Impaired arginine-induced insulin secretion in MODY2 patient with UGGT1R1526C variant

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Abstract

Arginine releases proinsulin from binding to UGGT1 in the endoplasmic reticulum (ER). PSIPRED analysis showed that the arginine/proinsulin binding domain (A/PBD) in the C-terminal region of UGGT1 forms a disordered region, which is flexible and outside of the main protein structure. Arginine and proinsulin may easily access UGGT1. Using the SNP library, two variants, Q1518* and R1526C, were identified in this region. Q1518* variant is a deficient form of A/PBD and ER-retention signal (ERRS). Q1518* mutated UGGT1 protein in cell analysis reveals Q1518* protein is mainly secreted protein, because of lack of ERRS. We found another UGGT1 variant, R1526C, among 489 Japanese maturity-onset diabetes of young (MODY) patients. In fact, a MODY2 (GCK^{WT/G261R}) patient with the UGGT1^{WT/R1526C} variant showed less arginine-induced insulin secretion than a MODY2 father with UGGT1^{WT/WT}. However, they have a similar response of glucose intolerance. At the molecular level, less interaction of proinsulin with UGGT1 was observed in both human UGGT1^{R1526C} and mouse UGGT1^{L1518C} with/without arginine. Collectively, the interaction of proinsulin with UGGT1 likely has a significant impact on the regulation of proinsulin processing and arginine-induced secretion in β -cells.

1 Introduction

Hypoinsulinemia leads to diabetes mellitus^[1, 2]. Maturity-onset diabetes of the young (MODY) is a rare (1–3%) form of diabetes mellitus. MODY is referred to as monogenic diabetes. In England, 0.01% MODY patients exist as an estimation^[3]. Specifically, MODY2 is due to glucokinase (GCK/HXK4, OMIM: *138079) variants^[4].

Arginine potently induces insulin secretion from β -cells^[1, 2, 5]. The arginine target proteins are CATs^[6–8], CASTOR1^[9, 10], and GPRC6A^[11]. In addition, we recently identified that arginine interacts with two target proteins regulating insulin secretion in β -cells^[12, 13]. One is UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1^[12], Gene ID: 56886, OMIM: *605897^[14–16]), which retains proinsulin in the endoplasmic reticulum (ER) in the absence of arginine. Another target is glucokinase (GCK), whose catalytic activity is upregulated by the interaction with arginine. Arginine also prevents the degradation of GCK^[13].

We hypothesized that mutations affecting these arginine targets will cause MODY if the interaction holds physiological significance. Here, we found impaired arginine-induced insulin secretion in a pancreatic beta cell line and MODY2 patient with the UGGT1 variant. Our data indicate that mutations affecting the interaction of proinsulin and UGGT1 may cause MODY and contribute to hypoinsulinemia in response to arginine.

2 Methods

2.1 Cell culture

Mouse NIT-1 cells (CRL-2055™, ATCC, Manassas, VA, USA) and human embryo kidney 293 FT (HEK293FT) cells (purchased from Invitrogen, Carlsbad CA USA) were maintained as described previously [5, 12, 13].

2.2 Secreted insulin analysis

Secreted insulin was measured as described previously with a commercial ELISA kit (Shibayagi, Gunma, Japan) [12, 13].

2.3 Immunoprecipitation (Ip) and Western blot (WB)

Insulin (L6B10 mAb, 8135S, Cell Signaling), and FLAG (F-1804, Sigma) antibodies were purchased. Ip and WB analyses were performed as described previously [12, 13]. Signals of WB signals were quantified with the LAS 4000 imaging system (GE Healthcare) [12, 13].

2.4 Protein structure analyses

Homology models of mouse and human UGGT1 from *Chaetomium thermophilum* were generated using the Swiss-Model server. Templates with the highest quality (PDB ID: 3WZT, 3WZS)^[15, 16] were selected for model building. Computer modeling was performed using the PyMOL mutagenesis algorithm wherein the backbone-dependent rotamers of glutamine were examined [12, 13]. Only the rotamer with the highest frequency of appearance in proteins is shown. Global and per-residue model quality was evaluated using the QMEAN4 scoring function. The obtained scores were – 5.83 and – 6.06 for WT and variants, respectively [12, 13].

