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Whole-exome sequencing in a Japanese family with highly aggregated diabetes identifies a candidate susceptibility mutation in ADAMTSL3

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ABSTRACT

Aims: The aim of this study was to clarify the genetic background of a family with multiple cases of diabetes accompanied by absolute insulin deficiency using whole-exome sequencing (WES).

Methods: In a Japanese family, WES was performed in four affected members with absolute insulin deficiency and two unaffected members. We focused on variants that were predicted to be disease-causing by bioinformatics and were shared by all of the four affected members but were not present in the two unaffected members. We assumed that the familial clustering of diabetes was caused by rare variants excluding those with allele frequency of more than 0.01 in the 1000 Genomes Project, the Human Genetic Variation Database, or a cohort of 105 normoglycemic controls in Japan. The rare variants were then genotyped in 2102 Japanese without diabetes and 119 Japanese with diabetes.

Results: Among the variants detected by WES and predicted to be disease-causing, 16 variants shared by all of the four of the affected members and not present in the two unaffected members were confirmed to be rare. Genotyping of the 16 rare variants revealed that only A137T in ADAMTSL3 (rs181914721) was observed more frequently in the 119 subjects with diabetes than in the 105 normoglycemic controls, and the allele frequency of the variant was significantly higher in the 119 subjects with diabetes than in another cohort of 2102 Japanese without diabetes.

Conclusions: We propose that A137T in ADAMTSL3 is a candidate mutation for susceptibility to diabetes in this family and in the Japanese population.

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1. Introduction

Diabetes is a disease of dysfunctional glucose regulation. The predominant types are type 1 diabetes (T1D) and type 2 diabetes (T2D). T1D is an organ-specific autoimmune disease that results from the progressive loss of insulin-producing β -cells in the pancreas. T1D represents a polygenic disorder [1]. Human leukocyte antigen (HLA) regions were first reported to be genetically associated with T1D; more than 50 loci have been linked to T1D susceptibility by genome-wide association studies (GWAS) to date [2].

There is compelling evidence that an individual's risk of T2D is strongly influenced by genetic factors. Currently, more than 80 association signals have been identified by GWAS. However, the alleles associated with T2D predominantly had frequencies of more than 0.05, which can explain only a minority of observed T2D heritability [3].

Next generation sequencing (NGS), in particular whole-exome sequencing (WES), which is the targeted sequencing of the human genome subset that is protein coding, has become a highly powerful and efficient strategy for identifying novel causative genes for various genetic disorders [4]. WES typically identifies tens of thousands of exonic variants; an appropriate selection strategy is required to facilitate identification of a single mutation that causes disease [4,5].

In the present study, to identify a causal variant in a Japanese family with highly aggregated diabetes, we combined two methods: (1) exome sequencing in the family and selection of candidate variants and (2) comprehensive genotyping of the identified variants in Japanese population cohorts.

2. Subjects, materials, and methods

2.1. Study subjects

2.1.1. The family studied with WES

We recruited a family with a two generation family history of diabetes in Japan (Fig. 1). Nine members donated their DNA. Clinical information was obtained at the time of enrollment and laboratory testing; HbA1c, glutamic acid decarboxylase antibody (GAD Ab), islet antigen-2 autoantibody (IA-2 Ab) and serum C-reactive protein (CPR) level tests were performed. GAD Ab was assayed with radioimmunoassay kit purchased from Cosmic Corporation, Japan. HLA genotyping was performed using the WAKFlow HLA Typing kit (Wakunaga Pharmaceutical, Hiroshima, Japan).

2.1.2. Hospital-based recruitment of subjects diagnosed with diabetes

We recruited another 119 subjects diagnosed with diabetes at Kyoto University Hospital and collaborating hospitals in Japan. If they had family history of diabetes, we attempted to recruit their family members. We obtained DNA, clinical information, and data of laboratory testing of the subjects and their family members.

