Deficiency of mitochondrial aldehyde dehydrogenase increases type 2 diabetes risk in males via autophagy dysregulation

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To the editor: The diabetes epidemic has increasingly become a major public health concern worldwide. In 2014, there were 102.9 million diabetic adults in China, representing 24.4% of the world's diabetic population, even though China only comprised 18.7% of the global population at the time.[1] Furthermore, the estimated overall prevalence of diabetes and prediabetes was 10.9% and 35.7%, respectively, indicating China as one of the countries with the highest prevalence of diabetes in the world.[2] Although factors, including sedentary lifestyles and energy-dense diets, drive the diabetes epidemic, genetic architecture may also contribute to the susceptibility of an individual's response to environmental challenges. Aldehyde dehydrogenase (ALDH)2 is a key enzyme that eliminates toxic aldehydes by catalyzing their oxidation to non-reactive acids. Emerging evidence has suggested that individuals with *ALDH2* deficiency have an increased risk of cardiovascular and metabolic diseases, in addition to alcohol intolerance, nitroglycerin tolerance, and carcinoma.[3] Notably, a unique *ALDH2* loss-of-function allele, *ALDH2**2, is found in approximately 50% of the East Asian and 8% of the global populations. It has been reported that this *ALDH2* mutation is associated with a higher prevalence of diabetes in coronary artery disease (CAD) patients, accompanied with increased C-reactive protein (CRP) levels.[4] *ALDH2* mutation is also related to various diabetes risk factors, but the direct correlation remains elusive. Herein, we have explored the potential pathogenicity and mechanisms of *ALDH2* deficiency in the development of type 2 diabetes in both laboratory and clinical settings.

To investigate the metabolic disorder caused by *ALDH2* loss-of-function, wild type (WT) and *ALDH2*-knockout (*ALDH2*-KO) mice were fed either normal diet (ND) or high-fat diet (HFD) for 26 weeks. Intraperitoneal glucose tolerance tests (IPGTTs) were used to measure fasting glucose and glucose tolerance. Our data demonstrated that the fasting glucose levels and IPGTT curves were similar between WT and *ALDH2*-KO mice at 6 weeks [Figure 1A–C]. However, at 32 weeks, the ALDH2-KO mice exhibited elevated fasting glucose levels and impaired glucose tolerance compared with the WT mice. These differences observed between the WT and KO mice were much more profound with HFD [Figure 1D–F], suggesting an independent role of *ALDH2* deficiency in diabetes that can be exacerbated by high fat intake. Skeletal muscle is the most insulin-sensitive, glucose-consuming organ. Therefore, we further tested whether *ALDH2* deficiency influenced skeletal muscle insulin sensitivity. The quadriceps of WT and KO mice were isolated after 32 weeks of HFD or ND, and GLUT-1 and GLUT-4 levels were evaluated. It is known that both proteins mediate glucose uptake in skeletal muscle; however, GLUT-1 is usually stably expressed, whereas GLUT-4 is the main regulatory target of insulin and is thus essential for insulin sensitivity. Immunoblot analysis revealed significantly decreased GLUT-4, but not GLUT-1, expression levels in the KO mice, and these levels were further decreased in the HFD-fed KO mice [Figure 1G–I]. Many reports have suggested that over activation of skeletal muscle autophagy, a cell renewal and catabolic process, exacerbates insulin resistance in diabetes, and *ALDH2* has been considered to be deeply involved in its regulation. Therefore, we evaluated the levels of autophagic end product, p62, as an autophagic marker as well as the expression and phosphorylation levels of the components of the Akt/AMPK/mTOR signaling pathway to illustrate the molecu-