2.5 UGGT1 mutants

Using mutated synthesized oligomers and PrimeSTAR mutagenesis basal kit (Takara, Kyoto, Japan), mutated UGGT1 expression vectors were produced. Cell lines were transfected with pCAGGS-UGGT1 (WT or mutants)-FLAG and pCDNA-insulin-Myc expression vectors with Lipofectamine 2000 (Thermo Fisher, Rockford, IL, USA).

2.6 Human MODY study

We examined the UGGT1 sequence of 489 subjects from 155 medical institutions throughout Japan who were suspected to have MODY (male/female, 266/223; age of onset, 15.4 ± 7.3 years; body mass index (BMI), 20.4 ± 3.3 kg/m²)^[17–19]. The inclusion criteria were age of onset-age of 35 years or less, anti-GAD antibody negativity and no obesity (BMI < 25 kg/m²). Family history was not included to avoid missing sporadic or low penetrant cases. We excluded obese subjects to limit the inclusion of early-onset type 2 diabetes as little as possible. Even mild obesity ($25 < \text{BMI} < 30$) was not included, as compensatory hyperinsulinemia can mask the phenotype of insulin deficiency^[17–19]. Family history was not included to ensure that sporadic or low-penetrant cases were not missed^[17–19]. Direct sequencing of exon 40 and flanking regions of UGGT1 was performed.

An arginine tolerance test in two MODY2 patients was performed at the Gifu University Hospital. Blood samples were obtained via a catheter inserted into a superficial forearm vein. An equivalent of 5 g arginine as 10% arginine hydrochloride (5 g) was administered intravenously over 30 s, the end of which was designated time point 0. Blood samples were withdrawn at 3 and 5 min for the determination of insulin levels^[13]. The test protocol was approved by the Institutional Review Board of Gifu University (Gifu-No. 25–153) in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants^[13, 17–19].

2.7 Statistical analysis

The values are reported as the means $\pm$ standard error ($n = 6$) with dots. Statistical significances (single-sided Student's t test) are indicated. All the data was shown in the graph, and there is no data set

3 Results

3.1 A human UGGT1 variant, UGGT1^{Q1518*}, is a secreted protein without an ER-retention signal (ERRS, Figures 1 and S1).

In our previous paper, we identified an arginine/proinsulin binding domain (A/PBD, Fig 1a^[12,13]). First, we screened the human single nucleotide polymorphism (SNP) library (<https://www.ncbi.nlm.nih.gov/snp>) and found two rare variants reported, human UGGT1^{Q1518*} (rs778170956, MAF 0.0000/1 [GnomAD]) and human UGGT1^{R1526C} (rs759864507, MAF 0.0000/5 [GnomAD]) in the C-terminal region (Fig. 1a). Truncated UGGT1^{Q1518*} protein lacks both A/PBD (red line)^[12] and ER-retention signal (ERRS). A/PBD is calculated as disordered region (DR) by PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred/>, Fig 1b, 1c, and S1).

When expressed in NIT 1 cells, WT-UGGT1F protein interacted with proinsulin-Myc only in the absence of arginine. In contrast, the Q1518*-UGGT1F protein showed hardly any interaction with proinsulin either in the presence or absence of arginine (Fig 1d). The impaired interaction between Q1518*-UGGT1F and proinsulin-Myc was associated with blunted insulin secretion in response to arginine (Fig 1e). These data are in agreement with our model that retention of proinsulin by UGGT1 in the absence of arginine and subsequent release in response to arginine is important for arginine induced insulin secretion. Considering that Q1518*-UGGT1 lacks ERRS, we analyzed the intracellular and extracellular distribution of Q1518*-UGGT1. As expected, secretion into the medium was observed only for Q1518*-UGGT1F, but not for WT-UGGT1F (Fig 1f and 1g).

