the age of 80 years old, they approached each other (78.6% vs. 100.0%). Figure 4 compares the estimated penetrance of bilateral PHEOs between C634R and non-C634R (i.e. C634Y, C634G and C634W combined) mutations**.** However, the onset of bilateral PHEO in C634R was not significant different from C634G (p=0.150) and C634W (p=0.110).

DISCUSSION

Our data showed that the estimated penetrance of PHEO due to codon 634 mutations reached almost 100% if a carrier could live until 80 years of age. By the age of 80 years, 95.2% of patients with codon 634 mutations would have developed a PHEO on at least one side. This finding is consistent with other populations[3,5,6]. A recent study from the MEN Consortium of Japan reported age-related penetrance of PHEO in carriers with a codon 634 mutation was 88% by the age of 77 years old[6]. Similarly, a high penetrance of PHEO (>80%) from Europe and the United States was reported in carriers with codon 634 mutations[12].

Our data also showed that there was no statistically significant difference in age-related penetrance of PHEO between C634R, C634Y, C634G and C634W (by age of 40, 69.8%, 55.2%, 25.0% and 56.2%, respectively) (p=0.529), even though the mean age of developing first PHEO in C634R and C634Y were slightly younger than C634G and C634W (36.1 ± 11.4 years and 36.0 ± 11.3 years vs. 45.4 ± 9.6 years and 40.2 ± 21.1 years, respectively, p=0.334). Interestingly, the latter finding was also observed in other studies focusing on C634 mutations[5,10,12-14]. A Japanese study[6] reported a significant earlier onset of PHEO in C634R than C634G/C634F ($p<0.01$) while a more recent Spanish study[10] also found the age-related penetrance of PHEO in C634R was higher than C634Y (by age 30, 50% vs. 16%, p=0.001). Another Spanish study found higher incidence of PHEO in C634R than C634Y and C634W mutations ($p<0.003$)[15], although this was not accumulative risk because the Kaplan-Meier method was not used.

One point worth highlighting was that despite being not statistically significant (because of the small number of patients), those with C634W had 3-15 times higher level of nor-metanephrines compared to other C634 mutations. Therefore, larger sized studies are required to confirm this. If confirmed, this could have important implications on intraoperative hemodynamics[16].

One of the most significant findings from our data was that the onset of developing bilateral PHEOs in C634R mutation was significantly earlier than those with C634Y (p=0.046) and those with other 3 non-C634R mutations combined (p=0.024). By the age of 40 years old, the risk of bilateral PHEOs in C634R mutation was more than two times higher than that of C634Y (59.3% vs. 25.2%, p=0.046) while it was also more than two times higher than that of the 3 other non-C634R mutations combined (59.3% vs. 21.5%, p=0.024). Although this age-related phenomenon

was not reported in more recent studies, an earlier study did find bilateral PHEOs to be more frequent in C634R than other non-C634R codons[6,10,15]. Nevertheless, it is important to note that the overall estimated penetrance or cumulative risk of bilateral PHEOs almost approached 100% regardless of which type of C634 mutations or amino-acid was substituted. In summary, therefore, although C634R mutation had significantly earlier onset of bilateral PHEOs, the cumulative risk of bilateral PHEOs was the same across all four C634 mutations. These findings have important implications on PHEO screening in MEN2A patients. For example, those with C634R mutation will benefit from earlier screening of contralateral PHEO than other C634 mutations after a unilateral adrenalectomy.

Another finding worth highlighting was that similar to other studies,[3,17] relative to sporadic PHEOs, both the risk of extra-adrenal tumors and malignancy appeared low in MEN2A patients. Our overall risk of malignant PHEO was 3.0% which appeared similar to larger series (1-3%)[3,6]. One reason for the low rates could be because these tumors tended to be diagnosed and managed earlier than sporadic tumors as almost half of our patient cohort (43.5%) with PHEO was relatively asymptomatic on presentation.

