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ORIGINAL ARTICLE

Homozygous *TMEM127* mutations in 2 patients with bilateral pheochromocytomas

K. Eijkelenkamp[1] | M.J.W. Olderode-Berends[2] | R.B. van der Luijt[3] | M. Robledo[4] | M. van Dooren[5] | R.A. Feelders[6] | J. de Vries[7] | M.N. Kerstens[1] | T.P. Links[1] | A.N.A. van der Horst-Schrivers[1]

[1]Department of Endocrinology and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[2]Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[3]Laboratory for Clinical Molecular Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

[4]Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre, Madrid, Spain

[5]Department of Clinical Genetics, Erasmus University Center, Rotterdam, The Netherlands

[6]Department of Endocrinology and Metabolic Diseases, Erasmus University Center, Rotterdam, The Netherlands

[7]Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Correspondence
Anouk N.A. van der Horst-Schrivers, MD, Department of Endocrinology and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands.
Email: a.n.a.van.der.horst@umcg.nl

Pheochromocytoma (PCC) and paraganglioma (PGL) are rare neuroendocrine tumors that are hereditary in up to 50% of patients. The gene encoding transmembrane-protein-127 (*TMEM127*) is one of the PCC/PGL-susceptibility genes with an autosomal dominant inheritance pattern. Here, we report 2 patients with bilateral PCC who both harbored a homozygous *TMEM127*-mutation. In a 31-year-old mentally retarded patient, the homozygous c.410-2A > G mutation was discovered during an update of DNA analysis. A 26-year-old mentally retarded patient was found to have a homozygous c.3G > A mutation. The parents of both patients were consanguineous. We reviewed previously reported clinical features of *TMEM127* mutation carriers and compared our findings with case descriptions of homozygous mutations in other PGL/PCC-susceptibility genes. Homozygosity for an autosomal dominant inherited disorder is an extremely rare phenomenon and has, to our knowledge, not been reported before for the gene encoding *TMEM127*. In the present cases, the clinical picture does not seem to be very different from heterozygous *TMEM127* mutation carriers, except for a relatively large tumor size and more pronounced plasma metanephrine concentration. It is unclear whether the mental retardation is causally related to homozygosity of the *TMEM127* mutations. Updating genetic screening in patients in whom PCC/PGL has been diagnosed in the past should be considered as it might provide clinically relevant information.

KEYWORDS

homozygous, paraganglioma, pheochromocytoma, *TMEM127* mutation carriers

1 | INTRODUCTION

Pheochromocytoma (PCC) and paraganglioma (PGL) are rare neuroendocrine tumors that may be hereditary in up to 50% of patients. Genetically determined PCC/PGL should be especially suspected in case of a positive family history, bilateral localization or presentation at a young age.[1] The continuous evolving field of clinical genetics has - until today - identified more than 20 different susceptibility genes[2] that are classified into 2 clusters depending on their gene expression profile. Cluster 1 mutations are involved with the pseudo-hypoxic pathway and include mutations in the following genes: *von Hippel-Lindau (VHL), succinate dehydrogenase complex (SDHx), prolyl hydroxylase domain protein 2 (PHD2), isocitrate dehydrogenase (IDH), hypoxia-inducible factor 2a (HIF2α), malate dehydrogenase 2 (MDH2)* and *fumarate hydratase (FH).* Cluster 2 mutations are associated with abnormal activation of kinase receptor and signaling regulators, including mutations in the following genes; *rearranged during transfection (RET), neurofibromin 1 (NF1), kinesin family member 1B (KIF1B), Harvey rat sarcoma viral oncogene (H-ras), myc-associated factor X (MAX)* and *transmembrane protein 127 (TMEM127).*[1]

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We here describe 2 patients with hereditary bilateral PCC, who were found to have a pathogenic homozygous mutation in the *TMEM127* gene. To our knowledge, homozygous *TMEM127* germline mutations have not been reported before. In this case report, we aim to review the clinical picture of heterozygous *TMEM127* mutation carriers and compare their phenotype with that of the 2 patients encountered by us with homozygous *TMEM127* mutations. In addition, we describe the clinical features of homozygous carriers of other PGL-susceptibility genes to gain more insight into the pathophysiological pathways of PCC/PGL tumor development. Informed consent was obtained for both patients. According to the Dutch Medical Research Involving Human Subjects Acts, because we used existing clinical data already collected for regular patient care, no further Institutional Review Board approval was required.

