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R46Q Mutation in the Succinate Dehydrogenase B Gene (*SDHB*) in a Japanese Family with both Abdominal and Thoracic Paraganglioma Following Metastasis

KAZUHIRO TAKEKOSHI, KAZUMASA ISOBE, HIROAKI SUZUKI*, SUMIKO NISSATO, YASUSHI KAWAKAMI, KOICHI KAWAI** AND NOBUHIRO YAMADA*

Molecular Laboratory Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8575, Japan

**Department of Internal Medicine (Endocrinology and Metabolism), Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8575, Japan*

***Tsukuba Diabetes Center, Kawai Clinic, Tsukuba*

Abstract. Recently, nuclear genes encoding two mitochondrial complex II subunit proteins, *SDHD* and *SDHB*, have been found to be associated with the development of familial pheochromocytomas and paragangliomas (hereditary pheochromocytoma/paraganglioma syndrome: HPPS). Growing evidence suggests that a mutation of *SDHB* is highly associated with abdominal (or thoracic) paraganglioma and the following distant metastasis (malignant paraganglioma). Previously, we identified a novel heterozygous G to A point mutation at the first base of intron 3 of the *SDHB* gene (IVS3+1G>A) in a malignant abdominal paraganglioma from a Japanese patient. In the present study, we report another case of *SDHB* mutation (R46Q) in a Japanese patient with both abdominal and thoracic paraganglioma following malignant metastasis. In addition, we identified an asymptomatic carrier of *SDHB* mutation in this family. Our report highlights the pathogenic role of the *SDHB* mutation (R46Q) in malignant paraganglioma. We also discuss the desired protocol that should be adopted to follow up an asymptomatic carrier of this mutation.

Key words: SDHB, Paraganglioma, Malignant pheochromocytoma

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IT has been recognized that pheochromocytomas are component tumors of von Hippel Lindau- disease, multiple endocrine neoplasia type 2 and neurofibromatosis type 1, occurring as a result of germline mutations in *VHL*, *RET* or *NF1*, respectively. More recently, nuclear genes encoding two mitochondrial complex II subunit proteins, *SDHD* and *SDHB*, have been associated with the development of familial pheochromocytomas and paragangliomas (hereditary pheochromocytoma/paraganglioma syndrome: HPPS) [1, 2]. Growing evidence suggests that a mutation of *SDHB* is highly associated with abdominal (or thoracic) paraganglioma and the following distant metastasis (malignant paraganglioma) [3–8]. Indeed, it has been found that malignant pheochromocytoma/paraganglioma is associated with 38 to 83% of patients with *SDHB* germline mutations, indicating a much higher rate of malignancy compared with other mutations or sporadic adrenal pheochromocytoma where the rate is <10%. Conversely, among all patients with malignant pheochromocytoma/paraganglioma the frequency of *SDHB* mutations is reported to be around one third, suggesting that *SDHB* mutations in these malignant tumors may not be as rare as expected [9]. Previously, we identified a novel heterozygous G to A point mutation at the first base of intron 3 of the *SDHB* gene (IVS3+1G>A) in a malignant abdominal paraganglioma from a Japanese patient. Interestingly, this was the first case report of a patient with the *SDHB* mutation from Japan [10].

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Correspondence to: Kazuhiro TAKEKOSHI, M.D. Ph.D., 1-1-1 Tennoudai, Tsukuba, 305-8575, Japan

Here, we report another case of a *SDHB* mutation (R46Q) in a Japanese patient with both abdominal and thoracic paraganglioma following metastasis. In addition, we identified an asymptomatic carrier of the *SDHB* mutation in this family. Although the pathogenic role of the R46Q mutation has been reported previously, this is the first case of an *SDHB* mutation (R46Q) in a Japanese patient.

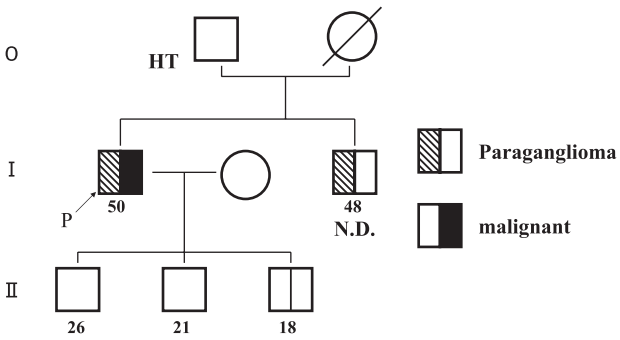

Fig. 1. Pedigree and clinical phenotype of family members.

