

## ORIGINAL ARTICLE

# A founder mutation of *CANT1* common in Korean and Japanese Desbuquois dysplasia

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Desbuquois dysplasia (DBQD) is a severe skeletal dysplasia of autosomal recessive inheritance. DBQD is classified into types 1 and 2 based on presence or absence of hand anomalies. In a previous study, we found a *CANT1* (for calcium-activated nucleotidase 1) mutation, c.676G>A in five DBQD families. They were all East Asians (Japanese or Korean). The high prevalence of the same mutation among Japanese and Korean suggested that it is a common founder mutation in the two populations. To examine a possible common founder, we examined the region around *CANT1* in chromosomes with c.676G>A mutation by genotyping polymorphic markers in the region for the families. We examined their haplotypes using the family data. We identified in all families a common haplotype containing the *CANT1* mutation that ranged up to 550 kb. The two unrelated carriers of the mutation in general populations in Korea and Japan could also have the haplotype. We estimated the age of the founder mutation as ~1420 years (95% CI=880–1940 years). The c.676G>A mutation of *CANT1* commonly seen in Japanese and Korean DBQD should be derived from a common founder.

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## INTRODUCTION

Desbuquois dysplasia (DBQD; MIM #251450) is a severe skeletal dysplasia of autosomal recessive inheritance that belongs to 'multiple dislocation group'.<sup>1</sup> DBQD is clinically heterogeneous and classified into two types on the basis of presence (type 1) or absence (type 2) of characteristic hand anomalies, which consist of an extra ossification center distal to the second metacarpal, delta phalanx, bifid distal thumb phalanx and dislocation of the inter-phalangeal joints.<sup>2</sup> Type 2 DBQD contained a further clinical subtype, Kim variant,<sup>3</sup> which is characterized by short metacarpals with elongated phalanges that result in nearly normal length of the fingers.

Recently, Huber *et al.*<sup>4</sup> identified mutations in the gene encoding the calcium-activated nucleotidase 1 (*CANT1*) in DBQD type 1, which is followed by Faden *et al.*<sup>5</sup> We also found *CANT1* mutations in DBQD type 2 and Kim variant. In our series, we identified *CANT1* mutations in all seven patients from five unrelated families with DBQD Kim variant, and all patients had c.676G>A (p.V226M).

The five families were Japanese or Korean. c.676G>A was also found in 1/754 Japanese and 1/187 Korean controls in a heterozygous state.<sup>6</sup> These results led us consider that the mutation may be inherited from a common founder(s) among Japanese and Korean. To test the hypothesis, we examined haplotypes of the patients around *CANT1* and found that the mutation was on a common haplotype background of ~500 kb.

## MATERIALS AND METHODS

### Subjects

Seven DBQD patients from five families (four Japanese and three Korean) and their parents were examined. One Japanese family (DB1) was consanguineous (1st cousin marriage) and two sib patients from the family had c.676G>A in a homozygous state. The other patients were of non-consanguineous and compound heterozygotes of the mutations.<sup>6</sup> Their phenotypes were all diagnosed as DBQD Kim variant as previously reported.<sup>3</sup> One Japanese (CT1) and one Korean (CT2) subject from general populations who were found to have c.676G>A in a heterozygous state in the previous study were also examined.<sup>3</sup>

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## Genotyping

Genomic DNA was extracted from blood by standard procedures or from saliva using Oragene DNA Self-Collection kit (DNA Genotek, Ottawa, Ontario, Canada). Affymetrix 10K Gene-Chip microarray (Affymetrix, Santa Clara, CA, USA) was used for family DB1 to determine homozygous regions containing *CANT1* inherited from a common ancestor in the consanguineous family. Single nucleotide polymorphisms (SNPs) for the founder haplotype determination were genotyped by direct sequencing. The regions containing targeted SNPs were amplified by PCR from genomic DNA, and then PCR products were diluted and sequenced by using an ABI Prism 3730 automated sequencer (PE Biosystems, Tokyo, Japan). We separated 5'-FAM-labeled PCR products containing microsatellites by sizes on an ABI Prism 3730 automated sequencer (PE Biosystems) against the Genescan-500LIZ size standard. PCR primers for the polymorphisms are listed in Supplementary Table 1.

## Haplotype analysis

We determined the mutation-containing haplotype of patients according to the genotypes of the trio (the patient and parents). We determined the homozygous region of DB1 using the microarray data of the family members. All the patients were genotyped for the markers selected from the homozygous region of DB1. The genotyping of the makers for their parents were examined only when the patients were heterozygotes. The boundaries of the common haplotype were determined by sequential genotyping of SNPs in flanking regions. The SNPs were selected from the HapMap database (Phase II+ III, release 27).