3.2 Intolerant arginine-induced insulin secretion in MODY patients with the UGGT1^{WT/R1526C} variant (Figure 2 and S2).

MODY is a rare form of diabetes caused by a single variant in an autosomal dominant gene. Considering that proinsulin mutants with the altered interactions with UGGT1 cause MODY, we hypothesized that UGGT1 genetic variant(s) might be found in MODY patients. We explored the genome sequences of 489 Japanese patients with MODY^[32-34] for the presence of mutations in the C-terminal region of human

UGGT1. One patient with MODY2 (called the proband) harbored a variant in human UGGT1^{WT/R1526C} (mouse UGGT1^{WT/L1518C}, Fig 1a, and S2a-c) along with heterozygote glucokinase^{WT/G261R} (GCK^{WT/G261R}). As the patient's father had only the GCK^{WT/G261R} variant, we compared their β cell functions. In comparison with the father, the proband displayed significantly lower homeostatic model assessment of β cells (HOMA β [20], proband=16.714, father=24.84, and Japanese range =50-80), indicating a reduced ability to secrete insulin after fasting (Fig. 2a). In an arginine tolerance test, circulating insulin was lower in the proband with the UGGT1^{WT/R1526C} genetic variant than in the father (Fig. 2b). Moreover, the other parameter of insulin secretion, the C-peptide-to-glucose ratio^[17-19] was lower in the proband (Fig. 2c). The reduced β -cell function in the proband with UGGT1^{WT/R1526C} is compatible with a model in which UGGT1 contributes to arginine-induced insulin secretion through the release of proinsulin. Surprisingly, higher glucose-induced insulin secretion of the proband was observed (Figs. 2d and S2d), suggesting that the UGGT1^{WT/R1526C} genetic variant contributed on to the only arginine challenge.

To dissect the impact of human UGGT1^{R1526C} protein on arginine sensitive interaction with proinsulin *in vitro*, human UGGT1^{R1526C} and a mouse UGGT1^{L1518C} mutant that corresponds to human UGGT1^{R1526C} (Fig. 1a) were analyzed in NIT-1 cells (Figs. 2e, 2f, S2e, and S2f). Neither human UGGT1^{R1526C} nor mouse UGGT1^{L1518C} mutant proteins increased insulin secretion in response to arginine while arginine-induced insulin secretion in the corresponding WT-UGGT1F cells (Fig. 2e). Human UGGT1^{R1526C} and mouse UGGT1^{L1518C} mutant proteins also had less interaction with proinsulin-Myc in the presence and absence of arginine in IP-WB analysis (Figs. 2f, S2e and f). UGGT1^{R1526C} mutated protein binds to arginine (Fig. 2g and h) but does not bind to proinsulin with/without arginine (Fig. 2i). Note that the UGGT1^{R1526C} mutated protein is predicted to be damaged by Polyphen (<http://genetics.bwh.harvard.edu/pph2/>); however, the UGGT1^{R1526C} protein exists and does not degrade, similar to WT-UGGT1.

4 Conclusion

We previously reported that proinsulin is retained in the ER through binding to the ER resident protein UGGT1 when the availability of arginine is low and that arginine releases proinsulin from the ER through competition with UGGT1^[12]. Here, we report a MODY-associated variant in UGGT1 that interferes with their interaction. Through the analysis of the variant, we further refined the molecular basis of the interaction between proinsulin and UGGT1.

Arginine administration increases the arginine intracellular concentration in the ER of pancreatic β -cells, and arginine disrupts the interaction between proinsulin and the C-terminal domain of UGGT1 and forms a new complex of arginine and UGGT1^[12]. This allows proinsulin to be further processed from the ER to the Golgi apparatus and mature in the secretory vesicles for insulin secretion^[12]. UGGT1^{WT/R1526C} genetic mutation associated with MODY2 causes impairment in arginine-inducing insulin secretion but causes a

distinct defect in the interaction between UGGT1 and proinsulin. On the other hand, glucose induces insulin secretion on the patient as well as cells showed UGGT1 does not concern to glucose signaling

5 Discussion

We previously reported that arginine regulates ER retention and the release of proinsulin from the ER^[12]. Proinsulin can be retained in the ER through its interaction with UGGT1 under conditions of arginine depletion. However, proinsulin retained in the ER can be released from UGGT1 when arginine becomes available to beta cells, leading to insulin secretion. These results demonstrate that the ER retention of proinsulin is one of the crucial regulatory steps in insulin secretion and that arginine plays an essential role in the release of proinsulin from retention in the ER. Notably, mature insulin is not expected to interact with UGGT1. Here, we provide further support for the physiological significance of the interaction between UGGT1 and proinsulin through the analysis of mutations associated with MODY. Our current study indicates that the altered interaction between UGGT1 and proinsulin may also contribute to the retention of proinsulin at the ER^[12]. In addition, UGGT1 is retained in the ER and will not be transported into secretory vesicles due to ERRS. Overall, the interaction between UGGT1 and proinsulin is limited in the ER and provides a mechanism to regulate proinsulin ER retention.

