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Letter to the editor

Transgenerational analysis of H3K4me3 and H3K27me3 by ChIP-Seq links epigenetic inheritance to metabolism

Histone methylation is a kind of important epigenetic modification which occurs on the lysine residue or arginine residue of histone tails (Zhang and Reinberg, 2001). It takes part in multiple biological processes, including gene expression, genomic stability, stem cell maturity, genetic imprinting, mitosis and development (Fischle et al., 2005). Abnormal histone methylation pattern may lead to a series of disorders, such as metabolic diseases, psychiatric disorders and cancer (Seligson et al., 2005; Peter and Akbarian, 2011). The transmission pattern of genetic information is extremely consistent with Mendel's law, while the epigenetic transmission pattern between generations remains to be elucidated. An increasing number of studies show that many diseases such as cancer, diabetes and obesity cannot be explained by genetics alone and may be related to epigenetic transmission (Jones and Baylin, 2007; Kaelin and McKnight, 2013; Radford et al., 2014). So it is of great importance to figure out the epigenetic transmission pattern through generations.

A number of researches have reported the inheritance pattern of DNA methylation during early embryonic development process in lower organisms and mammals (Wang et al., 2014). Fewer studies were carried out about histone methylation inheritance due to its complexity and difficulty. A study in mammals reported that reduction of H3K4 dimethylation in sperm impaired development and survivability of offspring and persisted transgenerationally (Siklenka et al., 2015). Another research showed the reprogramming of H3K4me3 in mouse early development that paternal H3K4me3 peaks were depleted in zygotes and reappeared at late two-cell stage while broad peaks of H3K4me3 exist at promoters and distal loci (Liu et al., 2016; Zhang et al., 2016a). They also found an extensive loss of H3K27me3 at the promoter region of developmental genes accompanied by global erasure of sperm H3K27me3 but inheritance of distal H3K27me3 from oocytes in mouse (Liu et al., 2016; Zheng et al., 2016). Researches above are about either the lower organisms or early embryos. However, in the study of pedigree, it is still unclear whether histone methylation could be inherited stably between generations, and what roles the stably inherited epigenetic states play in biological processes.

In this study, we collected 17 male volunteers' whole blood from seven families of CAO Cao descendants in Shandong (Rushan), Anhui and Zhejiang provinces (Wang et al., 2012) (Fig. S1A). The relationships of all volunteers are shown in Fig. S1A. There are five members at most and one member at least in each family. The oldest member is 86 years old and the youngest one is 16 years old. To investigate the genome-wide distributions of the two histone methylation H3K4me3 and H3K27me3, we chose chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) technology to obtain the raw data. All samples had two replicates and the accessible reads of each sample were greater than 20 million, which was sufficient for subsequent analysis (Tables S1, S2 and S3). Sequencing reads were aligned to the appropriate human reference genome (hg38) using BWA with default parameters. At the same time, we made statistical analysis of the enrichment level on the reads in different genomic regions and found that the peaks mainly distributed in intron and intergenic regions (Fig. S2).

Our previous study showed that DNA methylation was evolutionarily conserved on the Y chromosome (Zhang et al., 2016b). Here, we assessed whether active chromatin modification H3K4me3 and repressive chromatin modification H3K27me3 binding events also co-occurred across the human 17 samples. We performed genome-wide screen of these chromatin modification peaks to identify co-occurred peaks with overlapping at least 1 bp across 17 samples. There were 9757 common peaks of H3K4me3 among 17 samples and 1011 common peaks of H3K27me3 among 17 samples. We separately selected top 1000 peaks for two modifications according to standard deviation range from small to large and then annotated these peaks to associated RefGenes. About 80% of the common peaks located at distal regions from transcription start site (TSS) (at −500 kb to −5 kb and +5 kb to 500 kb from TSS), which is distinctly different from all peak distribution patterns around TSS (Fig. S1B). These results indicated that the relatively conserved peaks may play a particular role in biological functions at distal TSS.