Clinical implications for surgeons

To avoid life-long corticosteroid replacement and risk of Addisonian crisis, CSA (instead of bilateral total adrenalectomy) has become increasingly popular in the treatment of MEN-related bilateral PHEOs. However, the argument against CSA is that this procedure usually entails sparing as much of the cortex while removing as much medulla as possible on one side and therefore, the remaining medulla may result in remnant recurrence in the longer term. Although CSA was not performed in our series, our data showed that PHEO recurrence occurs uncommonly and only needs reoperation after an extensive long (>10 years) period of time. Therefore, in our opinion, CSA is a worthwhile procedure that should be encouraged. This opinion concurs with the finding of a recent multinational population-based study that showed PHEO recurrence was not different between CSA and bilateral total adrenalectomy (3% vs. 2%, p=0.57) and 57% of patients who underwent CSA did not become steroid dependent[18].

However, despite these findings, this was a relatively small study limiting the power to identify smaller effects. Also these were based mostly from two institutions and so results were subjected to institutional biases. Also we did not exclude the possible effect of *RET* polymorphism on PHEO penetrance[19].

Conclusion

Our data showed that the overall penetrance or cumulative risk of developing PHEO and bilateral PHEOs was almost 100% irrespective of which of the 4 codon 634 mutations (C634R, C634Y, C634G or C634W). Although our data did not find statistically significant difference in age-related penetrance of PHEO between C634R, C634Y, C634G and C634W (by age of 40, 69.8%, 55.2%, 25.0% and 56.2%, respectively) (p=0.529), the onset of developing bilateral PHEOs in C634R mutation was significantly earlier than those with C634Y (p=0.046) and other 3 C634 mutations combined (p=0.024). These findings implied that those with C634R mutation might benefit from earlier screening of contralateral PHEO than other C634 mutations after a unilateral complete adrenalectomy.

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Table 1. Patient characteristics of our patient cohort (n=67)

Parameters	**Mean +/- SD or No. (%)**
Age at MEN2A diagnosis (years)	
- Mean ± SD	32.2 ± 16.5
- Median (range)	32.5 (4.5 – 74.0)
Age at the time of analysis (years)	
- Mean ± SD	43.4 ± 19.5
- Median (range)	46.7 (13.5 – 85.5)
Sex	
- Male	27 (40.3%)
- Female	40 (59.7%)
Mean follow-up since diagnosis of MEN2A (years)	14.0 ± 9.4
Overall penetrance of PHEO	46/67 (68.7%)
Mean size of right PHEO (cm)	4.6 ± 4.2
Mean size of left PHEO (cm)	2.9 ± 2.6
Unilateral PHEO at the time of analysis	20/67 (29.9%)
Bilateral PHEOs at the time of analysis	26/67 (38.8%)
Malignant PHEO at the time of analysis	2/67 (3.0%)
Extra-adrenal PHEO at time of analysis	0/67 (0.0%)
Timing of PHEO relative to diagnosis of MTC	
- >6 months after MTC	18/46 (39.1%)
- within 6 months of MTC	23/46 (50.0%)
- >6 months before MTC	5/46 (10.9%)
Codon 634 (in exon 11) mutations	
- C634R	19/67 (28.4%)

- C634Y	36/67 (38.8%)
- C634G	4/67 (6.0%)
- C634W	8/67 (11.9%)
Incidence of PHEO by codon 634 mutations	
- C634R	16/19 (84.2%)
- C634Y	22/36 (61.1%)
- C634G	2/4 (50.0%)
- C634W	6/8 (75.0%)
Incidence of bilateral PHEOs by codon 634 mutations	
- C634R	13/19 (68.4%)
- C634Y	10/36 (27.8%)
- C634G	1/4 (25.0%)
- C634W	2/8 (25.0%)

Abbreviations: MEN2A = multiple endocrine neoplasia type 2A; MTC = medullary thyroid carcinoma; PHEO = pheochromocytoma; C634R = from cysteine to arginine at position 634; C634Y = from cysteine to tyrosine at position 634; C634G = from cysteine to glycine at position 634; C634W = from cysteine into tryptophan at position 634

Table 2. A comparison of clinical characteristics of pheochromocytoma (n=46) by mutations

