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RAPID COMMUNICATION

Gga3 gene-deleted C57BL/6J mice have elevated fasting blood glucose levels

Diabetes mellitus (DM) is one of the most common diseases in the elderly, and among DM patients, more than 90% have type 2 DM (T2DM). The etiology of T2DM is complex and associated with risk factors such as age, genetic disposition, and diet. The genetic factors underlying T2DM are currently the focus of intense research, and it has been established that the susceptibility genes associated with T2DM vary among different populations. A recent longitudinal exome-wide association study in the Japanese population has identified *GGA3* as a susceptible locus for T2DM.[1] GGA3 is one of the Golgi-localized γ-ear-containing ARF binding proteins (GGAs) that function widely in the transport of Golgi-derived vesicles and in endocytic trafficking pathways. However, although it is widely acknowledged that the control of glucose homeostasis via endocytosis and glucose metabolism plays a vital role in T2DM, the associations between intracellular trafficking and diabetes pathogenesis have not been sufficiently established. In this study, we used a *Gga3* gene knockout mouse strain to investigate the potential involvement of GGA3 in controlling blood glucose. The study was approved by the ethics committee of Jining Medical University (No. 2019-JS-005). Our results indicate that the *GGA3* gene might play a protective role against diabetes.

Conventional *Gga3* gene knockout mice were generated in a C57BL/6 J genetic background using CRISPR/cas9 endonuclease-mediated genome editing.[2,3] The targeting strategy and genotyping results are shown in Figure 1A. As C57BL/6 J mice show symptoms of glucose intolerance as young as 6 weeks of age and are highly susceptible to the development of obesity and overt T2DM when fed a high-fat diet (HFD), these mice are frequently used in the field of diabetes research.[4] Both $Gga3^{-/-}$ and wild-type (*WT*) mice were provided with a normal chow diet (NCD) containing 24% protein, 13% fat, and 63% carbohydrate (Beijing Keao Xieli Feed CO., LTD., Beijing, China). Fasting blood glucose (FBG) levels recorded in 6-week-old mice revealed that *WT* mice had FBG levels of 6.91 ± 1.35 mmol/L (x ± standard deviation, $n = 18$), whereas the $Gga3^{-/-}$ mice had significantly higher FBG levels of 8.18 ± 1.22 mmol/L ($n = 17$) (**$P = 0.006$) (Fig. 1B). These findings thus provide evidence to indicate that *Gga3* is involved in the control of mouse blood glucose levels. Notably, $Gga3^{-/-}$ mice reportedly reduce blood glucose levels on postnatal day 1[3]. As the newborn mice show symptomatic hypoglycemia or low blood glucose in the first hours or days after birth, this is speculated to reflect the relative fetal hyperinsulinism for balancing the high glucose levels induced by maternal diabetes, and thereafter, these mice will maintain normal blood glucose levels. Thus, the reduced blood glucose levels in newborn $Gga3^{-/-}$ mice might also indicate the occurrence of maternal diabetes or up-regulated blood glucose levels in adult mice. Conversely, the higher blood glucose levels in $Gga3^{-/-}$ mice may be associated with a distinct underlying mechanism, with the newborn mice being characterized by symptomatic hypoglycemia or low blood glucose. However, the role of *Gga3* in maternal diabetes or the blood glucose levels of elder mice needs to be further evaluated.

Littermate $Gga3^{-/-}$ ($n = 6$) and *WT* ($n = 6$) mice were subsequently used to compare the effects of the *Gga3* gene on FBG levels (Fig. 1C). As HFD can be used to induce a T2DM model,[5] after recording their FBG levels at 6 weeks of age, mice were switched from an NCD to an HFD containing 34% protein, 40% fat, and 26% carbohydrate to determine whether the blood glucose levels of $Gga3^{-/-}$ mice are more sensitive to an HFD. FBG levels in these mice were recorded once weekly for 5 weeks, which revealed that after initial fluctuation throughout the first 2 weeks (which could be attributable to the change in diet), the FBG levels remained