1.1 | Case I

In the year 1997, a mentally retarded Caucasian 31-year-old man was diagnosed with bilateral PCC. He had been admitted to our hospital (the University Medical Center Groningen) because of hypertension (blood pressure 160/90 mmHg, heart rate 100/minute), with symptoms of headache, nausea and dizziness. Laboratory investigations revealed high concentrations of urinary metanephrines: metanephrine 10 948 μmol/mol creatinine (reference range: 33-99 μmol/mol creatinine), normetanephrine 19 089 μmol/mol creatinine (reference range: 64-160 μmol/mol creatinine). An abdominal Computed Tomography (CT) scan identified bilateral adrenal masses (left 4.5 × 3.0 cm, right 7.0 × 6.0 cm). Bilateral adrenalectomy (by laparotomy) was performed after preoperative treatment with α- and β-antagonists. Pathologic examination confirmed the presence of a PCC in the right adrenal gland with a size of 7.0 × 6.0 × 5.0 cm (weight: 120 g) without signs of hyperplasia and 4 small PCC foci in the left adrenal gland. Postoperatively, the excretion of urinary metanephrines normalized. In 2008, the patient was found to have a recurrent PCC localized in the area of the right adrenal gland for which he was successfully operated. Since then, he has been free of recurrent PCC. Despite a negative family history, a hereditary cause of the PCC was suspected because of his young age and the bilateral presentation. In 1998, DNA analysis did not identify a mutation in the *RET*, *VHL*, *SDHB* or *SDHD* genes. In 2014, an update of the DNA analysis was negative for a mutation in the *MAX* gene but revealed a mutation in the *TMEM127* gene (c.410-2A > G, p?). Remarkably, sequencing results suggested that the *TMEM127* mutation, an A-to-G base substitution at nucleotide position c.410-2 in intron 3, was present in either the homo- or the hemizygous state in the patient, since no wild-type (A-) allele could be observed in the corresponding DNA sequence trace (Figure 1).

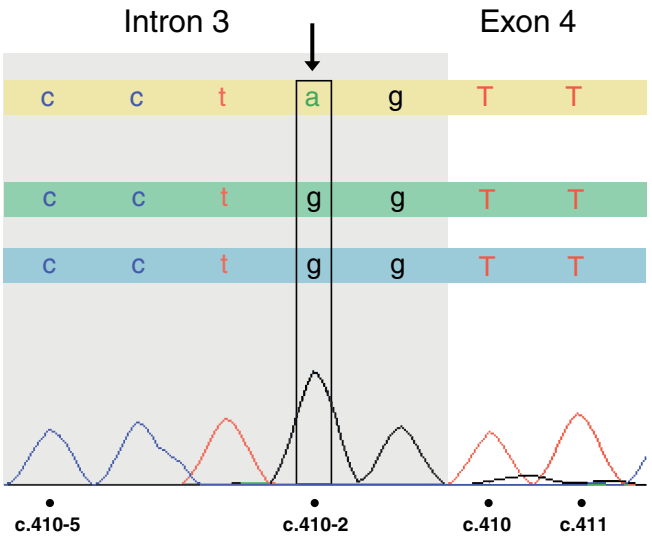

FIGURE 1 Shown is the nucleotide sequence (Sanger method) of the transmembrane protein 127 (*TMEM127*) gene between bases c.410-5 and c.411 of *TMEM127*. The DNA of the index patient shows homozygosity at position c.410-2 where the normal reference nucleotide (A) is replaced by a G, apparently on both *TMEM127* alleles. [Color figure can be viewed at wileyonlinelibrary.com]

A single-nucleotide polymorphism (SNP) array excluded a deletion on the other allele and showed various regions of homozygosity at other chromosomes. It was then confirmed by his mother that she and the patient's father were related, his mother did not know in which degree they were related. One of the homozygous regions was 24.4 Mb (chr2:95 395 757-119 772 352) and included the *TMEM127* gene. DNA mutation analysis was offered to the first-degree relatives of the patient (Figure 2). His mother, 1 sister and 1 brother were

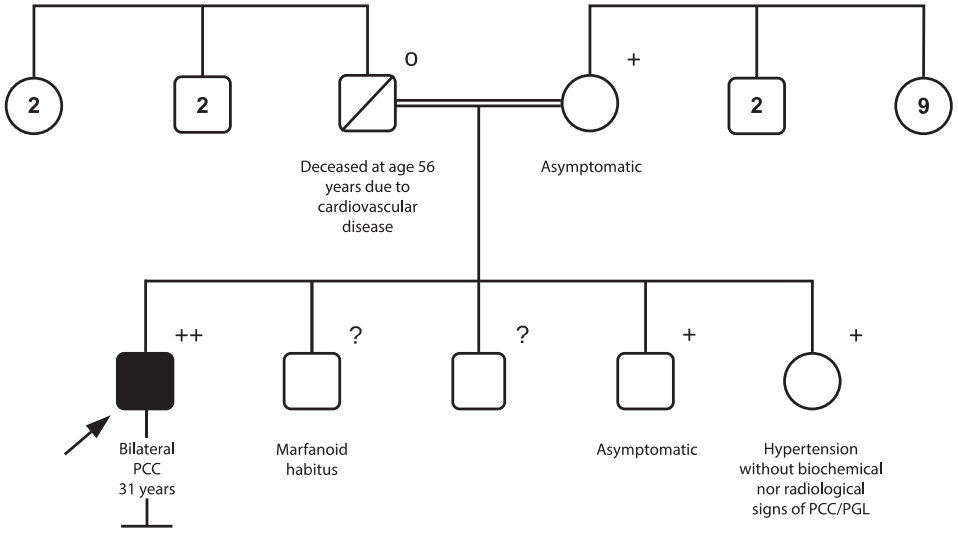

FIGURE 2 o = obligate carrier, + = carrier, ++ = double mutation,? = DNA analysis not performed.
PCC = pheochromocytoma, PGL = paraganglioma