2.1.3. Population samples in Japan

The Nyukawa Study: Nine hundred five local residents who participated in an annual medical check-up program in a district that belonged to the former Nyukawa village in Takayama city, Gifu, Japan, consented to donate their DNA [6]. Participants underwent anthropometric measures and blood tests including fasting plasma glucose and HbA1c every year. Subjects defined as normoglycemic controls had the following characteristics: both HbA1c and fasting plasma glucose below the screening standards for diagnosis of diabetes (HbA1c < 6.0% NGSP [<42.0 mmol/mol IFCC] and fasting plasma glucose < 5.5 mmol/l) during 5-year follow-up from 2002 to 2007, and age > 55. From these normoglycemic controls, 105 participants were randomly selected (52 men, 53 women).

The Nagahama Study: Study participants in this cohort were recruited from 2008 to 2010 from the general population of Nagahama city [7]. The inclusion criteria for this cohort was the following: community residents aged 30–74 years, living independently in the community, and without physical impairment or dysfunction. A total of 9804 participants were included. Of the participants with data of HbA1c and plasma glucose available at the time of enrollment, 2513 individuals were randomly selected for genotyping. After excluding ancestry outliers identified by principal component analysis with HapMap Phase 2 release 28 JPT data set as a reference and subjects estimated to have a first- or second-degree kinship within this population, 2179 subjects remained for further study. Diabetes in the Nagahama Study was defined as (1) receiving medication for diabetes or (2) HbA1c $\geq 6.5\%$ NGSP [≥ 47.5 mmol/mol IFCC], fasting plasma glucose ≥ 7.0 mmol/l, or (3) casual plasma glucose ≥ 11.1 mmol/l.

2.2. WES and variant calling

Genomic DNA was isolated from the participants' peripheral blood using the QIAamp DNA Blood Mini kit (QIAGEN Sciences, Maryland). For six family members (II-2, II-3, II-4, II-5, III-3 and III-4 in Fig. 1) and 12 young-onset subjects (onset age ≤ 30) without overweight selected from the 119 subjects diagnosed with diabetes described in Section 2.1.2, WES libraries were prepared using SureSelectXT2 Human All Exon v5 Kit (Agilent Technologies, California) and sequenced on Illumina HiSeq platform (Illumina Inc. California) as paired-end reads to 100 bp/read. Sequence reads were mapped to the reference human genome (UCSC GRCh37/hg19; <http://hgdownload.cse.ucsc.edu/downloads.html>) using Barrows-Wheeler Aligner [8]. Sequence variants were discovered using GATK (<https://software.broadinstitute.org/gatk/>). Functional annotation and effect prediction of the variants were performed using SnpEff [9] to integrate the information from public databases including UCSC hg19 and dbSNP 137 (<https://www.ncbi.nlm.nih.gov/SNP/>). Carriers of mutations ("HIGH" impact predicted by SnpEff) in genes involved in monogenic diabetes (HNF1A, HNF1B, HNF4A, GCK, INS, KCNJ11, ABCC8) were excluded from further studies.