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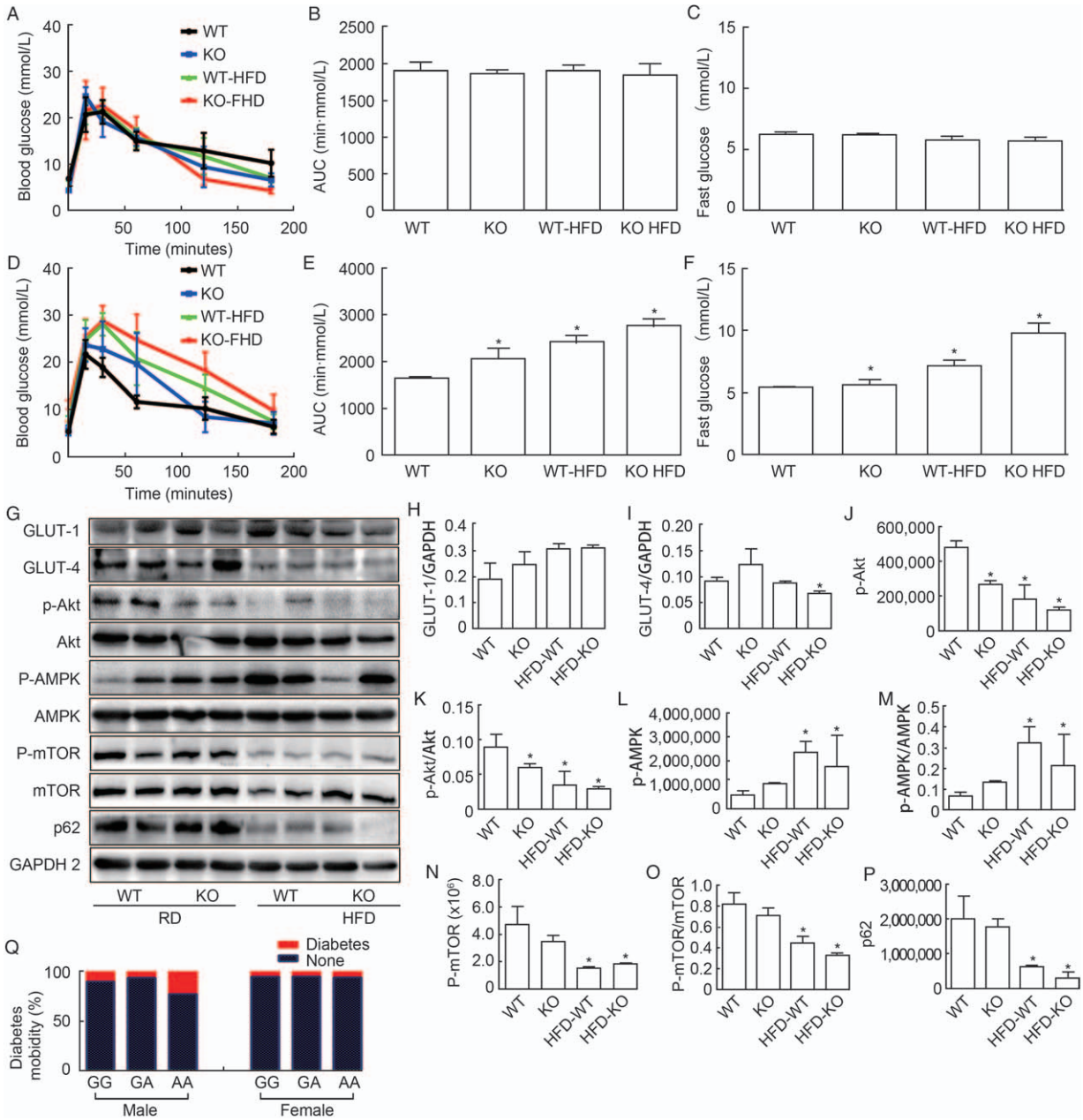

Figure 1: *ALDH2* deficiency contributed to the development of type 2 diabetes in both mice and humans. IPGTTs of WT and *ALDH2*-KO mice with or without HFD at 6 weeks (A) and 32 weeks (D). IPGTT AUC at 6 weeks (B) and 32 weeks (E). Fasting serum glucose levels at 6 weeks (C) and 32 weeks (F). $n = 6$, $^{*}P < 0.05$. (G) Representative Western blotting images of GLUT-1, GLUT-4, p-Akt, Akt, p-AMPK, AMPK, p-mTOR, mTOR, and p62. (H–P) Quantitative analysis of GLUT-1, GLUT-4, p-Akt, p-Akt/Akt, p-AMPK, p-AMPK/AMPK, p-mTOR, p-mTOR/mTOR, and p62. $n = 3$–4, $^{*}P < 0.05$. (Q) Diabetes prevalence in male and female study participants with the *ALDH2* GG, GA, or AA genotypes. Chi-square test, $P < 0.05$. ALDH2: Aldehyde dehydrogenase; *ALDH2*-KO: *ALDH2*-knockout; AUC: Area under the curve; HFD: High-fat diet; IPGTTs: Intraperitoneal glucose tolerance tests; WT: Wild type.