positive for heterozygous *TMEM127* mutations. His father's DNA could not be examined as he had already died at the age of 56 years, probably due to an aortic dissection. His 79-year-old mother showed no biochemical signs of a PCC or PGL, she declined imaging. Neither his 53-year-old brother nor his 47-year-old sister showed any biochemical or radiological signs (on head and neck and thoracic-abdominal Magnetic Resonance Imaging [MRI]) compatible with PCC/PGL. Two other brothers declined genetic testing as well as any further clinical examinations (Figure 2).

The medical file of the index patient showed that he had distinct dysmorphic features as a child, including microcephaly, hypertelorism, frontal bossing and divergent strabismus diagnosed by the general practitioner. His psychomotor development had been delayed; he could walk by himself at 2 years of age and he spoke his first words at 4 years of age. From the age of 12 years, he experienced short attacks of absence lasting a few seconds, where he turned away his eyes and became pale. Several neurologic examinations including electroencephalograms did not reveal an explanation. Later on, he developed complaints of headache and 2 years before the first diagnosis of bilateral PCC he developed hypertension. It is likely that his mental retardation had contributed to a delayed diagnosis of the PCC, but it is unclear whether the mental retardation is causally related to the homozygous *TMEM127* mutation. Further genetic analysis of the other homozygous areas in order to find an explanation for the mental retardation and dysmorphic features was declined by his family.

1.2 | Case II

A mildly mentally retarded 26-year-old Turkish man presented with bilateral PCC in 2013. He had been admitted to another hospital because of episodes of headache, dizziness and profuse perspiration. He was found to have severe hypertension (218/127 mm Hg). Laboratory investigation revealed elevated urinary metanephrines: metanephrine 14 513 μmol/mol creatinine (reference range: 35-150 μmol/mol creatinine), normetanephrine 4749 μmol/mol creatinine (reference range: 60-260 μmol/mol creatinine). CT scan of the abdomen showed a lobulated mass of the left adrenal gland with maximum size of 11 cm and an enlarged right adrenal gland of 2.5 cm. He underwent surgery at the Erasmus Medical Center Rotterdam where a left-sided adrenalectomy and right cortex sparing surgery was performed after preoperative treatment with α- and β-antagonists. Bilateral PCC were confirmed histologically. Pathologic examination revealed a multinodular PCC in the left adrenal gland (14.5 × 11.0 × 7.0 cm) and a PCC in the right adrenal gland (2.1 × 1.8 × 1.4 cm) with signs of hyperplasia of the adrenal medulla. Excretion of urinary metanephrines normalized postoperatively and the patient remained free of recurrent disease during follow-up.

The family history revealed consanguinity of the parents, who were cousins of each other. There were neither other family members with paragangliomas and/or developmental problems nor congenital anomalies (Figure 3). His father had been operated for a parathyroid gland adenoma at the age of 43 years. Because of the patient's mental retardation and because of the consanguinity of his

