A Large Deletion in the Succinate Dehydrogenase B Gene (SDHB) in a Japanese Patient with Abdominal Paraganglioma and Concomitant Metastasis

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Abstract. Recently, mutations in nuclear genes encoding two mitochondrial complex II subunit proteins, succinate dehydrogenase D (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 the mutation of SDHB is highly associated with abdominal paraganglioma and the following distant metastasis (malignant paraganglioma). In the present study, we used multiplex ligation dependent probe amplification (MLPA) analysis to identify a large heterozygous SDHB gene deletion encompassing sequences corresponding to the promoter region, in addition to exon 1 and exon 2, in a malignant paraganglioma patient in whom previously characterized SDHB mutations were undetectable. This is the first Japanese case report of malignant paraganglioma, with a large SDHB deletion. Our present findings strongly support the notion that large deletions in the SDHB gene should be considered in patients lacking characterized SDHB mutations.

Key words: Malignant pheochromocytoma, SDHB, MLPA, HPPS (hereditary pheochromocytoma/paraganglioma syndrome), Paraganglioma

The nuclear genes encoding two mitochondrial complex II subunit proteins, succinate dehydrogenase D (SDHD) and SDHB, have been reported to be associated with the development of familial pheochromocytoma and paraganglioma (hereditary pheochromocytoma/paraganglioma syndrome: HPPS) [1, 2]. Growing evidence suggests that point mutations in SDHB are highly associated with abdominal paraganglioma and the following distant metastasis (malignant paraganglioma) [3-8]. Indeed, it has been found that SDHB mutations are present in 40% to 50% of malignant pheochromocytoma/paraganglioma patients which far exceeds the incidence of mutations of other genes in these malignant tumors. By contrast, 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].

It should be noted, however, that these findings are based on mutations including point mutations as well as small deletions, which are detectable by direct sequencing method [3-9]. Since large deletions are rare and are undetectable using this method, neither the frequency nor the clinical manifestation associated with large SDHB deletions is well known. Recently, in addition to these mutations, the use of methods such as multiplex ligation-dependent probe amplification (MLPA) and quantitative multiplex PCR of short fluorescent fragments (QmPSF) have resulted in increased reports of large SDHB deletions [10-18].

Here we report a large heterozygous SDHB gene deletion encompassing sequences corresponding to the promoter region, in addition to exon 1 and exon 2, in a malignant paraganglioma patient in whom previously characterized SDHB mutations were undetectable. This is the first Japanese case report of malignant
paraganglioma with a large SDHB deletion.

**Subjects and Methods**

**Patient**

A 25 year-old Japanese female with a 2-year history of hypertension was referred to our hospital for treatment for abdominal and liver masses in July 1995. No familial incidence of pheochromocytoma or paraganglioma was known in her kindred.

On first admission in August 1995, the patient’s catecholamine level was significantly elevated as shown in Table 1. Enhanced abdominal computed tomography (CT) revealed a paraaortic tumor and a metastatic nodule on the right side of the liver (Fig. 1A). MIBG scintigraphy indicated abnormal uptake at both tumor sites (Fig. 1B).

An operation was performed in September 1995,
but the main tumor was unresectable because of severe invasion. Extraadrenal malignant paraganglioma was diagnosed through pathological investigation. After surgery in October 1995, her catecholamine level was slightly elevated, but hypertension was diminished postoperatively with doxazosin (1-2 mg/day). This subject was followed up annually along with measuring plasma catecholamine as well as urinary metanephrine. In May 2009, 14 years after the first operation at age 39, multiple nodules in the left sided paraaorta were detected by abdominal CT. Consistent with these nodules, MIBG scintigraphy showed abnormal uptake and her catecholamine level was again increased. Therefore, recurrence or remnant of malignant paraganglioma was strongly suspected.

Given the well-documented association of mutations in the \textit{SDHB} gene with malignant pheochromocytoma/paraganglioma, we decided to carry out germline analysis of the \textit{SDHB} gene.

\textit{Genetic analysis}

Subjects in this study were informed of the possibility of genetic study, its implications, and its purpose. 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).