Subjects and Methods

Patient and his family members

The proband, Japanese male, was previously reported by Kawai *et al.* as a case report [11]. In 1978, at the age of 22 yr, our subject (Fig. 1, proband) suffered from episodic headaches, truncal sweating, palpitations, and pallor. At this time, he presented with hypertension (systolic blood pressure >200 mmHg, diastolic blood pressure >140 mmHg) and was admitted to our hospital with a suspected catecholamine-secreting tumor. Urinary excretion of norepinephrine was increased during hypertensive attack as well as in 24-h collection. Preoperative chest and abdominal X-ray intravenous pyerography and pneumoretroperitoneum revealed an abnormal mass inside the cardiac shadow just above the midportion of the diaphragm. At surgery, mediastinal paraganglioma was removed. Postoperatively, however, blood pressure, as well as the 24-h urinary excretion of norepinephrine, did not fall within the normal range, indicating that another tumor still remained. Indeed, another tumor was found in the pelvic cavity. After removal of this second tumor, the 24-h urinary secretion of norepinephrine normalized and the patient was entirely free from further episodes. Subsequent histological investigations showed these two tumors to be pheochromocytomas (detailed findings of histopathological features as well as their microscopic appearance are available in the original case report [11]). Consequently, he was diagnosed with multiple paragangliomas (extra-adrenal pheochromocytomas).

Around the age of 30, the subject gradually became hypertensive. An abnormal shadow was detected by chest X-ray in the right midportion. After a second admission (1990, 38 yr), it was shown that both plasma and 24-h urinary excretion of norepinephrine were elevated, consistent with recurrent paragangliomas. After removal of a thoracic tumor, the 24-h urinary secretion of norepinephrine normalized. Histologically this tumor proved to be pheochromocytomas.

In 1994 (34 yr), ^{131}I-MIBG revealed increased uptake in the temporal skull, strongly suggesting a distant metastasis. At this time, the subject underwent an operation to remove a tumor.

At the age of 48 yr (2004) the subject presented with lower back pain. The 24-h urinary excretion of norepinephrine was elevated and ^{123}I-meta iodobenzylguanidine (MIBG) scintigraphy revealed discrete increased uptake in the lumbar vertebra. Magnetic resonance imaging showed lytic lesions within the lumbar vertebra (Th7, L4), consistent with distant metastasis of paragangliomas. These lesions were treated by local radiotherapy. The subject is currently alive and in good health.

The subject has three sons of whom only the third presented with hypertension (systolic blood pressure around 120–150 mmHg, diastolic blood pressure around 70–80 mmHg). After informed consent, we carried out genetic analysis of the proband as well as the three sons (details are given in *Genetic analysis* described below). Consequently, *SDHB* mutation (R46Q) was identified in the proband and his third son (see *Results* and Fig. 1). Given the clinical signs, together with the results of the genetic analysis, the third son was admitted to our hospital to exclude the possibility of having catecholamine-secreting tumors. The 24-h urinary excretion of norepinephrine, epinephrine, dopamine and their metabolites (*e.g.* metanephrine and normetanephrine) were found to be normal. Also, no tumor was detected in either CT or MIBG scintigraphy. Therefore, the subject's third son was diagnosed as an asymptomatic carrier of the *SDHB* mutation (R46Q).

Genetic analysis

Participants in this study were informed about the possibility of a genetic study, its implications, and its purpose. A written informed consent was obtained from those wishing to participate in the study, and the study was approved by the ethics committee of the Medical Faculty of Tsukuba University, Tsukuba, Japan. Blood samples were collected from the participants and DNA extracted using a Blood DNA extraction kit (WAKO, Osaka, Japan).

PCR and sSequence analysis

Peripheral blood for germline DNA analysis was drawn from the family members after written informed consent was obtained. Using blood DNA, the eight exons of the *SDHB* gene were screened with intronic primers [10]. PCR was carried out as described previously [10].

PCR amplicons were column purified and subjected to semi-automated sequencing using the above primers, dye terminator technology, and the Long-Read Tower DNA sequencer (Amersham Pharmacia Biotech) [10].

Results

SDHB mutation analysis

Direct sequencing of the eight exons of the *SDHB* gene in germline DNA of the subject (proband) showed a heterozygous G to A nucleotide transition in exon 2, changing arginine to glutamine at position 46 (R46Q) (Fig. 2). Furthermore, we carried out DNA analysis on his three sons, but the same R46Q mutation was only found in his third son. By contrast, no mutation of SDHB was found in the other two older sons. Although the third son presented with hypertension, his catecholamine level was normal and no tumor was detected by CT as well as MIBG, indicating that he is an asymptomatic carrier of *SDHB* (we defined 'asymptomatic carrier of *SDHB*' as a subject who carries the *SDHB* mutation but whose signs and/or symptoms are not derived from a catecholamine-producing tumor).