Variables	**C634R (n=16)**	**C634Y (n=22)**	**C634G (n=2)**	**C634W (n=6)**	***p*-value**
At presentation					
- Symptomatic (n=26)	8 (50.0)	17 (72.7)	0 (0.0)	1 (16.7)	**0.014**
- Unilateral (n=30)	8 (50.0)	17 (72.7)	1 (50.0)	4 (66.7)	0.354
- Bilateral (n=16)	8 (50.0)	5 (22.7)	1 (50.0)	2 (33.3)	0.152
Size of pheochromocytoma (cm)*	6.1 ± 3.3	4.8 ± 4.7	2.5 ± 1.0	4.1 ± 2.8	0.575
24-hour urinary catecholamines					
- Nor-epinephrine (N<440 nmol)	230.0 ± 130.8	290.7 ± 127.1	158.3 ± 41.9	432.7 ± 320.5	0.095
- Nor-metanephrine (N<240nmol)	346.5 ± 532.8	241.3 ± 91.8	100.3 ± 34.6	1582.6 ± 1826.4	0.134
- Epinephrine (N<110nnol)	327.2 ± 465.8	137.8 ± 124.0	197.0 ± 223.1	230.7 ± 269.1	0.345
- Metanephrine (N<145nmol)	1582.5 ± 3196.3	1053.0 ± 1712.3	211.7 ± 85.0	1470.7 ± 1660.9	0.432
- Dopamine (N<2570nmol)	2098.3 ± 1245.9	2615.9 ± 686.4	1833.3 ± 472.2	1685.7 ± 1066.0	0.674

* for bilateral cases, only the larger sized PHEO was recorded

LEGENDS

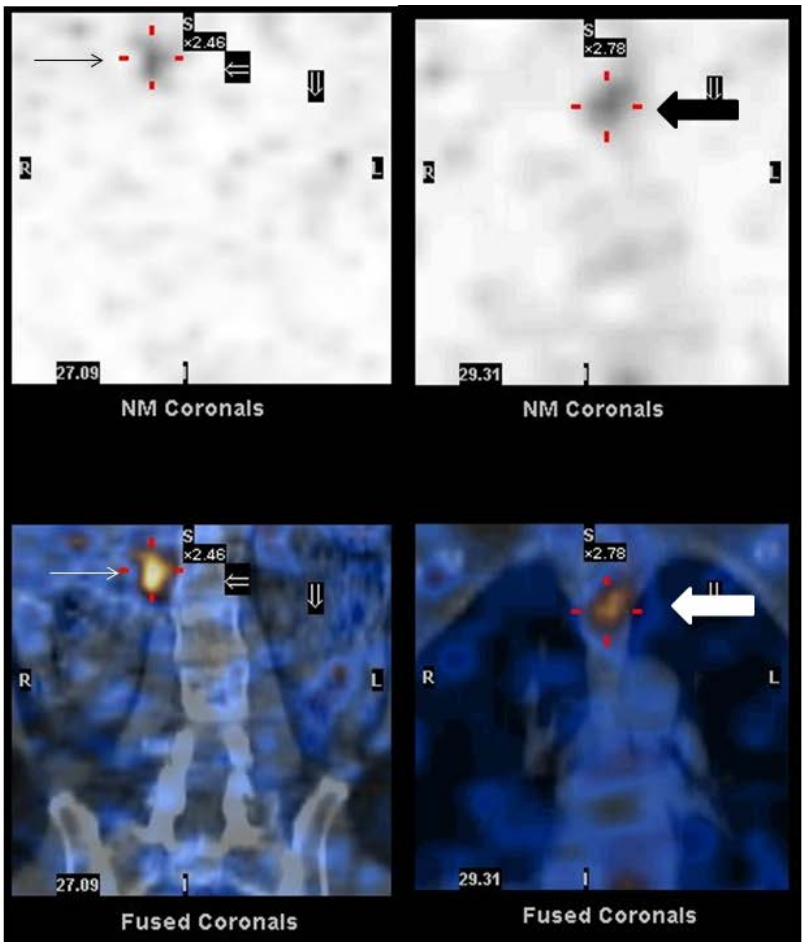

Figure 1. An index patient with C634R mutation who underwent an open right adrenalectomy for a 10cm PHEO at age 32 and developed PHEO recurrence 14.7 years later. 131I-MIBG scan confirmed right adrenal recurrence (*thin arrow*) and a solitary bone metastasis in T3 vertebra (*thick arrow*).