lar mechanisms of *ALDH2* deficiency in the development of diabetes. Our data showed that p62 levels were decreased in the HFD-fed WT mice and were further decreased in the HFD-fed KO mice [Figure 1G and P], indicating upregulated autophagy. Furthermore, the phosphorylation levels of Akt and mTOR were reduced in the KO mice and were further decreased in the HFD-fed KO mice [Figure 1G, J, K, M, and O]. Although AMPK phosphorylation levels were elevated in the KO mice, there was no significant difference between these levels in the HFD-fed WT and KO mice [Figure 1G, L, and M]. These results suggest that *ALDH2* deficiency may inhibit mTOR phosphorylation and thus activate autophagy via the suppression of Akt phosphorylation, and possibly partially via AMPK phosphorylation, which may mediate insulin resistance.

We also evaluated the correlation between *ALDH2* genotypes and the prevalence of diabetes mellitus in humans. A total of 850 consecutive patients who underwent routine, annual health examinations at the Health Management Center of the Huashan Hospital, Fudan University between November 2014 and December 2015 were enrolled in this study. The study protocol was approved by the ethical review board of the Huashan Hospital, Fudan University, and all participants provided written informed consent. The follow-

ing criteria were used to define diabetes: (1) the patient was previously diagnosed with diabetes or (2) the patient's serum fasting glucose levels were ≥7 mmol/L. Out of the 850 study patients, 70 had diabetes (8.2%). The patients were divided into non-diabetic and diabetic groups. Baseline data showed that the diabetic group exhibited a significantly higher rate of males (78.51% *vs.* 67.05%), an older average age (52.38 ± 1.18 *vs.* 47.66 ± 0.39 years), and a higher hypertension rate (52.86% *vs.* 22.31%) compared with the non-diabetic group [Supplementary Table 1, http://links.lww.com/CM9/A472]. Our results revealed that *ALDH2* AA homozygotes, particularly in males, displayed a higher rate in the prevalence of diabetes compared with those carrying at least one functional wild-type of ALDH2*1 allele. The prevalence of diabetes in the males with the *ALDH2* AA genotype reached 22.73%, which was two times more than that of the male patients with the other genotypes [Figure 1Q]. However, the prevalence of diabetes was lower and much more comparable among the female patients of all three *ALDH2* genotype groups. Therefore, these findings suggest that *ALDH2* mutation may contribute to diabetes risk in the Chinese population, particularly in males.

There have been several small clinical studies regarding the association of *ALDH2* mutation and diabetes; however, the conclusions of these studies are somewhat limited due to biological sex, alcohol consumption, and comorbidities. Recently, a meta-analysis of genome-wide association studies included 433,540 East Asian individuals and focused on the identification of loci associated with type 2 diabetes. A 2-Mb single-nucleotide polymorphism (SNP) near *ALDH2* (rs12231737) was found to be significantly correlated with the prevalence of type 2 diabetes. Interestingly, the SNP exhibited strong differences between sexes, with compelling evidence of this association in males but not in females.[5] Our study demonstrates that *ALDH2* possesses an intrinsic capacity to independently trigger type 2 diabetes or to interact with environmental factors, such as diet and age. These effects may possibly be through the unnecessary activation of autophagy via the Akt/AMPK/mTOR signaling pathway in skeletal muscle.

Furthermore, the ultimate goal of diabetes treatment has changed from simple glycemic control to simultaneous cardiovascular protection, and the use of sodium-glucose cotransporter-2 (SGLT-2), dipeptidyl peptidase 4 (DPP4) inhibitors, or glucagon-like peptide-1 (GLP-1) analogs strongly represents this shift. Therefore, *ALDH2* supplementation or activation represents a potential therapeutic target for the treatment of diabetes, because of its extensive cardioprotective role beyond insulin sensitivity regulation.

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Conflicts of interest

None.

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