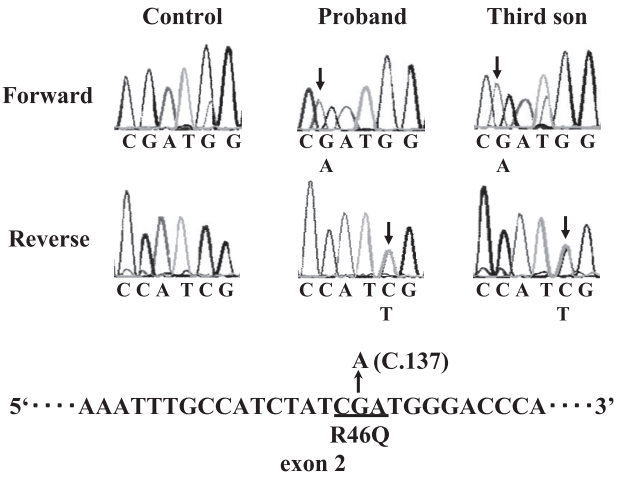

Fig. 2. Direct sequencing of the *SDHB* gene.
DNA was extracted from peripheral blood. Direct sequencing was performed for the eight exons using the described conditions. Sequencing reveals a heterozygous G to A nucleotide transition in exon 2, changing arginine to glutamine at position 46 (R46Q) in the patient (proband) as well as his third son.

Discussion

In this study, we identified a germline mutation of the *SDHB* gene (R46Q) in a malignant paraganglioma patient, as well as in his third son, who did not suffer from paraganglioma (Fig. 2). This is the first report of a patient with *SDHB* gene mutation (R46Q) and an asymptomatic carrier in a Japanese family.

Current evidence suggests that the mutation of *SDHB* is more frequently related to abdominal (or thoracic) paraganglioma and the following distant metastasis, in agreement with our case presented here [3–8]. Therefore, it is recommended that all patients with metastatic disease, especially from paraganglioma, be tested for *SDHB* mutations.

Recently, a detailed description of the clinical presentations, biochemical phenotypes and genotype-phenotype correlations of patients with mutations of *SDHB* has been presented by Timmers *et al.* [12]. The biochemical phenotype was consistent with hypersecretion of both norepinephrine and dopamine in approximately 50% of the cases. Hypersecretion of norepinephrine was only found in approximately 40% of the cases. Importantly, 10% of the cases had normal catecholamine (metabolite) levels, consistent with a biochemically silent paraganglioma. No obvious genotype-phenotype correlations were identified, in agree-

ment with previous reports [3–8]. Furthermore, clinical phenotypes were found to differ significantly between family members carrying the same mutation.

Indeed, it is reported that the same *SDHB* mutation, including R46Q, results in remarkable variations of clinical presentation. Initially, this mutation was reported by Gimenez-Roqueplo *et al.*, in a 55-year old female patient with a large, highly vascularized adrenal tumor, which extended into the heart with multiple pulmonary metastases [3]. An identical mutation was identified by the same author in a 28-year-old patient with a right adrenal tumor that had extensive vascular connections with the aorta and a tumor of the Zuckerkandl body [4]. Benn *et al.* reported five R46Q cases, which included the two cases described previously [7]. Four out of the five cases became malignant, despite the initial location of the tumors being different between family members with the same mutation.

In a population based study, Neumann *et al.* reported two *SDHB* (R46Q) cases [6]. Although the detailed clinical courses of these two cases remain to be clarified, the initial tumor was found as a neck paraganglioma and at the adrenal gland, both of which developed malignancy. In the present study, we identified a patient harboring the *SDHB* (R46Q) mutation with both abdominal and thoracic paraganglioma following distant metastasis. These findings reinforce the idea that *SDHB* (R46Q) mutations are closely related to malignant potential from paraganglioma/pheochromocytoma with divergent clinical phenotypes.

Another important finding of this study was to identify the same *SDHB* mutation (R46Q) in the third son of the proband as an asymptomatic carrier. At present, asymptomatic carriers of *SDHB* have been demonstrated in two large studies in the literature [6, 7]. Although there is little information available on how to follow-up and/or treat asymptomatic carriers, recommendations have been proposed as a result of these two studies [6, 7, 13]. The critical point of the recommended protocol is that all subjects must undergo periodic surveillance regardless of the presence of signs and symptoms. In the US, most *SDHB* mutation carriers are subject to follow-up by National Institutes of Health (NIH), where there are many clinicians experienced with pheochromocytoma and paraganglioma. Thus, we believe that in Japan *SDHB* mutation carriers, especially asymptomatic individuals, should be followed-up over an extended period of time at a restricted number of facilities where clinicians who specialize in pheochromocytoma and paraganglioma are working.

Further studies, especially prospective follow-up analyses of the *SDHB* mutation, are needed to establish routine screening for pheochromocytoma/paraganglioma, including asymptomatic carriers.

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