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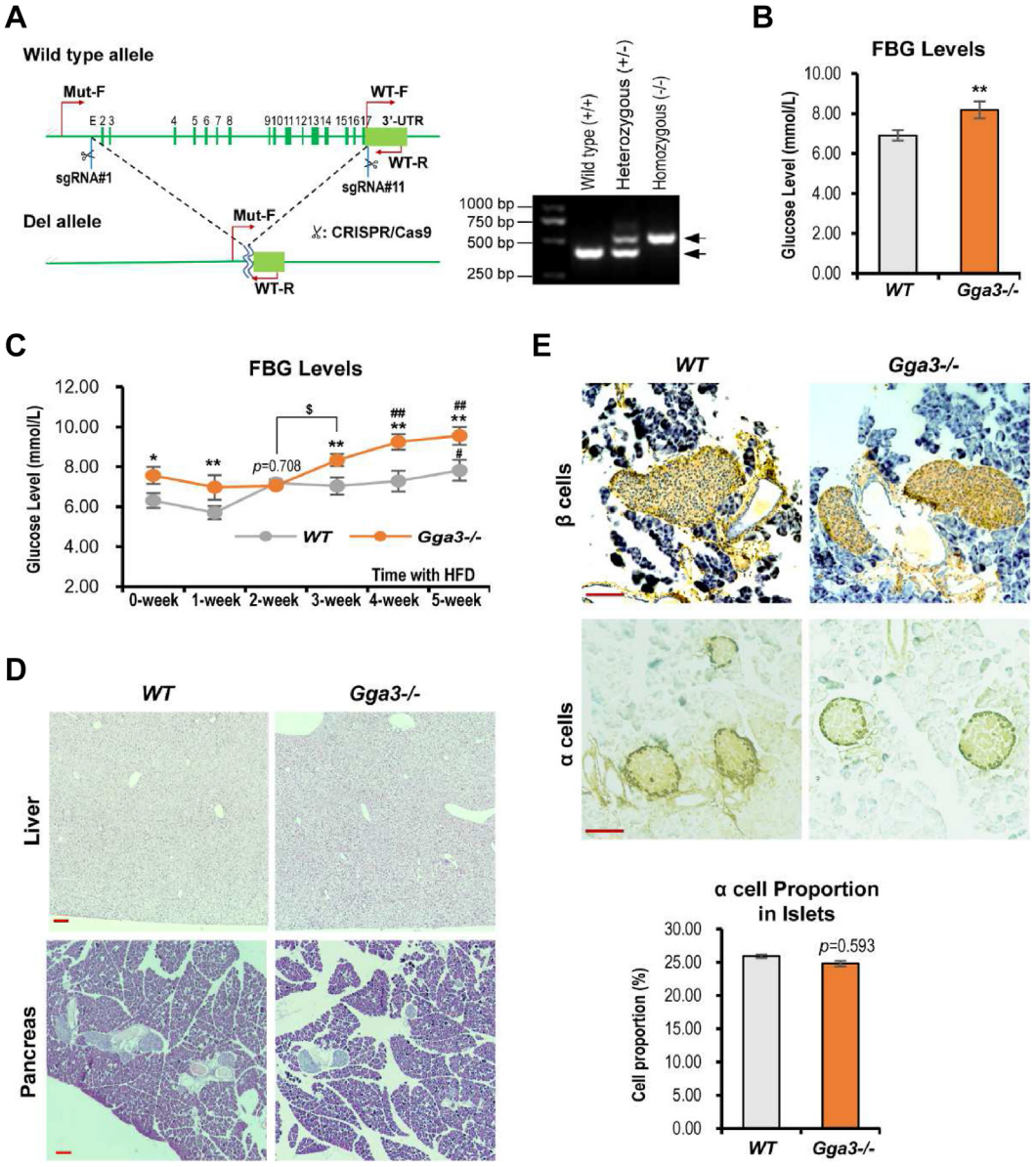