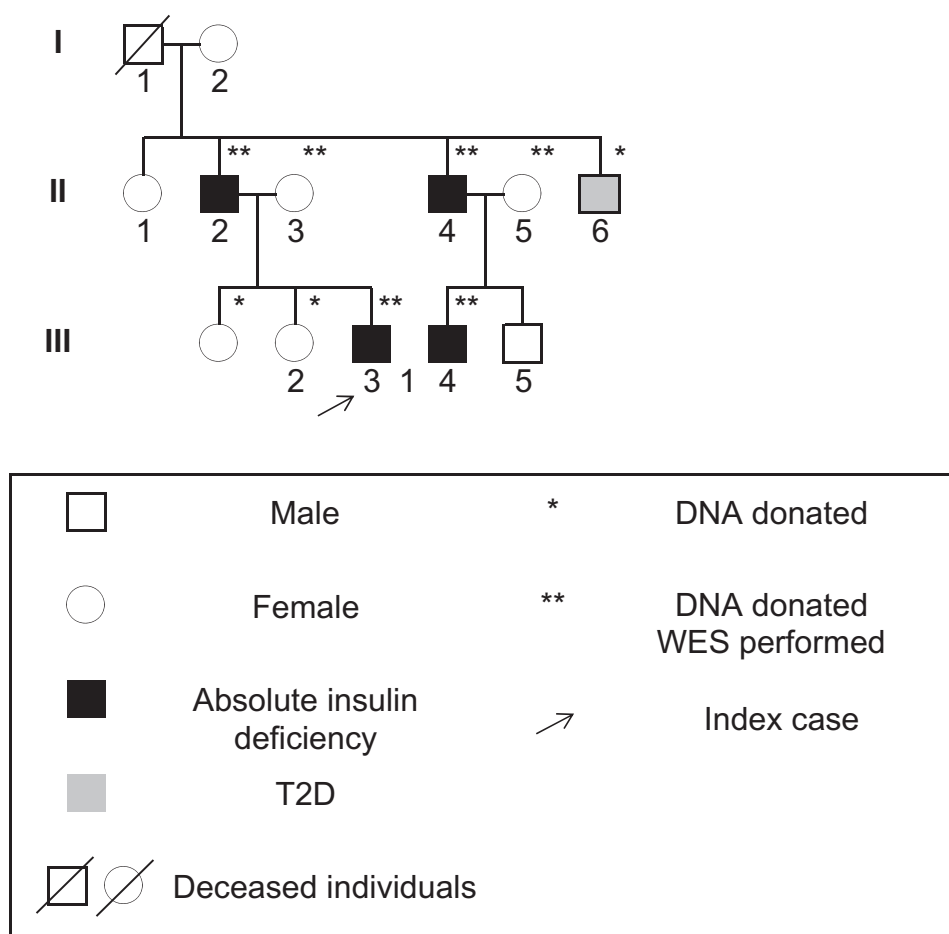


Fig. 1 – A family with family aggregated diabetes mellitus that was studied with whole-exome sequencing. (WES; Whole-exome sequencing, T2D; type 2 diabetes).

2.3. Selection of candidate variants

We focused on the non-synonymous exonic variants present in all of the four affected family members (II-2, II-4, III-3 and III-4 in Fig. 1) and not present in the two unaffected family members (II-3 and II-5 in Fig. 1). From the shared variants we selected variants that fulfilled either of the following two criteria (1) variants with the effect prediction of “SPLICE_SITE_ACCEPTOR,” “SPLICE_SITE_DONOR,” “START_LOST,” “STOP_GAINED,” or “STOP_LOST” by SnpEff (2) variants with the effect prediction of “NON_SYNONYMOUS_CODING” by SnpEff, which were predicted to be ‘probably damaging’ by PolyPhen-2 HumDiv [10].

As rare genetic variants often change the protein function and can be pathogenic, we checked the population frequency of the selected variants by using two databases: the 1000 Genomes Project (<http://www.internationalgenome.org/>) and a Japanese genetic variation database Human Genetic Variation Database [11], and narrowed down candidate variants by excluding variants with allele frequency of more than 0.01 in either databases.

2.4. Sanger sequencing and variant genotyping

We directly sequenced the candidate variants in an affected member of the family (II-2 in Fig. 1) by Sanger sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 3130 Genetic Analyzer (Thermo Fisher Scientific, Massachusetts). We genotyped the variants in the other family members, the subjects diagnosed with diabetes described in Section 2.1.2, and the normoglycemic controls from the Nyukawa Study using Taqman SNP genotyping assays and StepOnePlus system (Thermo Fisher Scientific). We again narrowed down candidate variants by excluding variants with allele frequency of more than 0.01 in the Nyukawa normoglycemic controls. For the participants in the Nagahama Study, genotyping was performed using Infinium Omni2.5–8 BeadChip (Illumina Inc.).

2.5. Statistical analysis

Frequencies of nucleotide changes in control subjects from population samples and in subjects with diabetes were compared by the Fisher’s exact test.

2.6. Ethics

Ethical approval for this study was given by the Ethics Committee of Kyoto University School of Medicine (approval No. G572 and G1032). Written informed consent was obtained from each participant. This study was carried out in accordance with the Declaration of Helsinki as revised in 2000.