In summary, we demonstrated that arginine competes for an interaction between proinsulin and UGGT1 and contributes to the release of proinsulin. More importantly, we identified variants in proinsulin and UGGT1 that disrupt their interaction in subjects with MODY, indicating that the interaction of proinsulin and UGGT1 contributes to arginine-induced insulin secretion.

Declarations

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Author Contributions

JC, YH, MY, YT, ME, HM, and DY, performed the experiments, YH, and TI designed the experiments, analyzed the data, and wrote the manuscript.

Author Information

Readers are welcome to comment on the online version of the paper. Correspondence should be addressed to TI (timai@ncgg.go.jp).

Data Availability

Requests for data and materials should be addressed to TI (timai@ncgg.go.jp). The two UGGT1^{Q1518*} (rs778170956, MAF 0.0000/1 [GnomAD]) and human UGGT1^{R1526C} (rs759864507, MAF 0.0000/5 [GnomAD]) are from the human single nucleotide polymorphism (SNP) library (<https://www.ncbi.nlm.nih.gov/snp>).

Competing interest

The authors declare no competing financial interests.

Supplemental information is available in the online version of the article.

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Figures

Figure 1

A human UGGT1 variant, UGGT1Q1518*, lacking an arginine/proinsulin binding domain and ER retention signal, is a secreted protein.

(a) Alignment of C-terminal amino acid sequences of UGGT1 wild-type (WT) (mouse 1,506–1,544 a. a., human 1,514–1,556 a. a.) and mutants (L1518C, R1526C, and Q1518*). Mouse UGGT1^{Q1510-Q1538} corresponds to human UGGT1^{Q1518-Q1551}, and human UGGT1^{R1526C} corresponds to mouse UGGT1^{L1518C}.

(b and c) Structures of UGGT1^{WT} **(b)** and UGGT1^{Q1518*} **(c)**.

(d) The UGGT1^{Q1518*} mutant failed to interact with proinsulin in the absence or presence of arginine. UGGT1 flag-tagged analyzed by proinsulin-IP-WB.

(e) UGGT1^{Q1518*} mutated protein interfered with arginine-induced insulin secretion analyzed using an ELISA kit. Data are presented as the mean ± SE. n=6

(f and g) Intracellular and secreted UGGT1 were detected using WB (f) and ELISA (g). The UGGT1^{Q1518*} mutant protein lacking ERRS was secreted.

Figure 2

Impaired arginine-induced insulin secretion in a MODY2 patient with UGGT1^{WT/R1526C}.

(a–c) Arginine-induced insulin secretion in the father and the child with MODY2 who both had GCK^{WT/G261R} [32–34]. HOMA β ^[20] was analyzed after fasting in the father and the child with MODY2 (GCK^{WT/G261R}), of which a child had UGGT1^{WT/R1526C} (a). Impaired insulin concentration and C-peptide-to-glucose ratio was observed in a patient with UGGT1^{WT/R1526C}-mutated MODY2 (GCK^{WT/G261R}) following the arginine challenge (b and c).

(d) UGGT1^{WT/R1526C} variants stimulate insulin secretion after glucose challenge.

(e) Impaired arginine-induced insulin-Myc secretion in cells expressing mouse UGGT1F^{L1517C} and human UGGT1F^{R1526C} mutations.

(f) Impaired arginine competitive interaction of arginine with human UGGT1F^{R1526C} and mouse UGGT1F^{L1517C} variants. Data are presented as the mean $\pm$ S.E. n=6 *p < 0.05; **p < 0.005; ***p < 0.0001.

(g and h) UGGT1WT and UGGT1R1526C mutated proteins also bind to arginine.

(i) Proinsulin is reduced after arginine administration with UGGT1^{WT}. Proinsulin is reduced with/without arginine by the UGGT1^{R1526C} protein.

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