Figure 1 Effects of the *Gga3* gene on blood glucose levels in C57BL/6 J mice. **(A)** Construction of *Gga3* gene knockout C57BL/6 J mice. The strain was generated by microinjection using the Extreme Genome Editing system developed by Biocytogen Pharmaceuticals (Beijing, China) based on CRISPR/Cas9 gene editing. A pair of sgRNA oligos (5'-guide sgRNA#1: AGATATCCTTGTGTTACGAGTGG; 3'-guide sgRNA#11: ACGGCTACTTCAAGTCACACAGG) was selected to delete a sequence containing exons 2—17 of the mouse *Gga3* gene (NCBI ID: 260302). Mice were genotyped by PCR using the following primers: Mut-F (AGCCCAGAAAGCAGGATTTGTCTG), WT-F (CTTGGTGTGGATCGGAGCCCTGG), and WT-R (GCTGCTTGCAGAAAGTGAAGACTACAG). The PCR products were as follows: a 430-bp band for wild-type mice (WT-F/WT-R), a 579-bp band for knockout mice (Mut-F/WT-R), and the presence of 430- and 579-bp bands for heterozygous mice (WT-F/WT-R and WT-F/WT-R). **(B)** Comparison of fasting blood glucose (FBG) levels. WT mice ($n = 18$) and $Gga3^{-/-}$ mice ($n = 17$) were fed with a normal chow diet to 6 weeks of age, at which time fasting glucose levels in mice were measured. Independent sample *t*-test, $^{**}P \leq 0.01$. **(C)** Comparison of the FBG levels in mice fed a high-fat diet (HFD). Littermate $Gga3^{-/-}$ ($n = 6$) and *WT* ($n = 6$) mice were switched to HFD after recording FBG levels at 6 weeks of age. The FBG levels were thereafter recorded weekly for 5 weeks. The FBG levels were compared between groups at the same time point ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$), between the FBG levels at each time point and the FBG levels at 6 weeks of age ($^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.01$), or between the FBG levels at sequential time points in the same group ($^{\$}P \leq 0.05$). Analyses were performed using a one-way

constitutively elevated from the third week on, particularly in the *Gga3*$^{-/-}$ mice, in which there was a significant increase in FBG levels during the third week compared with those in the second week ($^{\$}P = 0.043$). When measured after 5 weeks on the HFD, the FBG levels in *Gga3*$^{-/-}$ mice had increased from 7.57 ± 0.37 mmol/L at 6 weeks of age to 9.55 ± 0.52 mmol/L ($^{\#\#}P = 0.002$); however, the levels in the *WT* mice had increased from 6.30 ± 0.43 mmol/L at 6 weeks of age to 7.82 ± 0.44 mmol/L ($^{\#}P = 0.017$). However, differences in the changes in FBG levels between the two mouse groups over the 5-week experiment were found to be non-significant (1.98 ± 1.03 mmol/L in *Gga3*$^{-/-}$ mice *vs*. 1.52 ± 0.61 mmol/L in *WT* mice, $P = 0.326$). As an alternative approach for comparing the changes in FBG levels, the levels in each mouse were normalized to the level at 6 weeks of age to obtain an amplification value. However, we detected no significant differences between the two groups (1.27 ± 0.14 times in *Gga3*$^{-/-}$ mice *vs*. 1.26 ± 0.15 times in *WT* mice, $P = 0.717$) (Fig. S1). These findings thus indicated that the *Gga3*$^{-/-}$ and *WT* mice had similar sensitivity to the HFD-induced increase in blood glucose. As controls, we established further two groups of mice of the same age (non-littermates), which were continuously fed the NCD, and at 11 weeks, the FBG levels in each mouse were compared with those measured at 6 weeks of age. We accordingly found that in both *Gga3*$^{-/-}$ and *WT* mice, the FBG levels at 11 weeks of age were lower compared with those measured at 6 weeks of age (amplification: 0.93 ± 0.19 times in *Gga3*$^{-/-}$ mice *vs*. 0.93 ± 0.10 times in *WT* mice, $P = 0.982$) (Fig. S1). In this regard, it has been suggested that circulating levels of glucose in C57BL/6 J mice decline throughout growth, and it appeared that glucose levels in C57BL/6 J mice fed with HFD (58% fat, 16.4% protein, 25.6% carbohydrate) markedly increase during the first few weeks on the diet but thereafter underwent a gradual reduction.[5] Overall, these observations indicate that *Gga3* gene knockout C57BL/6 J mice have higher blood glucose levels than the corresponding *WT* mice, providing evidence that *GGA3* might play a protective role against DM.