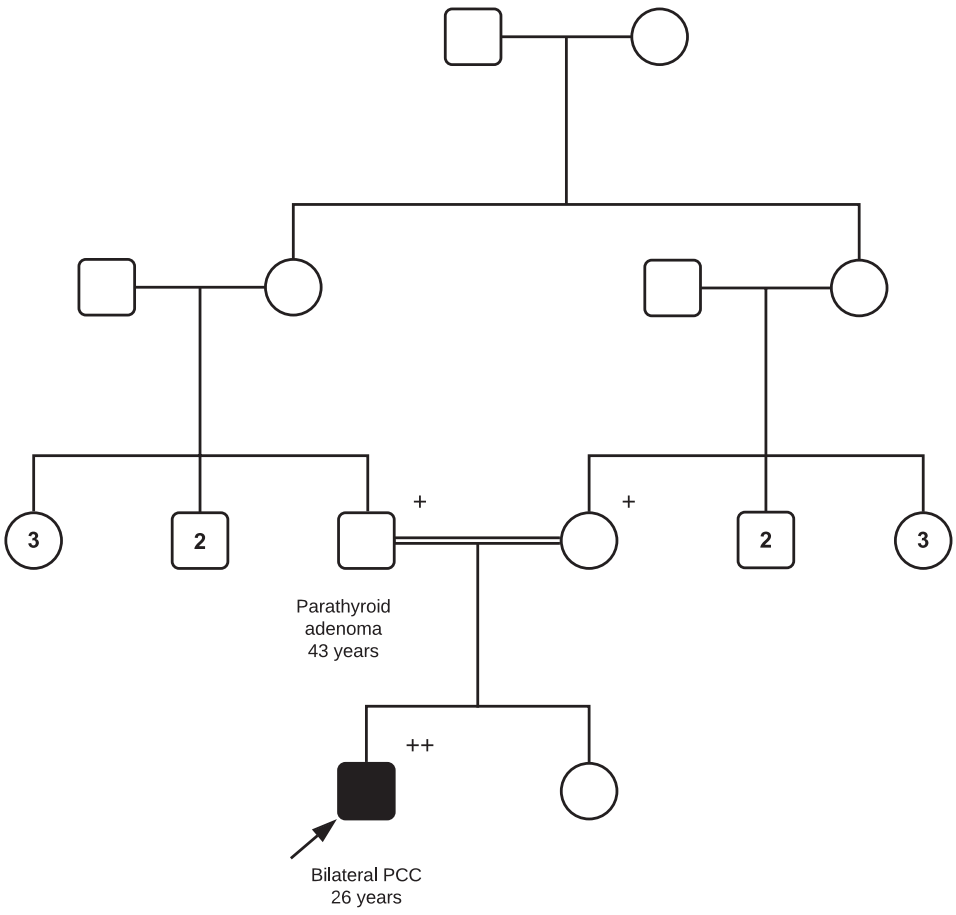

FIGURE 3 + = carrier, ++ = double mutation, PCC = pheochromocytoma

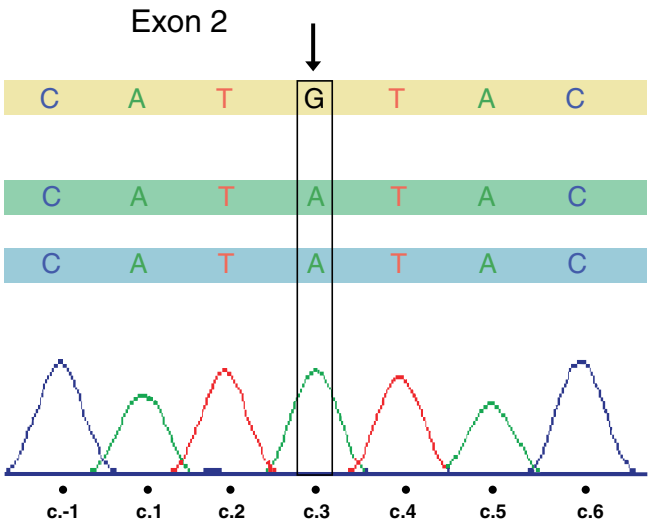

FIGURE 4 Shown is the nucleotide sequence (Sanger method) of the transmembrane protein 127 (*TMEM127*) gene between bases c.-1 and c.6 of *TMEM127*. The DNA of the index patient shows homozygosity at position c.3, where the normal reference nucleotide (G) is replaced by an A apparently on both *TMEM127* alleles. [Color figure can be viewed at wileyonlinelibrary.com]

parents, an SNP array analysis was performed, showing a normal male profile with large regions of homozygosity. One of the genes in this region was *TMEM127*. Sanger sequencing of the *TMEM127* gene showed a homozygous variant of uncertain clinical significance c.3G > A, p.(Met1?) (*chr2:94 900 727-100 417 149 (5 Mb)*) (Figure 4). Both parents were recently identified as heterozygous carriers of this variant in *TMEM127*. They had no suggestive clinical signs or symptoms, diagnostic analysis for PCC was not performed. Immunohistochemistry of the tumor showed normal staining of the *SDHA* and *SDHB* proteins. Next-generation targeted sequencing in tumor tissue with a filter for genes involved in PCC and PGL (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *VHL*, *KIF1B*, *NF1*, *RET*, *HIF2α*, *MAX*, *TMEM127* and *PHD2*) revealed also a homozygous variant in *TMEM127* (c.3G > A p. (Met1?)). There were no abnormalities in other genes.

