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LETTER TO EDITOR

Whole-genome methylome analysis reveals age-related diabetes risk factors

Dear Editor,

Diabetes was one of the most common health problems around the world, with increasing morbidity.[1] Previous studies showed that the major pathogenesis contained a lack of insulin secretion and the occurrence of insulin resistance, which was caused by both genetics and epigenetics factors.[2] As the genetic factors of diabetes, some diabetes susceptibility genes had been demonstrated to have more risky single nucleotide polymorphisms (SNPs) in Asians than Africans.[3] However, it still could not explain why Asian babies were less likely to get diabetes, although they had the same risky SNPs as adults.

Here, we studied this enigma from the perspective of epigenetics. As shown in Figure S1, we collected the whole blood sample from many pedigrees, which covered approximately all ages, from 0 to 88 years old (Figure S2). Based on each sample's known age, we calculated the correlation between DNA methylation level and age for each CpG site. Then 3690 age-related autosomal methylated CpG sites (Pearson correlation > 0.8) were identified, and most of them showed age-related hypomethylation trends (Figure 1A(a)). Besides, approximately 27% and 30% of these CpG sites located in the GpG island and promoter region, respectively (Figure 1A(b)). The kyoto encyclopedia of genes and genomes (KEGG) database revealed that most of them were involved in diseases whose prevalence increased with age, such as diabetes (Figure S3). Specifically, it indicated that age-related DNA methylation change in healthy individuals might contribute to the risk of diabetes.

To further investigate the relationship between age-related DNA methylation and diabetes, we identified the significantly differentially methylated sites (DMSs) based on different divisions of age groups. First, we made the two age groups comparison and found the DMSs between "age < 6" and "age ≥ 6" group (Figure 1A(c) and (d)). Results suggested that hexokinase-1 (*HK1*) and hexokinase-2 (*HK2*) encoded key proteins in the glycolysis were hypomethylated in children. Further analysis demonstrated that *HK1* (Figure 1B(a)) and *HK2* (Figure 1B(d)) were hypermethylated with age and keep stable in adults. Next, we made the five age groups comparison and found the continuous hypermethylated CpG sites among all the age ranges (Figure 1A(e) and (f)). We found insulin receptor substrate-2 (*IRS2*), which was responsible for transporting the signal from insulin or insulin-like growth factor-1 to the downstream pathways such as PI3K/Akt and ERK/MAPK signaling.[4] *IRS2* (Figure 1B(g)) showed a continuous hypermethylation trend among the five age groups. Previous reports had demonstrated that dysregulation or mutation of *IRS2* could lead to diabetes.[5,6] Based on previous and our data, we could hypothesize that the rising methylation level of the promoter region with age might downregulated *IRS2*'s expression in older people and increased the risk of diabetes. Interestingly, these three CpG sites showed similar hypermethylation trend in parents compared with their babies in six families with newborn twins (Figure 1B(j)).[7] Based on the previous statistics and our data, we could hypothesize that the hypermethylation of these genes in the *HK1*, *HK2*, and *IRS2* genes, which were all responsible for insulin function, might cause Asian adults' high incidence of diabetes compared with children.

To further validate our hypothesis, we built locally weighted regression models for the three identified CpG sites. We found an individual with abnormal hypermethylation of *HK1* (Figure 1B(c)), *HK2* (Figure 1B(f)), and *IRS2* (Figure 1B(i)) compared with the older people. So, we subsequently did a clinical examination for him. As expected, he was a diabetes patient. This result further validated our hypothesis.