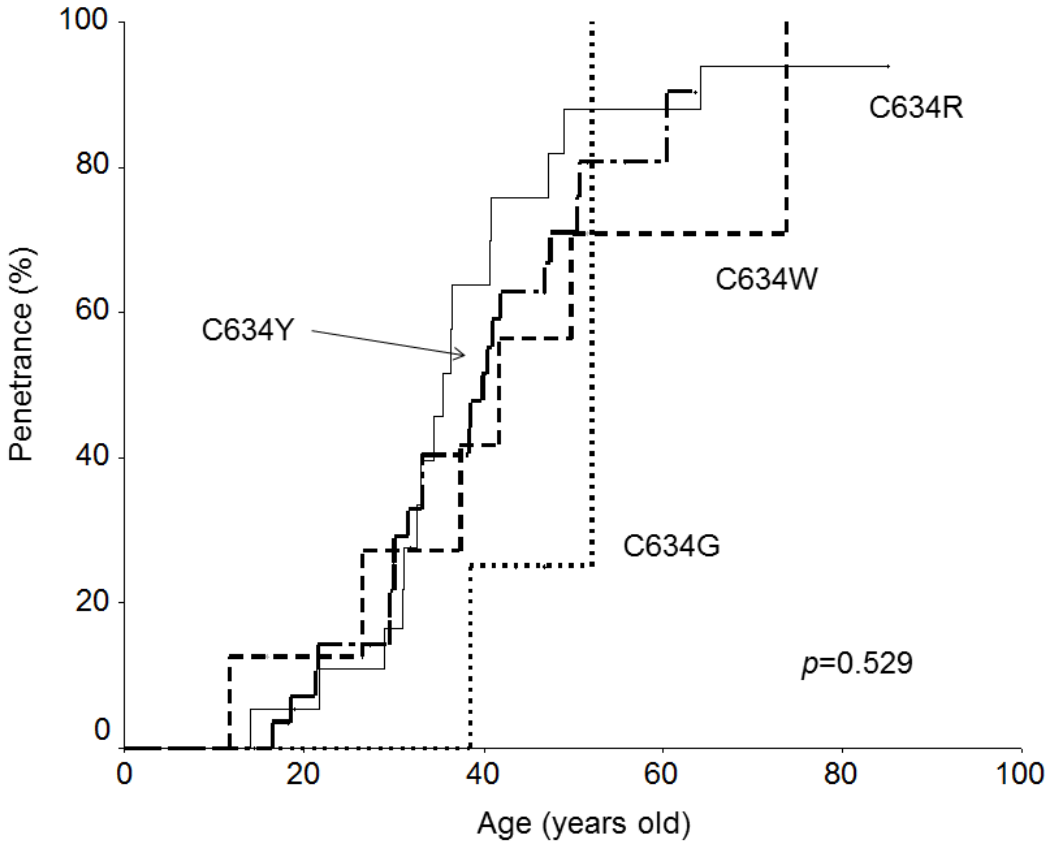

Figure 2. The age-related penetrance of PHEO between the 4 different mutations.