2 | DISCUSSION

The relationship between germline mutations in the *TMEM127* gene and the development of PCC/PGL was first described in 2010 by Qin et al.[3] The *TMEM127* gene is located at chromosome 2q11.2 and encodes a transmembrane endosomal protein of 238 amino acids. Microarray analysis of *TMEM127* mutations revealed a cluster 2 transcriptome, associated with increased kinase receptor signals. This results in inhibition of mammalian target of rapamycin (mTOR), an important regulator of protein synthesis and cell survival.[3] *TMEM127* thus acts as a tumor suppressor gene. *TMEM127* germline mutations are transmitted in an autosomal dominant fashion.[4] Yao et al already hypothesized a low penetrance since only a 1 out of 4 of the cases described, had a clear positive family history, suggesting a penetrance much lower than 100%.[5] Toledo et al estimated a cumulative penetrance varying from 0% at 0 to 20 years to 32% at 51 to 65 years.[4]The parents of both patients here described, as well as the siblings of the first patient, were asymptomatic carriers, which is also compatible with a low penetrance. In the study of Yao et al *TMEM127* mutations were identified in 2% (20/990) of the patients with a PCC.[5] Until now, 104 *TMEM127* germline mutation carriers have been described in the literature (Table 1). The majority of these mutation carriers develop (bilateral) PCC, but extra-adrenal PGL and head and neck paraganglioma (HNPGL) may also occur. According to the current literature, the median age at presentation of disease is 44 years, with a range from 16 to 80 years of age.[3–19] This is clearly older than the median age at diagnosis in other PCC/PGL-associated germline mutation carriers and is more comparable with the age distribution of sporadic PCC/PGL. Malignant PCC/PGL, defined as the presence of metastases, seems to be very rare.[4,11] Renal cell carcinomas, however, have been described in patients with *TMEM127* germline mutations.[12,15,19] The optimal follow-up and surveillance for mutation carriers has not been established yet. In the Netherlands, *TMEM127* mutation carriers are periodically examined according to the familial paraganglioma surveillance protocol including annual measurement of plasma metanephrines and MRI scanning of head and neck region once every 3 years (Dutch guideline for detecting hereditary tumors 2017, www.stoet.nl). Since the majority of *TMEM127* mutation carriers develop a bilateral PCC which is in accordance to patients with multiple endocrine neoplasia type 2, adrenal-sparing surgery should be considered.[20]

Both *TMEM127* mutations observed in our patients are predicted to present loss-of-function mutations and are highly likely to have a deleterious character. Either because they lead to a so-called null allele (ie, no *TMEM127* protein produced at all) or because they result in the synthesis of a truncated, non-functional *TMEM127* protein. The c.410-2A > G p.(?) mutation (Case I) is located near the natural splice donor acceptor site of exon 3. The substitution abolishes this splice site, while strongly activating a nearby cryptic splice acceptor site, located at position c.418 within exon 4. Use of this cryptic splice acceptor site is expected to lead to the deletion of the first 8 bases from exon 4 in the mutant transcript. This deletion creates a frame shift starting at codon Leu138. The new reading frame ends in a stop codon, 11 positions downstream. Because the premature stop codon would be located in the last exon of the *TMEM127* gene, non-sense-mediated mRNA-decay is unlikely to occur. We therefore postulate that the splice-site mutation would result in the synthesis of a C-terminally truncated, non-functional *TMEM127* protein. The mutant protein could contain the normal N-terminal amino acids 1 until 137, followed by 11 missense amino acids. The shortened, 148-amino acid protein would lack the normal N-terminal amino acids 138 until 238. It is noteworthy that the effect of this c.410-2A > G p.(?) mutation on the natural and cryptic splice sites is predicted by all of the 5 splice prediction programs tested (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder). An identical effect on splicing is expected for the proven pathogenic *TMEM127* mutation previously reported by others.[2] The mutation c.410-2A > G p.(?) has not been described in literature before, although a mutation in the same nucleotide, but in a different substitution (ie, c.410-2A > C and c.410-1G > C) has been reported in 14 *TMEM127* carriers.[4,5,18] The clinical features of these carriers are shown in Table 2.

TABLE 1 Studies describing the clinical phenotype of heterozygous *TMEM127* mutation carriers