Members in the same family may have the similar diet and living habits, which may have a significant influence on epigenomes of human bodies. To detect family-specific binding regions, we defined the peak that existed in all members within a family and not appeared in other families as family-specific binding regions. For H3K4me3 modifications, we detected 3999, 49, 65 and 313 family-specific binding regions for RS_FamilyA, RS_FamilyB, RS_FamilyC and RS_FamilyD, respectively (Fig. S3A). After classifying above enriched regions to associated genes, these peaks were associated with 1741, 22, 33 and 91 family-specific genes in families (Fig. S3B). In order to study their functions, we used the GREAT (version 3.0) to perform gene ontology (GO) analysis for the

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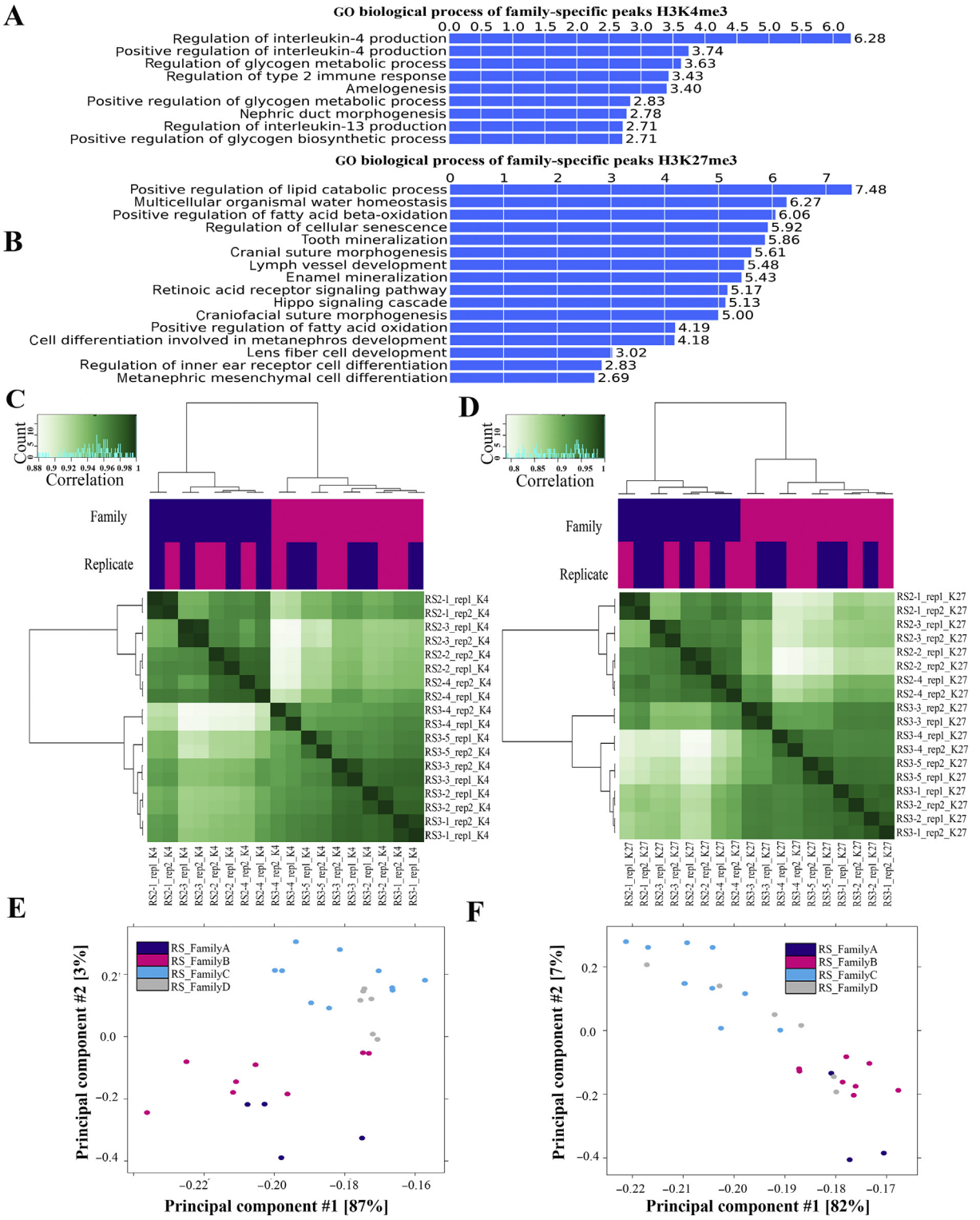

