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First Korean Case of Partial D *DBS-1*

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Dear Editor,

RHD and *RHCE*, encoding Rhesus proteins, are highly homologous genes located adjacently on the same chromosome (chromosome 1). Therefore, hybrid *RHD* genes, in which some portions are substituted with the *RHCE* sequence can change the extracellular loop of the RhD antigen, leading to variable reactivity to anti-D reagents [1].

Partial D phenotypes have historically been classified using epitope studies [2] and, recently, using genetic studies. DBS is a partial D phenotype characterized by c.[676G>C]+[697G>C] (NM_016124.4) and has been named based on its positive reactivity with the D monoclonal antibodies (MoAbs) BS228 and BS233 (Biotest, Dreieich, Germany) [3]. Three DBS subtypes have been reported till date: *DBS-0* [1], *DBS-1* [3, 4], and *DBS-2* [5]. Molecular studies have identified *DBS-1* in an Arabian [3] and a Japanese family [4]; however, it is unknown whether *DBS-1* correlates with the same hybrid gene of a single evolutionary origin in other, unrelated individuals from different ethnic groups. To the best of our knowledge, this is the first report of a *DBS-1* case in a Korean family. This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, Korea (SMC-2019-11-160), and written informed consent was obtained from the proband and all family members.

The proband was a Korean woman with fibrocystic breast changes, who was admitted to the Samsung Medical Center. D typing using anti-D Bioclone (MAD2 clone; Ortho Clinical Diagnostics, Raritan, NJ, USA) was negative. Weak D testing using anti-D Bioclone and human IgG/IgM monoclonal anti-D (Millipore, Livingston, UK) yielded a result of grade 2+, while the result of partial D testing using D-screen (Diagast, Loos, France) was consistent with *DBS-1*. The phenotypes of the current case and previously reported DBS cases are summarized in Table 1.

The RhCE phenotype was ccEe (anti-C, -c, -E, and -e antibodies were obtained from Bioclone, Ortho Clinical Diagnostics, Buckinghamshire, UK), and *RHD* genotyping was carried out according to a previously described method [6]. Exon 5 was not amplified by the primer sets used in this study, but the region from exon 4 to exon 6 was amplified and the PCR product was sequenced using *RHCE* exon 5-specific primers; the Rhesus box was also PCR-amplified (Fig. 1A). The proband harbored a hybrid *RHD-cE(5)-D* (*DBS/d*) allele involving the following amino acid changes: F223V, A226P, E233Q, V238M, V245L, G263R, and K267M. *RHD* genotypes of other family members are shown in Fig.1B. The breakpoints were confirmed between exon 4 and exon 6 by intron analysis [3, 4]. The 5′ breakpoint region was the same as that reported by Omi, *et al.* [4], whereas the 3′ breakpoint region was novel (Fig. 1C).

In various partial D phenotypes, such as DIIIa, DVa, DVI, DAR,

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Table 1. Comparison of serologic characteristics based on analysis using MoAbs between previous DBS cases and the present DBS case. Partial D testing was performed using D-screen (Diagast, Loos, France)

Type	RhD phenotype											RhCE phenotype	Ethnicity	Reference
	D epitope	epD2	epD3	epD5		epD6/7			epD6/7, epD9*	epD6/7, NA†	epD8	epD9			
	MoAbs	P3X249	P3X290	P3X241	P3X35	HM10	HM16	P3X61	TH28, MS26	MAD2, Polyclonal	P3X21211F1	P3X21223B10			
DBS-0		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	CDe	NA	[1]
DBS-1		Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg (IS) Pos (AHG)	Neg (IS) Pos (AHG)	Neg	Pos	cDE	Korean	Current study
		Pos (1+)	Pos (3+)	Neg	Neg	Neg	Neg	Neg	NA	NA	Neg	Pos (2+)	cDE	Arabian	[3]
		Pos (1+)	NA	Neg	Neg	NA	Neg	NA	NA	NA	Neg	Pos (1+)	cDE	Japanese	[4]
DBS-2		Neg	Neg	Neg	Neg	Neg	Neg	Neg	NA	NA	Trace	Pos (1+)	cDE	Chinese	[5]

*The results using human IgG/IgM monoclonal anti-D (Millipore, Livingston, UK); †The results using anti-D Bioclone (MAD2 clone; Ortho Clinical Diagnostics, Raritan, NJ, USA).
Abbreviations: ep, epitope; MoAbs, monoclonal antibodies; IS, immediate spin; AHG, antihuman globulin; Pos, positive; Neg, negative; NA, not available.

DFR, DBT, and DBS, *RHD* exon 5 is substituted with a part of *RHCE* exon 5 [7]. Exon 5 is predicted to encode the fourth extracellular loop of the D polypeptide. In DBS, the coexistence of two amino acid changes (A226P and E233Q) caused by c.676G>C and c.697G>C point variants is required for the characteristic phenotype [4]. A226P is observed in the fourth loop of antigen E [4] and is thought to have a considerable effect on D antigen density [1]. E233Q is also observed in the fourth loop as part of D^{w} (RH23) [8]. *DBS-1* and *-2*, which share F223V, A226P, and E233Q, exhibited different reactivity to the MoAbs P3X249, P3X290, and P3X21211F1 (*DBS-1*, +/+/– and *DBS-2*, –/–/trace). This difference might have been caused by the change in the extracellular amino acid, V238M, which is found in *DBS-0* and *DBS-1*, but not in *DBS-2*. Other amino acid changes observed in *DBS-1*, including V245L, G263R, and K267M, are located in the intracellular or transmembrane regions; however, they might affect the RhD phenotype.