\textit{PCR and sequence analysis}

Peripheral blood for germline DNA analysis was drawn from the patient after written informed consent was obtained. Using blood DNA, the eight exons of the \textit{SDHB} gene were screened with intronic primers [19-21]. PCR was carried out as described previously [19-21].

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) [19-21].

\textit{MLPA analysis [22]}

We used a commercially available kit, SALSA MLPA P226 (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer’s instructions. Multiplex ligation-dependent probe amplification (MLPA) is a semiquantitative method designed to detect deletions/duplications of one or more exons at the genomic level. The P226 kit includes nine specific probes for \textit{SDHB}, six for \textit{SDHC}, and five for \textit{SDHD} to be used in one single reaction.

\textit{Results}

\textit{SDHB mutation analysis}

Mutations in the eight exons of the \textit{SDHB} gene in the subject’s germline DNA were undetectable by direct sequencing. While the majority of patients undergoing \textit{SDHB} mutation analysis have missense and nonsense mutations, some mutation negative patients have been reported to carry either large partial or total deletions of the \textit{SDHB} gene [10-18]. To investigate this possibility, we carried out MLPA and identified a heterozygous \textit{SDHB} gene deletion encompassing sequences corresponding to the promoter region, exon 1 and exon 2 (Fig. 2A, B).

\textit{Discussion}

Current evidence suggests that mutations in \textit{SDHB} are frequently associated with abdominal paraganglioma and the following distant metastasis [3-9]. Therefore, it is recommended that all patients with metastatic disease, especially from paraganglioma, be tested for \textit{SDHB} mutations. It should be noted, however, that these findings are largely based on mutations which are detectable by direct sequencing [3-9].

While many \textit{SDHB} mutation such as missense/non-sense mutations have been identified, some cases with large deletions have been described (Fig. 2B) [10-15]. The precise frequency of \textit{SDHB} deletion across all \textit{SDHB} positive cases remains to be established. Two occurred in exon 1 of \textit{SDHB}, including one of approximately 20 kb, previously demonstrated by Cascon \textit{et al.} [12]. Consequently, the frequency of \textit{SDHB} deletion across all French \textit{SDHB} positive cases was estimated at 8.3% (8/96).

In a large cohort of Spanish patients, seven independent families were found to carry deletion in the \textit{SDHB} gene, representing 28% of positive \textit{SDHB} cases [17]. The presence of founder mutations in the Spanish population could also explain the high rate of \textit{SDHB} mutations in this series. Taken together these recent results indicate that large \textit{SDHB} deletions represent approximately 10% of all \textit{SDHB} mutations and sug-
classification. For example, the French cohort included only paraganglioma patients, and excluded a single pheochromocytoma (adrenal catecholamine secreting tumor) patient. In the present study, we have carried out MLPA analysis in five malignant cases (initial location of tumor, four: extra-adrenal, one: adrenal), in which point mutations in the \textit{SDHB} gene were undetectable. A large deletion in the \textit{SDHB} gene was detectable in only one patient (the case presented here), corresponding to a deletion frequency of 20% (1/5), in agreement with previous studies \cite{16, 17}.

Given that only a small number of \textit{SDHB} deletion cases have been described \cite{10-18}, it is difficult to meaningfully compare the phenotypes and pene-
trance of patients with either point mutations or large deletions in \textit{SDHB}. The combined findings described above, however, indicate that they share almost similar phenotype as well as penetrance. Indeed, in a previous study of 82 \textit{SDHB} point mutation carriers, an estimated 45\% developed paraganglioma by age 40 [7]. In a recent report, with a smaller number of subjects in large family (23/41), the penetrance of paraganglioma related to exon 1 large \textit{SDHB} deletion based on a Kaplan-Meier analysis was slightly lower and estimated to be 35\% by age 40 [16].

In conclusion, we have identified a large \textit{SDHB} gene deletion, which includes the promoter region, in a patient with abdominal paraganglioma concomitant with distant metastasis. This is the first Japanese case report of its type and our findings strongly support the notion that large deletions in \textit{SDHB} should be considered in patients in which mutations of \textit{SDHB} genes are undetectable.

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\section*{References}