3. Results

3.1. Characteristics of the family studied with WES

The family pedigree is shown in Fig. 1. Four out of the five members with diabetes were diagnosed with T1D and treated with insulin (II-2, II-4, III-3, and III-4). All of the members diagnosed with T1D were lean (Body Mass Index (BMI) <22 kg/m²) and showed absolute insulin deficiency (fasting serum CPR <0.3 ng/ml). Control, unaffected family members (II-3 and II-5) were aged 50 or over and their HbA1c levels were <6.0% NGSP [<42 mmol/mol IFCC]. Detailed characteristics of the family are shown in Table 1. For members diagnosed with T1D, GAD Ab was negative excepting member III-4. Member II-6 was diagnosed with T2D and treated with oral antidiabetic drugs; his serum CPR was preserved at the age of over 50.

3.2. WES and selection of the candidate variants

WES was performed in the four family members with absolute insulin deficiency (II-2, II-4, III-3 and III-4 in Fig. 1) and the two unaffected family members (II-3 and II-5 in Fig. 1). WES generated >30 million sequence reads for each sample and the vast majority (>97%) of paired reads was uniquely mapped to the reference human genome UCSC GRCh37/hg19. More than 75% of the uniquely mapped reads were on the target regions and >96% of the target regions were covered with at least 10x depth.

Around 20,000 non-synonymous exonic single nucleotide variants were detected in each family member (Supplementary Table 1); we selected variants shared by all of the four affected members that were absent in two unaffected members of the family. Among the shared variants, we found 105 non-synonymous variants that fulfilled either of the following 2 criteria: 1) variants with effect prediction of “SPLICE_SITE_ACCEPTOR,” “SPLICE_SITE_DONOR,” “START_LOST,” “STOP_GAINED,” or “STOP_LOST” by SnpEff, and 2) variants with effect prediction of “NON_SYNONYMOUS_CODING” by SnpEff, which are predicted to be ‘probably damaging’ by PolyPhen-2 HumDiv (Supplementary Table 2). After excluding variants with allele frequency of more than 0.01 in the 1000 Genomes Project and the Human Genetic Variation Database, we narrowed down the candidate variants to 18 (Table 2). These 18 variants were directly sequenced with Sanger method in an affected member of the family (II-2, Fig. 1) and all variants in the member were confirmed to be heterozygous. We genotyped the 18 variants in the rest of the family members with Taqman method and the variants were confirmed to be heterozygous in all members with absolute insulin deficiency. We also found that the variants were

heterozygous in some of the other family members excepting members II-3 and II-5 (Table 2).

3.3. Frequencies of the candidate variants in population samples and subjects with diabetes

The 18 candidate variants were genotyped in the 105 normoglycemic controls in the Japanese population cohort (The Nyukawa Study, detailed data in Supplementary Table 3). Sixteen of the candidate variants were found to be rare (minor allele frequency; MAF <0.01) in the normoglycemic controls (Table 3). We then genotyped the 16 rare variants in 119 subjects diagnosed with diabetes recruited in hospitals (Table 3, Supplementary Table 4). The only variant that was observed more frequently in subjects with diabetes than in normoglycemic controls was A137T in ADAMTSL3 (rs181914721). Further we sequenced all exons of the ADAMTSL3 gene in (1) the family member III-13 in Supplementary Fig. 1 (also a young-onset member without overweight) who is included in the 119 subjects in Supplementary Table 4 and (2) other 11 young-onset subjects without overweight selected from the 119 subjects diagnosed with diabetes. One subject from the 11 young-onset subjects was excluded from further study because he carried a heterozygous p.V246Gfs mutation in HNF1A. As a result, seven missense variants of ADAMTSL3 were detected and the rare variant that was predicted to be disease-causing by PolyPhen-2 was only the A137T in ADAMTSL3 (Supplementary Table 5).