Author	Number of carriers	Age in years (range)[a]	PCC (bilateral)	sPGL	HNPGL	Malignant	Other neoplasms	Biochemical profile[b]		Tumor size (PCC/PGL)
								MN	NMN	
Qin et al (2010)[3]	7	43 (25-72)	7 (4)	0	0	0	-	-	-	-
Yao et al (2010)[5]	13[c]	43 (21-61)	13 (3)	0	0	1	Breast carcinoma, papillary thyroid carcinoma	-	-	-
Neumann et al (2011)[6]	2	34, 51	1 (1)	1	1	0	AML	13.3	1.5	-
Burnichon et al (2011)[7]	1	20	1 (1)	0	0	0	-	-	-	4.0 × 2.6 cm and 15.0 × 5.0 cm
Elston et al (2012)[8]	1	33	1	0	0	0	-	8	2.5	4.0 × 3.6 cm
Takeichi et al (2012)[9]	2	40, 48	2 (2)	0	0	0	-	17	3.7	2.9 × 3.7 cm and 2.5 × 1.3 cm, 12 × 2.0 cm
Abermil et al (2012)[10]	6	43 (16-80)	6 (1)	0	0	0	-	Range: 2.6-18	Range: 1.3-22	-
Lefebvre and Foulkes (2014)[11]	5	46 (16-80)	5	0	0	2	-	-	-	-
Toledo et al (2015)[4]	34	43 (22-55)	11 (5)	0	0	0	-	-	-	Median: 1.0 cm, range: 0.11-10.5 cm
Qin et al (2014)[12]	4	47 (45-67)	0	0	0	0	RCC, breast carcinoma, prostate carcinoma	-	-	-
Welander et al (2014)[13]	1	55	1	0	0	0	-	-	-	5.5 × 4.0 × 2.5cm
Curras-Freixes et al (2015)[14]	2	28, 33	2	0	0	0	-	-	-	-
Hernandez et al (2015)[15]	1	47	1	0	0	0	RCC	2.8	1.2	5.2 × 5.1cm
Patocs et al (2016)[16]	3	40 (22-51)	3 (2)	1	1	0	-	-	-	-
Saitoh et al (2017)[17]	1	42	1	0	0	0	-	19	0.4	6.0 × 4.0cm
Bausch et al (2017)[18]	21	47 (18-76)	20 (10)	2	6	3	AML, pancreatic adenocarcinoma, melanoma, colon carcinoma	-	-	-
Deng et al (2017)[19]	1	47	1	0	0	0	RCC	-	-	-

Abbreviations: AML, acute myeloid leukemia; HNPGL, head and neck paraganglioma; MN, metanephrines, NMN, normetanephrines; PCC, pheochromocytoma; RCC, renal cell carcinoma; sPGL, sympathetic paraganglioma.

[a] Median age at diagnosis.

[b] Urinary metanephrines, ratio of measured value divided by upper reference level.

[c] Yao et al without cohort of Qin et al.

TABLE 2 Clinical features of carriers harboring a heterozygous *TMEM127* c.410-2A > C mutation[4,5,18]

	Sex	Age at diagnosis (y)	Hypertension	Location PGL	Bilateral	Malignant
1 (index)	F	33	+	PCC	+	–
2	F	43	+	PCC	+	–
3	M	52	+	PCC	+	–
4	M	47	+	PCC	–	–
5	F	54	–	PCC	–	–
6	M	37	+	PCC	+	–
7	M	47	+	PCC	+	–
8	F	35	–	PCC	–	–
9	F	55	–	PCC	–	–
10	F	33	+	PCC	–	–
11	M	22	+	PCC	–	–
12	F	34	ND	PCC	+	–
13	F	37	ND	PCC	–	–
14[a]	M	45	ND	PCC	+	–

Abbreviations: F, female; M, male; ND, not described; PCC, pheochromocytoma; PGL, paraganglioma.

[a] c410-1G > C mutation.

The c.3G > A p.(Met1?) mutation (Case II) is located at the first methionine codon in the *TMEM127* protein coding sequence, which represents the translation initiation codon in the open reading frame. The G-to-A nucleotide substitution replaces the ATG (methionine) codon by ATA in exon 2, the latter triplet encoding the amino acid isoleucine. Importantly, because the translation initiation codon is lost, no full-length *TMEM127* protein is expected to be produced from the mutant allele. Either does this mutation lead to a so-called null allele (no protein synthesized) or to the use of the next ATG (methionine) codon in the normal open reading frame as the translation initiation codon. In the wild-type protein reference sequence, the second in-frame methionine codon is located at amino acid position 85 in exon 3. Use of this methionine would result in the synthesis of an N-terminally truncated *TMEM127* protein which lacks the first 84 amino acids. In either case, no full-length, functional *TMEM127* protein is likely to be synthesized from alleles harboring the c.3G > A p.(Met1?) mutation. This mutation c.3G > A p.(Met1?) is not described in the ExAC data set which contains more than 60 706 unrelated individuals. This supports a pathogenic nature of the c.3G > A p.(Met1?) mutation, well as the fact that this mutation has been recently described in 4 patients.[18]

To our knowledge, homozygous *TMEM127* germline mutations have not been reported before. Our findings raise the question to which extent the phenotype differs between patients harboring a heterozygous or a homozygous *TMEM127* germline mutation. The homozygous *TMEM127* mutation in these 2 patients did not result in a more severe clinical picture or earlier presentation of PCC/PGL. However, compared to heterozygous *TMEM127* mutation carriers, both our cases demonstrated a more markedly elevated excretion of urinary metanephrines and 1 patient harbored a relatively large-sized PCC (Table 1). The age at diagnosis of the present cases was not different from the reported age distribution in heterozygous mutation carriers. Remarkably, both patients suffered from mental retardation. It remains unclear, however, whether mental retardation is causally related to homozygosity of the *TMEM127* gene. Mental retardation occurs in up to 50% of the children of consanguineous parents and might therefore also be explained by homozygosity of other DNA regions. The dysmorphic features of the first patient also raise the question whether these might be associated with homozygosity of the *TMEM127* mutation. Notably, the second patient did not have any dysmorphic features. Unfortunately, the family of both patients declined further genetic research. The fact that the endocrine phenotype does not differ much from heterozygous mutation carriers could theoretically be explained by imprinting; however, this phenomenon has not been described thus far for mutations of *TMEM127*. Moreover, chromosomal region 2q11.2 which harbors the *TMEM127* locus is not known as an imprinting region. The genealogy of Toledo et al did not support a parent-of-origin inheritance effect.[4] In view of loss of *TMEM127* leads to increased mTOR signaling[3] contributing to the pathogenesis of PCC and PGL, one would expect a more severe presentation in our 2 cases. However, a recent study by Oudijk et al showed a relatively low expression of the mTOR1C pathway in tumor tissue,[21] implying that other until now unknown factors are important in the pathogenesis.