## Estimation of the mutation age

We estimated the age of the founder mutation by the method previously described.<sup>7,8</sup> We used the following equation:

$$g = \log[(p_d - p_n)/(1 - p_n)]/\log(1 - \theta),$$

where  $p_d$  and  $p_n$  denote frequencies of the ancestral allele at the marker locus on the chromosomes with A and G alleles at the disease locus.  $\theta$  denotes the recombination fraction between the marker and the disease locus. The age of the mutation was corrected by taking the population growth into account using the following equations:

$$g_c = g + g_0,$$

$$g_0 = -(1/d)\ln[\theta \times e^d / (e^d - 1)],$$

where  $d$  denotes the population growth rate. Physical and genetic map positions in bp and  $c_M$  for SNP loci were obtained from the HapMap database (Phase II, release 21).

## RESULTS

### Genotyping and identification of a common haplotype

Using the SNP microarray data, we first determined a homozygous region of the genome that contained *CANT1* in the consanguineous family, DB1. The family had a stretch of SNPs that was homozygous in the patients and heterozygous in the parents. The homozygous stretch flanked by rs2934226 and rs2306755 extended up to ~9.8 Mb. Of 48 SNPs that composed the stretch in the microarray data, we selected 10 SNPs around *CANT1*; six were at the 3' to *CANT1* and four were at the 5' to *CANT1*. We genotyped them for the five families. A possible common haplotype was restricted in the region between rs6501224 and rs2377402.

To confirm the common haplotype and define its more detailed boundary, we selected 13 SNPs in the boundary regions from a database and genotyped them for the five families. In the SNPs we could get a definite haplotype result, all in a region from rs10512617 to rs2045660 showed a common haplotype. The nearest SNPs to rs10512617 and rs2045660 that defined the different haplotypes in any of the five families were rs11077391 and rs2377309, respectively. At the SNPs between

**Table 1 Haplotypes of markers with the c.676G>A mutation in the Desbuquois dysplasia (DBQD) families and control subjects**

Marker	Distance <sup>a</sup>	Subject							
		DBQD Family					Control		
		DB1	DB2	DB3	DB4	DB5	CT1	CT2	
rs7405591	1 712 736	C	T	T	T	C	T	T	
rs4789523	978 405	T	T	T/C <sup>b</sup>	T	T	T/C	T/C	
rs4129767	587 275	C	C	C	C	T	C	C/T	
rs6501224	488 804	G	G	C	G	G	G	G	
rs16971539	434 643	A	G	G/A <sup>b</sup>	G	G	G	G	
rs9899295	402 575	A	G	A	G	G	A/G	G	
rs9896451	347 233	G	G	A	G	G	G	G/A	
rs11077391	330 052	G	A	A	G	A	G	G	
rs10512617	297 708	G	G	G	G	G	G	G	
rs4103047	254 069	C	C	C	C	C	C	C/G	
rs3744801	197 531	C	C	C	C	C	C/T	C	
rs2889529	93 784	C	C	C	C	C	C/T	C/T	
c.676G>A	0								
rs8077024	2 287	C	C	C	C	C	C/T	C	
D17S1847	33 602	185	185	185	185	185	185/191	185/189	
rs2707047	132 355	G	G	G	G	G	G/A	G/A	
rs2612787	132 449	T	T	T	T	T	T/C	T/C	
rs9302889	168 892	T	T	T	T	T	T/A	T	
rs12600665	180 703	G	G	G	G	G	G/A	G	
rs2045660	185 092	C	C	C	C	C	C	C	
rs2377309	201 821	C	C	C	C	C/T <sup>b</sup>	C	C/T	
rs2612753	220 795	G	G	G	G	T	G/T	G	
rs1000791	274 619	T	A	T	T	T	T	T/C	
rs2377402	340 187	A	C	C	A	C	C	C	

<sup>a</sup>Base number between the marker and the disease locus.

<sup>b</sup>Equivocal because of the double heterozygosity of the parents.

rs10512617 and rs2045660, the two carriers of the mutation (CT1 and CT2) had all the alleles that composed the common haplotype. Therefore, they could also have the same haplotype (Table 1).

### Estimation of the mutation age

The markers that defined the haplotype of the families were used for estimation of the mutation age. The disease chromosomes of DB1 were counted as one because of its consanguinity.  $p_d$  was calculated in five chromosomes with A allele at the disease locus from the five DBQD families. As frequency of the mutation (A allele) was quite low in control subjects, we regarded the allele frequency of markers in the general Japanese population as  $p_n$ , and the allele frequencies were obtained from NCBI dbSNP database. The population growth rate,  $d$  was set to 0.08 as previously described.<sup>7</sup> The allele, frequency of which was higher in the five chromosomes than that in the general population was regarded as the ancestral allele. We excluded the markers in the region less than 60 kb from the disease locus.<sup>9</sup> The estimated ages by different markers ranged from 30 to 246 generations (mean=71; 95% CI=44–97; median=41; standard deviation=58) (Table 2). If we assumed an intergenerational time of 20 years, the age of the mutation was estimated to be 1420 years (95% CI=880–1940 years; median=820 years; standard deviation=1160 years).

