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Short Communication

Deep pedigree analysis reveals family specific "fingerprint" pattern of DNA methylation for men

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DNA methylation plays an essential role in mammalian development [1]. However, how DNA methylation is inherited between generations and if there is family-specific DNA methylation pattern remains to be elucidated [2]. In this study, we collect male blood samples including a big pedigree of the descendants of an ancient Chinese empire and samples from different haplogroups to study their whole genome DNA methylation pattern. We find 115 male family-specific methylation sites from three families. The difference of whole genome DNA methylation pattern correlates with the remote divergence time between samples. Overall, we showed a high correlation between the DNA methylation profile and the blood relationship within male family members, but not female family members.

To address if there is family specific DNA methylation pattern, it is very important to establish a big pedigree. We took the advantage of the haplogroup platform we established previously. Haplogroup is a group of haplotype on Y chromosome that share the same SNP ancestor. Because Y chromosome has unique paternal heredity pattern and 95% region is non-homologous recombinant area, Y chromosome DNA haplogroup is a powerful tool for genetic research. In previous work, we analyzed epigenetic pattern of the Y chromosome in 72 healthy male members who belong to 12 different haplogroups [3]. Our work showed that the DNA methylation pattern on the Y chromosome is highly conserved among all haplogroups [3].

In this study, we further investigated the whole genome DNA methylation pattern of different haplogroup samples. Interestingly, the whole DNA methylation pattern showed very different pattern than DNA methylation pattern of Y chromosome. We found that the DNA methylation profile of family male members correlated with their blood relationship. According to deep-rooted pedigrees and the latest Y chromosome phylogenetic tree, we collected 11 healthy male samples from Rushan, Shandong, China. They came from three families (families A, B, and C) and they were all the descendants of Emperor CAO Cao (155 AD–220 AD), one of the most famous Emperors in China (Figs. 1a and S1a). The DNA methylation levels of all samples were detected using the Illumina 450 K methylation microarray (Illumina, Inc., San Diego, CA, USA). After removing the CpG sites containing missing values or with detection *P*-values greater than 0.05, 47.5 million sites on the whole genome were obtained for further analysis.

First, we found that the methylation pattern of immediate relatives was more similar than that of other male samples (Fig. S1b). Next, we further analyzed the methylation sites with one or more of the sites in the panel changed remarkably ($|\Delta\beta\text{-value}| \geq 0.2$). Results indicated that the methylation profile was correlated with the family members within family tree (Fig. S1c, Online). In specific, among each family's members, the DNA methylation pattern of sons was more similar with their father than with their uncles (Fig. S1b and d, Online).

Moreover, we identified some family specific methylation sites (Wilcoxon Rank-sum Test, FDR-adjusted $P < 0.05$, and $|\text{beta\_difference}| \geq 0.1$) by comparing the mean β-value between different families (Figs. 1b, c, S1e and Table S1, Online). Moreover, these

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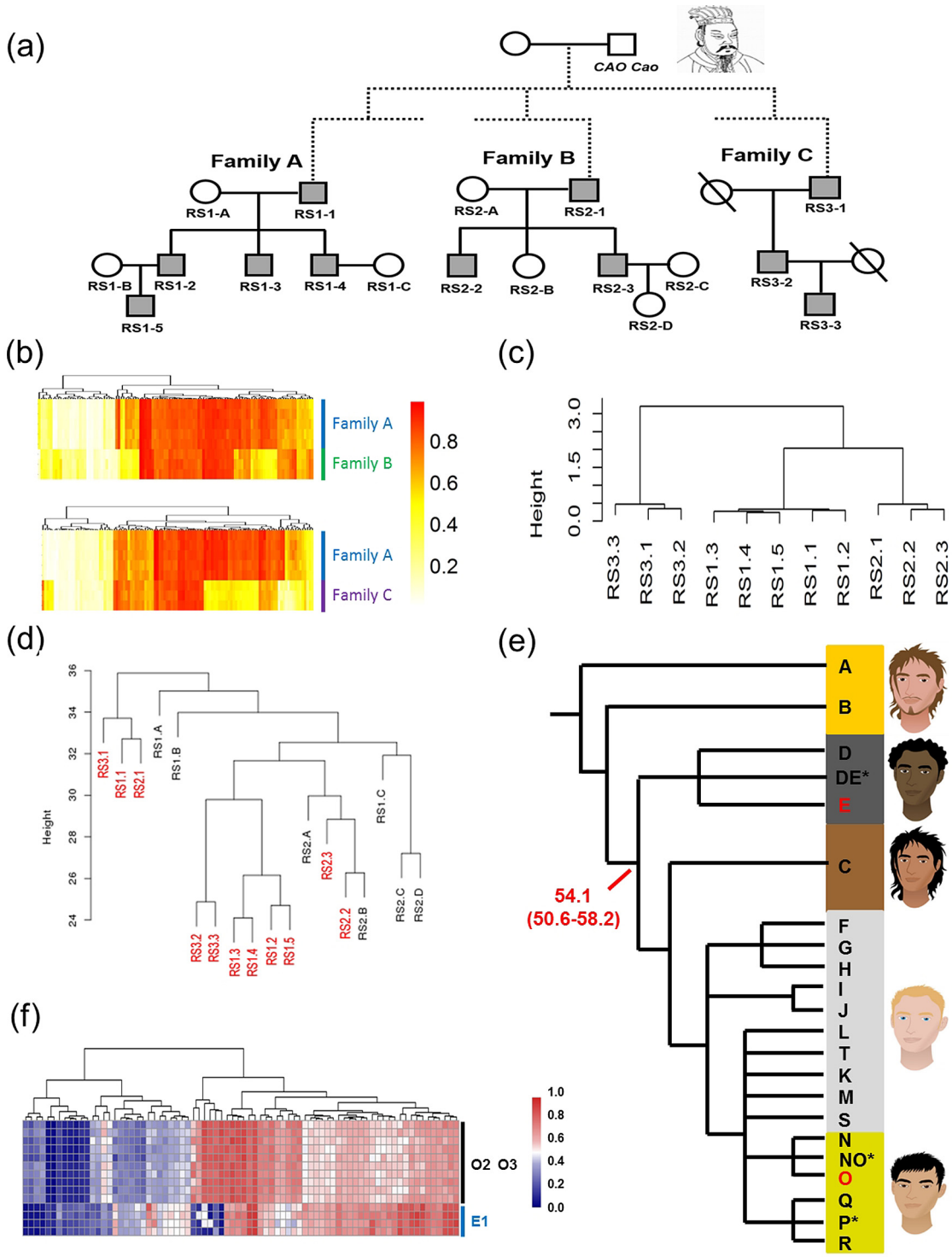