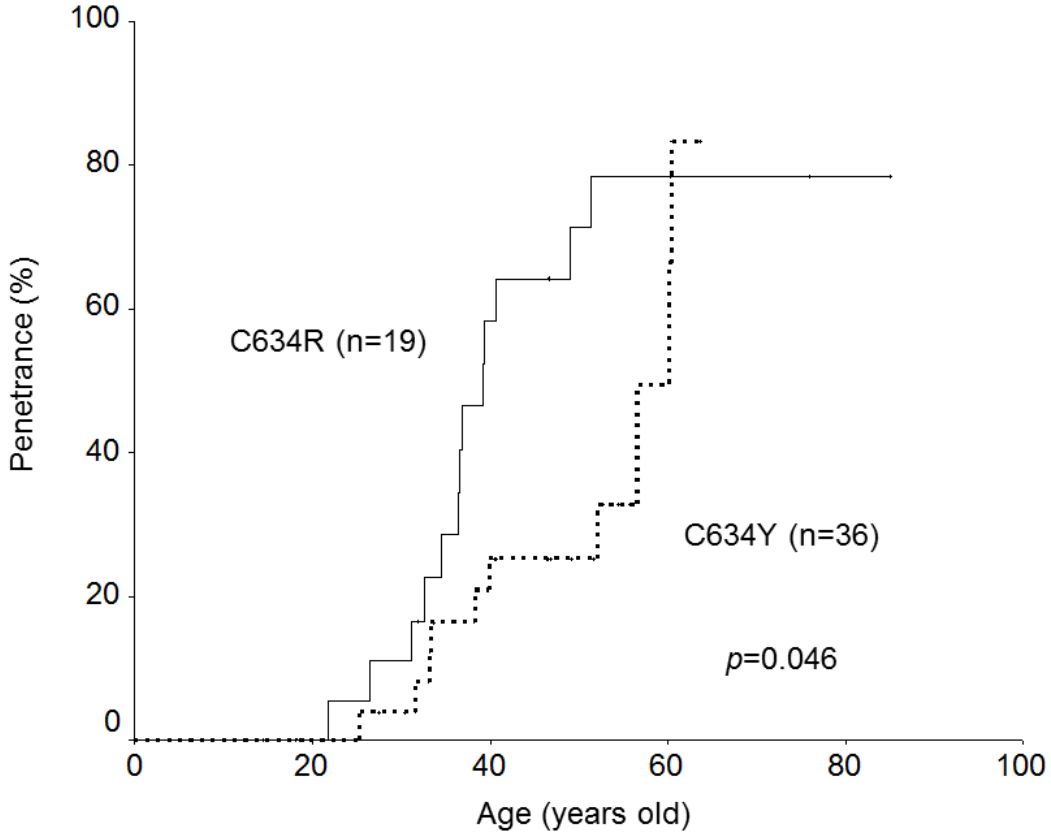

Figure 3. The age-related penetrance of bilateral PHEOs between C634R and C634Y mutations.

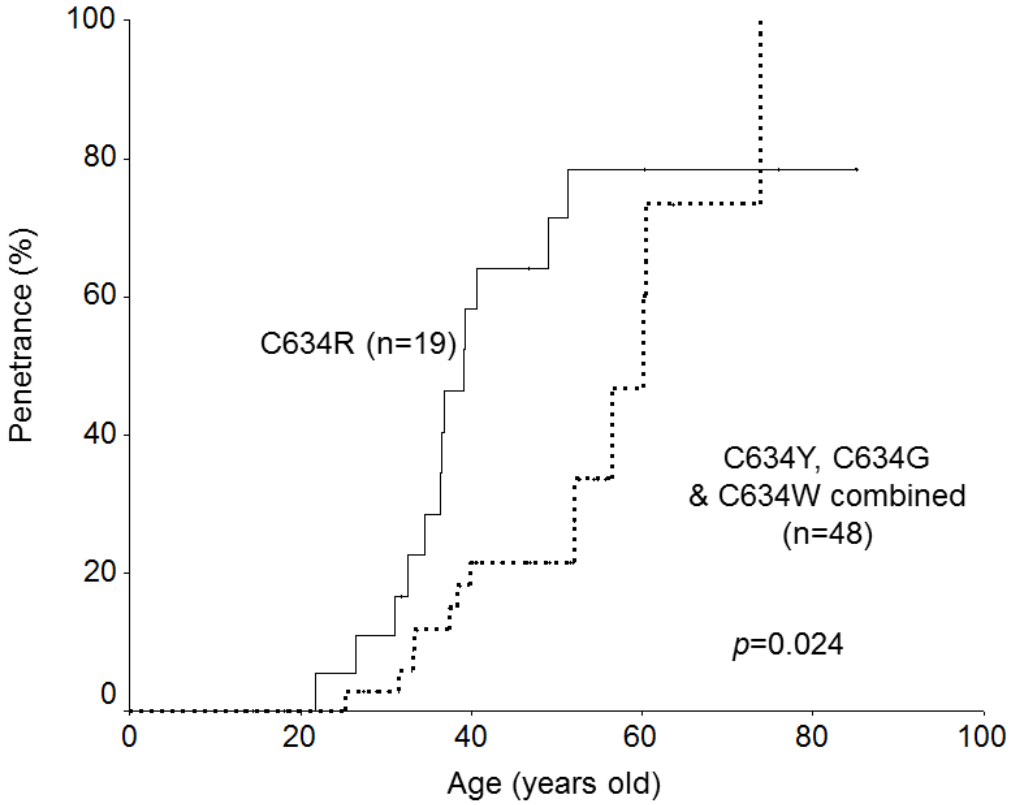

Figure 4. The age-related penetrance of bilateral PHEOs between C634R and non-634R mutations.