## DISCUSSION

We have demonstrated that the c.676G>A mutation in Japanese and Korean had a common founder because all five chromosomes with

**Table 2** Estimated age of the c.676G>A mutation

Marker	<i>Pd</i>	<i>Pn</i>	$\theta$	<i>g</i>	<i>gO</i>	<i>gc</i>
rs7405591	0.4	0.295	0.0545	34.0	4.3	38.3
rs4129767	0.8	0.488	0.0191	25.7	17.4	43.1
rs6501224	0.8	0.698	0.0145	74.5	20.9	95.4
rs9899295	0.6	0.430	0.00998	120.6	25.5	146.1
rs9896451	0.8	0.488	0.00432	114.3	36.0	150.3
rs11077391	0.6	0.295	0.00401	208.6	37.0	245.6
rs10512617	1	0.400	0.00394	0	37.1	37.1
rs4103047	1	0.659	0.00391	0	37.2	37.2
rs3744801	1	0.523	0.00385	0	37.4	37.4
rs2889529	1	0.233	0.00187	0	46.5	46.5
c.676G>A			0			
rs2707047	1	0.145	0.00407	0	36.7	36.7
rs2612787	1	0.024	0.00408	0	36.7	36.7
rs9302889	1	0.727	0.00691	0	30.1	30.1
rs12600665	1	0.500	0.00722	0	29.6	29.6
rs2045660	1	0.576	0.00727	0	29.5	29.5
rs2612753	0.8	0.589	0.0106	62.8	24.8	87.6
rs1000791	0.8	0.625	0.0159	47.6	19.7	67.3
rs2377402	0.4	0.157	0.0201	61.3	16.8	78.1

c.676A in DBQD patients had a common haplotype composed of nine markers around this disease locus. In addition, the genotype of the two unrelated normal subjects with this mutation contained all the alleles that composed the common haplotype, suggesting that the founder mutation has widely spread among Korean and Japanese populations. The haplotype region ranged 480–550 kb. We estimated the age of this mutation at ~1400 years (95% CI=880–1940 years). Although the calculated CI is quite wide, we can estimate the c.676G>A mutation dates back to a time around the late Kofun era. Because the allele frequency of c.676G>A in Korean controls is much higher than that in Japanese controls, we speculated that the common founder was a Korean, and this mutation spread from Korea to Japan. This speculation is not contradictory to migration from Korea to Japan in the ancient history.

Although the exact prevalence of DBQD is difficult to determine, DBQD is generally considered as a very rare disease. However, the carrier frequency of the common mutation is considerably high in Korean. From our experience,<sup>6</sup> the frequency of the *CANT1* mutation is speculated as about double of that of the common mutation. Thereafter, prevalence of DBQD is ~1/35 000 in Korean and ~1/600 000 in Japanese; the former is far higher than our impression in daily practice. Many DBQD patients caused by *CANT1* mutations may be pre- or peri-natally lethal, although all the East Asian cases of DBQD with *CANT1* mutations so far reported had good prognosis. Alternatively, many DBQD may be undiagnosed and/or be put into the waste box of diagnosis as unknown ‘multiple dislocation group’ of skeletal dysplasia. Diagnosis of DBQD based on clinical and/or radiographic information is sometimes very difficult because of the overlapping phenotype with other diseases.<sup>10</sup>

In DBQD patients, the founder mutation was identified in all the seven Kim variant patients, and only in the Kim variant patients to our

knowledge. Therefore, this mutation may be closely related with specific phenotypes of the Kim variant; that is, accelerated carpal bone ages in childhood, short metacarpals, elongated appearance of phalanges and absence of accessory ossification center distal to the second metacarpal and thumb anomalies.<sup>3</sup> Further accumulation of Korean and Japanese DBQD patients and examination of their *CANT1* mutations are necessary to conclude relation of the founder mutation and the specific phenotype. The founder mutation may present with other phenotype. Moreover, further collection of DBQD patients and screening of *CANT1* mutations in other populations would help confirming the phenotype–genotype relation. Other mutation (s) may present with the Kim-type DBQD phenotype. The diagnosis of DBQD is sometimes very difficult at present; however, we think identification of the specific phenotypes of hands followed by examination of the founder mutation in *CANT1* would efficiently led us to definite diagnosis in Japanese and Korean patients suspected for having DBQD.

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