We genotyped A137T in ADAMTSL3 in another, larger set of Japanese subjects without diabetes (from the Nagahama study, $n = 2179$). Genotyping was successful in 2173 subjects and 71 of the subjects were defined as having diabetes. We compared the allele frequencies of the variant in 2102 subjects without diabetes from the Nagahama study and in 119 subjects diagnosed with diabetes recruited in hospitals; the allele frequency of A137T in ADAMTSL3 was significantly higher in the 119 diabetes subjects recruited in hospitals than in the 2102 subjects without diabetes from the Nagahama study (Odds ratio, 4.92; 95% confidence interval, 1.35–17.86; $P = .0354$; Table 4, Supplementary Table 6).

Among the diabetes subjects recruited in hospitals with A137T in ADAMTSL3, DNA samples of family members were available for one subject. We genotyped the variant in the family members and it was observed in 6 out of the 9 family members with diabetes treated with oral drugs and/or insulin (Supplementary Fig. 1, Supplementary Table 7).

4. Discussion

In this study, we investigated a Japanese family with highly aggregated diabetes. This family was distinctive because it had four closely related affected members with absolute insulin deficiency, suggesting the possibility of finding a causative gene using WES. The four affected members and two unaffected members were selected for WES and 16 rare non-synonymous coding variants shared only between affected members were identified. The variants are predicted to be probably disease-causing and all of them had MAF of <0.01 in normoglycemic controls (Tables 2 and 3).

Table 1 – Characteristics of the family members as of 1st Jan 2014.

ID	Age	Sex	BMI	HbA _{1c} (%) NGSP [mmol/mol IFCC]	Age when diagnosed	Current therapy	GAD Ab (U/ml)	IA-2 Ab (U/ml)	sCPR (ng/ml)	HLA genotype
II-2	63	M	17.0	7.2 [55.1]	55	Insulin	<0.4	<0.1	<0.2	
II-3	60	F	20.0	5.7 [38.7]	–	–	<0.4		2.71	
II-4	60	M	21.8	7.4 [57.3]	36	Insulin	0.7		<0.03	
II-5	58	F	18.9	5.1 [32.2]	–	–	<0.4		0.90	
II-6	57	M	21.1	6.8 [50.8]	35	Oral Drug	<0.4		8.03	
III-1	32	F	22.6	5.3 [34.4]	–	–	<0.4		1.25	
III-2	30	F	20.4	5.7 [38.7]	–	–	<0.4		1.09	
III-3	28	M	20.6	7.6 [59.5]	23	Insulin	<0.4	<0.1	0.28	
III-4	27	M	19.0	6.7 [49.7]	16	Insulin	890		<0.03	DRB1 * 04:05 DRB1 * 12:01 DQB1 * 03:03 DQB1 * 04:01

Table 2 – The genotype of the 18 candidate variants in the family members.

Chromosome	Position (hg19)	SNP ID	Amino Acid Change	Gene Name	Genotypes (M; major allele m; minor allele)								
					II-2	II-3	II-4	II-5	II-6	III-1	III-2	III-3	III-4
1	156918273	NA	R608H	ARHGEF11	Mm	MM	Mm	MM	MM	MM	MM	Mm	Mm
1	227968355	NA	R459Q	SNAP47	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
1	231048479	rs79413990	E653K	TTC13	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
1	240286526	NA	P555S	FMN2	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
3	47050773	NA	S2743L	NBEAL2	Mm	MM	Mm	MM	Mm	MM	MM	Mm	Mm
6	38810443	rs78877915	R1537C	DNAH8	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
6	52870063	NA	R595Stop	ICK	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
8	73849154	NA	E522K	KCNB2	Mm	MM	Mm	MM	MM	Mm	MM	Mm	Mm
8	133945809	NA	C1607F	TG	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
10	99995805	rs201839212	R124H	R3HCC1L	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
11	17542914	NA	R355I	USH1C	Mm	MM	Mm	MM	MM	MM	MM	Mm	Mm
11	121037391	rs189181502	V1830M	TECTA	Mm	MM	Mm	MM	MM	Mm	MM	Mm	Mm
12	66620588	NA	G186R	IRAK3	Mm	MM	Mm	MM	MM	Mm	MM	Mm	Mm
12	114282527	NA	D911N	RBM19	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
15	84488608	rs181914721	A137T	ADAMTSL3	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
15	90630791	NA	A180V	IDH2	Mm	MM	Mm	MM	Mm	Mm	Mm	Mm	Mm
16	70285773	NA	P11S	EXOSC6	Mm	MM	Mm	MM	Mm	Mm	MM	Mm	Mm
17	53155439	NA	E397K	STXBP4	Mm	MM	Mm	MM	Mm	MM	Mm	Mm	Mm