In contrast to our patients, homozygous or compound heterozygous carriers of mutations in other PCC/PGL-susceptibility genes demonstrated a severe clinical picture (Table 3). Homozygous mutations of the *SDHx* and *FH* genes result in neurodegenerative

TABLE 3 Clinical presentation homozygous germline mutations in PCC/PGL susceptibility genes

	Clinical features
SDHA	Leigh syndrome[22]
SDHB	Leukodystrophia with clinical signs of hypotonia[23]
SDHD	Encephalomyopathy[24]
VHL	Erythrocytosis, Chuvash polycythemia[25]
FH	Neurodegeneration, dystonia, seizures[23]
RET	Medullary thyroid carcinoma[26]

Abbreviations: FH; fumarate hydratase, PCC; pheochromocytoma, PGL; paraganglioma; RET; rearranged during transfection; SDH (A, B, D); succinate dehydrogenase subunit A, B, D; VHL; von Hippel Lindau.

disorders.[23] Homozygous *SDHA* mutations may result in Leigh syndrome, a progressive neurodegenerative disorder that is characterized by subacute necrotizing encephalomyelopathy during infancy resulting in epilepsy, psychomotor retardation and spasticity.[22] Alston et al reported a patient with a homozygous *SDHB* mutation (c.143A > T; pAsp48Val) presenting with neurological complications of leukodystrophia with clinical signs of hypotonia due to accumulated succinate demonstrated by magnetic resonance spectroscopy.[22] Homozygous *SDHD* mutations have been associated with encephalomyopathy.[24] Furthermore, homozygous mutations in the *FH* gene may result in neurodegenerative disorders, such as progressive encephalopathy with dystonia and seizures.[23] Homozygous mutations in the *VHL-gene* have been associated with congenital polycythemia.[25] Lecube et al described 4 patients with homozygous *RET* mutation, 2 patients presented with medullary thyroid carcinoma.[26] To our knowledge, homozygous mutations in the *PHD2*, *IDH*, *HIF2α*, *MDH2*, *MAX*, *H-RAS*, *KIF1B* genes have not been described.

In conclusion, homozygosity for an autosomal dominant disorder is an extremely rare phenomenon and has not been reported previously for the gene encoding *TMEM127*. The clinical picture in these 2 patients seems to be different from heterozygous mutation carriers with respect to biochemical profile and tumor size, but it is not accompanied by a younger age at diagnosis. It is unclear whether the mental retardation in both cases is causally related to the homozygous *TMEM127* mutation. The present case furthermore illustrates the importance of updating genetic screening in patients who were diagnosed with a PCC/PGL in the past.

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Conflict of interest

The authors do not have any financial or other conflict of interest to declare.