Additionally, we downloaded some public whole blood data from diabetes patients.[8] Combined with our data collected from healthy individuals, we found the methylation levels of *HK1*, *HK2*, and *IRS2* increased with age in

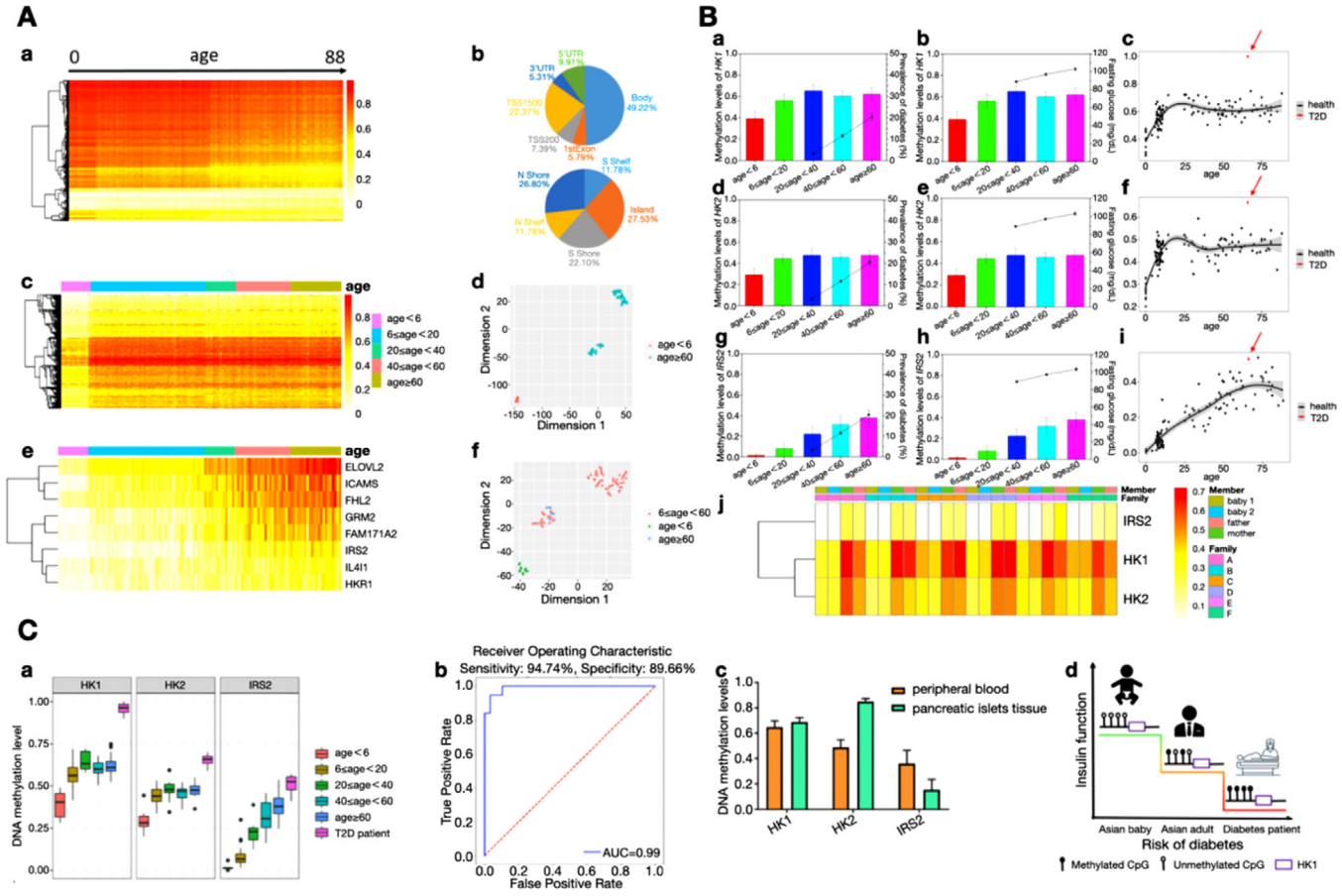

FIGURE 1 **A,** The age-related DNA hypermethylated genes were related to insulin function. **(a)** Heatmap showing the 3690 age-related autosomal methylated CpG sites (Pearson correlation > 0.8). The individuals were sorted by age. **(b)** Pieplots showing the function region distribution of these age-related CpG sites. **(c)** Heatmap showing the DNA methylation profile of hypomethylated CpG sites in the people aged <6 years compared with the people aged >6 years. These sites obtained by the two age groups comparison were methylated in the age ≥6 group. **(d)** tSNE results of individuals using methylation levels of CpG sites identified by two age groups comparison. **(e)** Heatmap showing the DNA methylation profile of gradually hypermethylated CpG sites with five age groups ($0 \leq$ age < 6, $6 \leq$ age < 20, $20 \leq$ age < 40, $40 \leq$ age < 60, age ≥ 60). **(f)** tSNE results of individuals using methylation levels of CpG sites identified by five age groups comparison. **B,** Age-related methylation of *HK1*, *HK2*, and *IRS2* in the healthy population and aberrant hypermethylation of them in diabetes patients. The correlation of diabetes prevalence (point and line) and methylation levels change (bar plot) of *HK1* **(a)**, *HK2* **(d)**, and *IRS2* **(g)** with age. The correlation of fasting glucose (point and line) and methylation levels change (bar plot) of *HK1* **(b)**, *HK2* **(e)**, and *IRS2* **(h)** with age. The locally weighted regression models for the increased methylation levels of *HK1* **(c)**, *HK2* **(f)**, and *IRS2* **(i)** with age in the healthy population (black points and line) and diabetes patients (red points and arrows). **(j)** The methylation levels of *HK1*, *HK2*, and *IRS2* in six healthy families contained twins. **C,** Age-related methylated *HK1*, *HK2*, and *IRS2* were correlated with the risk of diabetes. **(a)** Boxplots showing the comparison of age-related methylated *HK1*, *HK2*, and *IRS2* in healthy controls with the previously reported aberrant high methylation levels of them in diabetes patients. **(b)** The receiver operating characteristic analysis of the diabetes risk model constructed using our and previously reported data. **(c)** The methylation profile of *HK1*, *HK2*, and *IRS2* in Europeans' peripheral blood and pancreatic islets tissues. **(d)** Model showing the relationship of age-related epigenetics regulation of *HK1*, which is involved in the insulin function and the risk of diabetes