Among the 16 rare variants the only variant observed more frequently in 119 diabetes subjects than in the cohort of 105 subjects aged over 55 who retained fasting normoglycemia for five years (the Nyukawa Study) was A137T in ADAMTSL3. The allele frequency of the variant in the 119 diabetes subjects was significantly higher than in 2102 subjects without diabetes from another Japanese population cohort (the Naga-hama Study). These results suggest that A137T in ADAMTSL3 could be a susceptibility variant for diabetes in the Japanese population.

Furthermore, the fact that A137T in ADAMTSL3 was also detected in another large Japanese family with diabetes (Supplementary Fig. 1) supports the involvement of the A137T in ADAMTSL3 with diabetes susceptibility. The mutation was detected in six family members; two members with young-onset diabetes (onset age ≤ 30 , III-2 and III-13 in Supplemen-

tary Fig. 1) and four with late-onset diabetes (onset age from 50 to 65) (Supplementary Table 7).

The superfamily of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) includes 19 distinct ADAMTS that are metalloproteases and at least six ADAMTS-like proteins without metalloprotease activity [12]. ADAMTSL-3/punctin-2 is a member of the ADAMTS-like subfamily [12–16]. The protein is homologous to the ADAMTS ancillary domains but lacks the protease domain and metalloprotease activity. Like ADAMTS, it is a component of the extracellular matrix. In previous studies, the ADAMTSL3 locus was reported to be associated with human height [15] and schizophrenia [16].

The 137th amino acid residue alanine in ADAMTSL-3 is completely conserved among vertebrates and a mutation changing the residue is predicted to be pathogenic [10]. As

Table 3 – Allele frequencies of the 18 candidate variants in Nyukawa normoglycemic controls and subjects with diabetes from hospital-based recruitment. (M; major allele, m; minor allele, MAF: minor allele frequency).

Amino acid change	Gene name	Genotypes (Normoglycemic, Nyukawa) n = 105				Genotypes (Diabetes, Hospital-based) n = 119			
		MM	Mm	mm	MAF	MM	Mm	mm	MAF
R608H	ARHGEF11	105	0	0	0	119	0	0	0
R459Q	SNAP47	105	0	0	0	119	0	0	0
E653K	TTC13	105	0	0	0	119	0	0	0
P555S	FMN2	105	0	0	0	119	0	0	0
S2743L	NBEAL2	105	0	0	0	119	0	0	0
R1537C	DNAH8	102	3	0	0.014				
R595Stop	ICK	105	0	0	0	119	0	0	0
E522K	KCNB2	98	7	0	0.033				
C1607F	TG	105	0	0	0	119	0	0	0
R124H	R3HCC1L	103	2	0	0.009	117	2	0	0.008
R355I	USH1C	105	0	0	0	119	0	0	0
V1830M	TECTA	103	2	0	0.009	119	0	0	0
G186R	IRAK3	104	1	0	0.004	119	0	0	0
D911N	RBM19	105	0	0	0	119	0	0	0
A137T	ADAMTSL3	105	0	0	0	116	3	0	0.012
A180V	IDH2	105	0	0	0	119	0	0	0
P11S	EXOSC6	105	0	0	0	119	0	0	0
E397K	STXBP4	105	0	0	0	119	0	0	0

Table 4 – Comparison of the allele frequencies of rs181914721 (A137T in ADAMTSL3) in population samples. (M; major allele, m; minor allele, MAF: minor allele frequency, *: Fisher's exact test).