We subsequently determined whether there is a developmental deficit in the two key organs involved in the control of blood glucose, namely, the liver and the pancreas. Reportedly, the mice in which *Gga* genes have been deleted have no evident histological abnormalities on postnatal day 1.[3] Figure 1D shows a comparison of the histological structures of the liver and pancreas of littermate *Gga3*$^{-/-}$ and *WT* mice after the fifth week of feeding on the HFD. Hepatocytes in the livers of *Gga3*$^{-/-}$ and *WT* mice were observed to be normal in size, orderly and clear in structure, and the pancreatic islet cells of mice in both groups were of regular shape and evenly distributed, with clear boundaries between the islet cells and acinus. The histology of the pancreatic islets was further investigated via immunohistochemical staining. Sections of the pancreas were incubated with insulin antibodies to detect β cells or with glycogen antibodies to identify α cells (Fig. 1E). We detected no evident differences in the distribution or shape of the β and α cells. As the α cells are clearly distributed at the islet boundary, we counted these cells and detected no significant difference between the two groups with respect to the proportions of α cells (24.82% ± 7.48% in the *Gga3*$^{-/-}$ group *vs*. 25.94% ± 6.22% in *WT* group, $P = 0.593$). The islets in adult mice generally comprise 65% − 85% β cells for the secretion of insulin and 10% − 25% α cells for the secretion of glycogen, and thus, the proportions of α cells detected in both the *Gga3*$^{-/-}$ and *WT* mice are within the generally accepted range for adult mice. These observations indicate that there are no marked histomorphological lesions in the liver or pancreas of *Gga3*$^{-/-}$ mice. Thus, the elevated glucose levels in *Gga3*$^{-/-}$ mice might not be associated with histological dysfunction.

Collectively, the present findings provide evidence to indicate that the *GGA3* gene plays a protective role in controlling blood glucose levels, and this gene is associated with the pathogenesis of DM. Accordingly, our results provide support for *GGA3* as a novel risk gene for diabetes. However, considerably more research is necessary to further confirm the role of *GGA3* in the pathology of diabetes and to establish the underlying mechanisms.

Author contributions

XZ and XL designed the study. XZ, SY, and DJ did the experiments. GZ and JL analyzed the histology results. XZ, SY, and XL analyzed all the results and wrote the manuscript.

Conflict of interests

The authors declare that they have no competing interests.

ANOVA with the least significant difference test for analysis of significance among groups. **(D)** Comparison of the histology of the liver and pancreas of *Gga3*$^{-/-}$ and *WT* mice. Livers and pancreases were collected from the mice used in (C) after the final measurement of FBG levels for hematoxylin and eosin (HE) staining. The tissues were observed and imaged under an optical microscope with a × 4 objective lens. Scale bar = 100 μm. **(E)** Comparison of pancreatic β and α cell distributions. Left: sections from the same tissues used in (D) were incubated with insulin antibodies (1:100, #ab181547, Abcam) to mark β cells or glycogen antibodies (1:100, #sc-514592, Santa Cruz) to mark α cells. Sections were then stained using a DAB kit and HE. The tissues were observed and imaged under an optical microscope with a × 20 objective lens. Scale bar = 100 μm. Right: numbers of α cells from each islet section were normalized to the total cell number of each islet section [cell proportion = (100 *α cell number/total cell number) %, $n > 20$]. Student's *t*-test, $^{*}P \leq 0.05$. Statistical analyses were performed using IBM SPSS Statistic 23 software. A *P*-value of 0.05 was used as the significance threshold throughout this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.031.

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