the healthy population. In contrast, those in the diabetes patients whose age was <60 were obviously higher than age-matched or healthy older controls (Figure 1C(a)). Next, we randomly divided our and this public data into independent training and test dataset, respectively. Then a diabetes risk model was constructed using a random forest algorithm and training dataset. The test result showed its sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were 94.74%, 89.66%, and 0.99, respectively (Figure 1C(b) and Table S1). Additionally, our result showed that the methylation level of *HK1* identified from blood was conservative in pancreatic islets tissues, while those of *HK2* and *IRS2* was not (Figure 1C(c)). It enabled the methylation level of *HK1* to be considered as a potential biomarker for the risk of diabetes in healthy people (Figure 1C(d)).

In summary, this study provides a new perspective on the relationship between insulin function and age-related DNA methylation. Furthermore, the methylation levels of these CpG sites in the *HK1*, *HK2*, and *IRS2* genes were positively correlated with age and the risk of diabetes. Therefore, we could hypothesize that reducing methylation levels of these risk CpG sites might delay the age at onset of diabetes. Moreover, the *HK1*'s methylation level in the whole blood can be considered as a potential biomarker for the risk of diabetes in healthy individuals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data and materials are available upon reasonable request.

ETHICAL APPROVAL

Our study was approved by the Ethics Committee of Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. All individuals were adequately informed and signed an informed consent form before participating in the study.

AUTHOR CONTRIBUTIONS

Yingli Sun and Jie Qiao conceived the study and interpreted the data. Luo Hai, Zongzhi Liu, Wei Chen facilitated its designs. Luo Hai and Zongzhi Liu wrote the paper with the assistance of Yingli Sun. All authors revised the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.