	MM	Mm	MAF	P vs. Nagahama [*]	P vs. Nyukawa [*]
Nagahama, Without diabetes (HbA1c < 6.5% NGSP [47.5 mmol/mol IFCC])	2091	11	0.0026		
Nyukawa, Normoglycemic (HbA1c < 6.0% NGSP [42 mmol/mol IFCC])	105	0	0		
Diabetes, Hospital-based	116	3	0.012	0.0354	0.2496

the presence of ADAMTSL-3 protein was detected in human pancreatic islets using immunohistochemistry [14], there is a possibility that the function of ADAMTSL-3 is linked to fragility of insulin-producing β -cells.

Of the four affected members with absolute insulin deficiency in the family described in Fig. 1, one member had positive GAD Ab, another member had a weak GAD Ab level (below the cut-off level 1.5U/ml), and the other two members had no evidence of islet autoimmunity. Although the GAD Ab levels and the disease-onset ages of the affected members were different, based on the fact that as many as four closely related members were affected with absolute insulin deficiency, we propose that A137T in ADAMTSL3 could be a genetic factor that leads to fragility of insulin-producing β -cells and when islet autoimmunity and/or other environmental and genetic factors caused stress in β -cells all four family members became deficient in insulin secretion [17]. For the family member III-4 with positive GAD Ab, considering that the odds ratio of the T1D susceptibility haplotype (heterozygous DRB1*04:05 and DQB1*04:01) is 3.2 [18], A137T in ADAMTSL3 might be involved in the depletion of insulin secretory capacity, not the onset of T1D, together with the susceptibility HLA haplotype.

Our study has a few limitations. First, not all subjects heterozygous for A137T in ADAMTSL3 in our study have developed diabetes accompanied with absolute insulin deficiency. Regarding the young family members (III-1 and III-2) in Fig. 1 we thought that the possibility they develop absolute insulin deficiency in the future cannot be excluded. Regarding the fact that the member II-6 in Fig. 1 and six family members in Supplementary Fig. 1 were heterozygous for the mutation and that their serum CPR is preserved, we cannot exclude the possibility that some additional factors other than A137T of ADAMTSL3 also affected the cause of diabetes in members with absolute insulin deficiency in Fig. 1. In addition, 11 control subjects from the Nagahama Study were heterozygous for A137T in ADAMTSL3. This might be because the average age of controls from the Nagahama Study was below 55 and the controls might develop late-onset diabetes in the future. There is a possibility that other unknown genetic factors are also associated with absolute insulin deficiency in the family described in Fig. 1.

Second, in the family described in Fig. 1, the GAD Ab levels and the disease-onset ages of the affected members with absolute insulin deficiency were different and genetic or environmental factors that explain the difference of the phenotypes of the affected members remains unclear. The family

member III-4 with positive GAD Ab can be diagnosed with T1D. To elucidate genetic factors shared by three affected family members with negative GAD Ab (II-2, II-4, and III-3) but not shared with III-4 and two unaffected members in Fig. 1, we analyzed the WES data to select disease-causing variants rare in population databases (Supplementary Table 8). No such variants in known genes associated with diabetes were shared by the three affected members, but (1) nine variants shown in Supplementary Table 8 and (2) 16 out of the 18 variants shown in Table 3 (excluding E522K in KCNB2 and R1537C in DNAH8 that were not rare in controls), totally 25 variants including A137T in ADAMTSL3, should still be considered as possible candidate variants involved in the pathogenesis of diabetes for three affected family members with negative GAD Ab (II-2, II-4, and III-3).

However, considering the results of the genotyping in Japanese families and population cohorts, we propose that A137T in ADAMTSL3 may be a susceptibility mutation for diabetes. A family-based approach with exome sequencing might be a useful strategy to elucidate the complex genetic background of diabetes.

Conflict of interest

The authors have no conflicts of interest to report relative to this manuscript.

Authors' contribution

Conceived and designed the study: BJ, DT and NI. Researched data: BJ, DT, SH, NH, TH, YF, YW, YL, YT, FM, and AK. Analyzed the data: BJ, DT, and YT. Wrote the manuscript: BJ and DT. Edited the manuscript: KN, AK and NI. NI is the guarantor of this work. All authors have reviewed and approved the final version of this manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.diabres.2017.11.012>.

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