Fig. 1. Identification of family-specific binding sites of H3K4me3 and H3K27me3 modification in Rushan samples. **A** and **B:** GO biological process enrichment of family-specific binding sites of H3K4me3 (**A**) and H3K27me3 (**B**). The length of blue horizontal histogram represents binomial *p* value ($-\log_{10}$). **C** and **D:** Correlation heat map shows the clustering of two big Rushan family samples using only differential binding sites of H3K4me3 (**C**) and H3K27me3 (**D**). The names of samples are shown at right and below. **E** and **F:** Principal component analysis of H3K4me3 (**E**) and H3K27me3 (**F**) of all Rushan samples. Each dot presents a sample and the legend indicates family names.

detected family-specific regions (McLean et al., 2010). Results indicated that the peaks were enriched on the genes associated with three glycogen metabolic pathways (Fig. 1A). Additionally, we also analyzed the family-specific peaks for H3K27me3. We found that there were 3045, 420, 412 and 1914 family-specific binding regions for RS_FamilyA, RS_FamilyB, RS_FamilyC and RS_FamilyD, respectively (Fig. S3C). These family-specific peaks of H3K27me3 were annotated to 492, 82, 211 and 1296 family-specific genes, respectively (Fig. S3D). Through the GO analysis, we found that the peaks of H3K27me3 associated genes were related to three lipid metabolic processes (Fig. 1B). We used DiffBind R package to analyze the differential binding sites. We did sample cluster analysis of H3K4me3 (Fig. 1C and E) and H3K27me3 (Fig. 1D and F) using Rushan samples and all 17 samples (Fig. S4). We found that the histone modification pattern of members in the same family can be clustered together but cannot be clustered according to the distance of genetic relationship.

Through the bioinformatic analysis of histone modification pattern of H3K4me3 and H3K27me3, we found that both H3K4me3 and H3K27me3 histone modifications had a family-dependent conservation. Additionally, H3K27me3 was more conservative compared with H3K4me3. Transgenerational epigenetic inheritance is a topic of great interest with many unclear questions. It has been reported in animal experiments that the impact of nutrition, smoking and irradiation may affect the children's phenotype, and parent and offspring have similar epigenetic profiles (Radford et al., 2014). The family specific methylation pattern of H3K4me3 and H3K27me3 between generations indicated that both of the two histone methylations may be transgenerationally inheritable. Several studies have found that some small molecules *in vivo*, such as miRNA, tRNA and prions, play important roles in regulating the epigenetic modification inheritance between generations (Uptain and Lindquist, 2002; Halfmann and Lindquist, 2010; Gapp et al., 2014; Huypens et al., 2016). Here, we firstly used the haplogroup samples as research model to study the inheritance of histone modification between generations.

Our results also showed that the conservative modifications of H3K4me3 and H3K27me3 were separately enriched in three metabolic glycogen pathways and three metabolic lipid pathways, indicating that both of the two histone methylations were important for the normal physiological functions. Histone modifications can not only influence the structure of chromatin but also serve as recognition elements for proteins binding particular modifications. Each of these modifications is closely related to the metabolic state and catalytic processes of the cell. It is interesting that the family specific histone methylation pattern is linked with metabolism. Our result indicated that inheritable epigenetic variations may also make contribution to inheritable metabolic abnormalities.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jgg.2017.11.004.

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