ORCID

K. Eijkelenkamp http://orcid.org/0000-0003-3597-1803

REFERENCES

1. Pillai S, Gopalan V, Smith RA, Lam AK. Updates on the genetics and the clinical impacts on phaeochromocytoma and paraganglioma in the new era. *Crit Rev Oncol Hematol*. 2016;100:190-208.
2. Toledo A, Burnichon N, Cascon A, et al. Consensus statement on next-generation-sequencing-based diagnostic testing of hereditary phaeochromocytomas and paragangliomas. *Nat Rev Endocrinol*. 2017;13(4):233-247.
3. Qin Y, Yao L, King EE, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat Genet*. 2010;42(3):229-233.
4. Toledo SP, Lourenco DM, Sekiya T Jr, et al. Penetrance and clinical features of pheochromocytoma in a six-generation family carrying a germline TMEM127 mutation. *J Clin Endocrinol Metab*. 2015;100(2):E308-E318.
5. Yao L, Schiavi F, Cascon A, et al. Spectrum and prevalence of FP/-TMEM127 gene mutations in pheochromocytomas and paragangliomas. *JAMA*. 2010;304(23):2611-2619.
6. Neumann HP, Sullivan M, Winter A, et al. Germline mutations of the TMEM127 gene in patients with paraganglioma of head and neck and extraadrenal abdominal sites. *J Clin Endocrinol Metab*. 2011;96(8):E1279-E1282.
7. Burnichon N, Lepoutre-Lussey C, Laffaire J, et al. A novel TMEM127 mutation in a patient with familial bilateral pheochromocytoma. *Eur J Endocrinol*. 2011;164(1):141-145.
8. Elston MS, Meyer-Rochow GY, Prosser D, Love DR, Conaglen JV. Novel mutation in the TMEM127 gene associated with phaeochromocytoma. *Intern Med J*. 2013;43(4):449-451.
9. Takeichi N, Midorikawa S, Watanabe A, et al. Identical germline mutations in the TMEM127 gene in two unrelated Japanese patients with bilateral pheochromocytoma. *Clin Endocrinol (Oxf)*. 2012;77(5):707-714.
10. Abermil N, Guillaud-Bataille M, Burchinon N, et al. TMEM127 screening in a large cohort of patients with pheochromocytoma and/or paraganglioma. *J Clin Endocrinol Metab*. 2012;97(5):E805-E809.
11. Lefebvre M, Foulkes WD. Pheochromocytoma and paraganglioma syndromes: genetics and management update. *Curr Oncol*. 2014;21(1):e8-e17.
12. Qin Y, Deng Y, Ricketts CJ, et al. The tumor susceptibility gene TMEM127 is mutated in renal cell carcinomas and modulates endolysosomal function. *Hum Mol Genet*. 2014;23(9):2428-2439.
13. Welander J, Andreasson A, Juhlin C, et al. Rare germline mutations identified by targeted next-generation-sequencing of susceptibility genes in pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab*. 2014;7:E1352-E1360.
14. Curras-Freixes M, Inglada-Perez L, Mancikova V, et al. Recommendations for somatic and germline genetic testing of single pheochromocytoma and paraganglioma based on findings from a series of 329 patients. *J Med Genet*. 2015;52:647-656.
15. Hernandez KG, Ezzat S, Morel CF, et al. Familial pheochromocytoma and renal cell carcinoma syndrome: TMEM127 as a novel candidate gene for the association. *Virchows Arch*. 2015;466(6):727-732.
16. Patocs A, Lendvai NK, Butz H, et al. Novel SDHB and TMEM127 mutations in patients with pheochromocytoma/paraganglioma syndrome. *Pathol Oncol Res*. 2016;22(4):673-679.
17. Saitoh K, Yonemoto T, Usui T, et al. Novel germline variant of TMEM127 gene in a patient with familial pheochromocytoma. *Endocrinol Diabetes Metab Case Rep*. 2017;2017.17-0014.
18. Bausch B, Schiavi F, Ni Y, et al. Clinical characterization of the pheochromocytoma and paraganglioma susceptibility genes SDHA, TMEM127, MAX, and SDHAF2 for gene-informed prevention. *JAMA Oncol*. 2017;3(9):1204-1212.
19. Deng Y, Flores SK, Cheng Z, et al. Molecular and phenotypic evaluation of a novel germline TMEM127 mutation with an uncommon clinical presentation. *Endocr Relat Cancer*. 2017;24(11):L79-L82.
20. Castinetti F, Taieb D, Henry JF, et al. Management of endocrine disease: outcome of adrenal sparing surgery in heritable pheochromocytoma. *Eur J Endocrinol*. 2016;174(1):R9-18.
21. Oudijk L, Papathomas T, de Krijger R, et al. mTORC1 complex is significantly over-activated in SDHX-mutated paragangliomas. *Neuroendocrinology*. 2017;105(4):384-393.
22. Alston CL, Davison JE, Meloni F, et al. Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. *J Med Genet*. 2012;49(9):569-577.
23. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. *Nat Rev Cancer*. 2003;3(3):193-202.
24. Jackson CB, Nuoffer JM, Hahn D, et al. Mutations in SDHD lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. *J Med Genet*. 2014;51(3):170-175.
25. Pastore Y, Jedlickova K, Guan Y, et al. Mutations of von Hippel-Lindau tumor-suppressor gene and congenital polycythemia. *Am J Hum Genet*. 2003;73(2):412-419.

26. Lecube A, Hernandez C, Oriola J, et al. V804M RET mutation and familial medullary thyroid carcinoma: report of a large family with expression of the disease only in the homozygous gene carriers. *Surgery*. 2002;131(5):509-514.
27. Papathomas TG, Duregon E, Korpershoek E, et al. Sarcomatoid adrenocortical carcinoma: a comprehensive pathological, immunohistochemical, and targeted next-generation sequencing analysis. *Hum Pathol*. 2016;58:113-122.
28. Papathomas TG, Oudijk L, Persu A, et al. SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a Multinational Study of the European Network for the Study of Adrenal Tumors (ENS@T). *Mod Pathol*. 2015;28(6):807-821.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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