The genetic basis of the RhD blood group differs across races and ethnicities. For example, the RhD-negative phenotype mainly results from *RHD* deletion in Caucasians, whereas *RHD* alterations, such as *RHD*D-CE*(4-7)*-D*, are common in Africans [9]. Asians exhibit a high prevalence of c.1227G>A (NM_016124.4), known as "Asia type DEL." These ethnicity-specific trends are used not only to diagnose RhD variants, but also for transfusion protocols [10]. The 3′ breakpoint observed in our case differed from that found in a Japanese family [4], suggesting that the *DBS-1* cases have different genetic origins.

In conclusion, we reported the first case of *DBS-1* in a Korean family. To understand the RhD characteristics specific to Korean ethnicity, further evaluation of RhD variants is required.

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AUTHOR CONTRIBUTIONS

DC conceived the study; SC and HBY contributed to the interpretation of the results; all authors contributed to writing the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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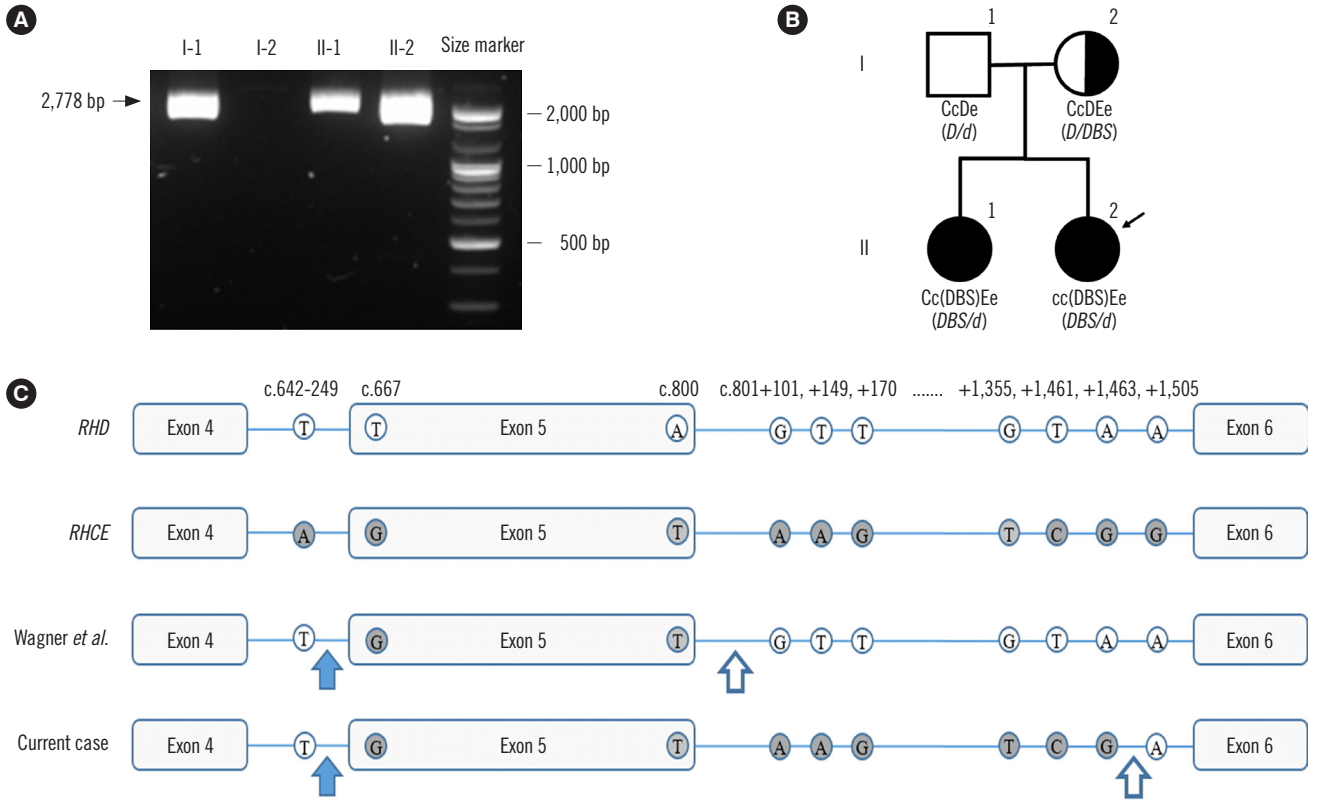

Fig. 1. Results of the genetic analysis of the proband and her family members. (A) Long-range PCR with primers located in non-Rhesus box sequences. A 2,778-bp fragment was amplified by PCR, indicating the presence of a hybrid *RHD* gene (lanes I-1, II-1, and II-2). (B) Pedigree, Rh phenotypes, and *RHD* genotypes of the Korean DBS-1 family. The genotypes and phenotypes of the DBS-1 family were determined using combined data from sequencing analysis, hybrid Rhesus box PCR, and serological analysis. Black circles indicate the DBS-1 phenotype. The proband is indicated by a black arrow. Total *RHD* deletion is denoted as "*d*" in the genotype. (C) Part of the *RHD* nucleotide sequence in DBS reported by Wagner, *et al*. [3] and this case. In both cases, the 5′ breakpoint region was located between the *RHD*-specific c.642-249T and the first *RHCE*-specific nucleotide, c.667G (blue arrow). The 3′ breakpoint region, located between the last *RHCE*-specific nucleotide and the first *RHD*-specific nucleotide of intron 5, differed for each case; it was located between c.800 and c.801+101 in the case reported by Wagner, *et al*. [3] and between c.801+1463 and c.801+1505 in the current case (white arrow).

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