Fig. 1. Only males', but not females' DNA methylation profiles are positively correlated with their blood relationship. (a) A pedigree of three families with both male and female samples. (b) Heatmap showing cluster result of 11 male family members using methylation pattern of only 115 family specific sites. (c) Similar dendrogram of hierarchical clustering of 11 male family members using methylation pattern of only 115 family specific sites. (d) Comparison of hierarchical clustering of 11 male (red) and 7 female (black) family samples using the methylation pattern of 47.5 million DNA methylation sites. (e) The phylogenetic tree showing the coalescence time for different haplogroup samples. Numbers in red indicate the coalescence time (in years) and 95% confidence interval of the node. The DNA methylation pattern of haplogroup E and haplogroup O male samples were studied. (f) Heat map showing the methylation level of haplogroup O and E samples' specific methylation sites.

family-specific methylation sites were mostly located in non-CpG Islands (46%). About 33% family-specific methylation sites located within the promoter region of genes (23% for TSS1500 and 10% for TSS200) (Fig. S1f, g). Interestingly, our results indicated that the DNA methylation profile correlated with family members' blood relationships.

To further investigate if the family specific DNA methylation pattern was associated with their similar genetic background, we studied the DNA methylation of both maternal and paternal members. Interestingly, we found that only the methylation profile of male samples, but not of the female samples, correlated with their family tree distance.

We collected some female samples of family A and family B to test whether their methylation profile correlated with the family tree (Fig. 1a). Results showed that the methylation pattern of female samples could not be properly clustered into their family tree respectively (Fig. 1d). Further, through analyzing the methylation sites with one or more sites in the panel changed remarkably ($|\Delta\beta\text{-value}| \geq 0.2$), we also found that only the methylation profile of male samples, but not of the female samples, is correlated with their family tree, through principal component analysis (PCA) (Fig. S2). We also performed another analysis using bootstrapping, K-means clustering and *M* value. The *M* value of males is significantly less than females (one-tail *t* test, $P < 0.001$), which also means males', but not females' methylation profile is correlated with the family tree (Table S2, Online). This conclusion is consistent with hierarchical clustering (Fig. 1d) and PCA (Fig. S2, Online).

We then showed that the DNA methylation not only correlated with blood relationship within a family but also clustered with haplogroup. The whole genome DNA methylation profile of different sub-haplogroup samples also correlated to their blood relationships. According to deep-rooted pedigrees and the latest Y chromosome phylogenetic tree, we collected different sub-haplogroup male samples (Fig. S3a, Online). All these samples came from China, and most of the samples belonged to sub-haplogroup O2 and sub-haplogroup O3. Results showed that all the young samples could be clustered depend on the different sub-haplogroup tree (Fig. S3d, Online), no matter where they came from and what kind of environment they lived in. Moreover, through comparison the methylation pattern of sub-haplogroup O2 and O3 samples, some sub-haplogroup specific methylation sites were discovered (Fig. S3c, Table S3, Online). These sub-haplogroup specific methylation sites were mostly located in non-CpG Islands (37%) (Fig. S4a, Online). Further, about 15% sites located within the promoter region of genes (9% for TSS1500 and 6% for TSS200) (Fig. S4b, Online). Interestingly, 11% of these sites located in human housekeeping genes (Fig. S4c, Online), which indicated that these different methylation sites would play an important role in specific sub-haplogroup samples.

According to above findings, we proposed that the DNA methylation pattern would be different for different haplogroup samples. To test our hypothesis, we analyzed the DNA methylation pattern of haplogroup O2, O3 and haplogroup E samples. The divergence time between these samples was about 54.1 thousand years ago (kya) (Fig. 1e). The results showed that the methylation pattern of the same haplogroup samples was more similar than that of different haplogroup male samples (Fig. S5a, Online). Moreover, we further found that the more remote relationship it is, the more differential methylation sites there were (Fig. S5b, Online). In our work, we also include haplogroup E from Africa. We found more significant difference between haplogroup O and E samples than between sub-haplogroup O2 and O3 samples (Figs. 1f, S5b, Table S4, Online), indicating that with the increasing divergence time, the degree of different methylation level was more significant.

Several studies and our results have revealed that DNA methylation pattern would change with age [4,5]. Next, we further study the different methylation pattern between senior and young male samples. Results showed there was bigger difference between senior and young male samples (Figs. 1c, S6a, Online). However, the levels of methylation difference were weak (Fig. S6a, Online). Therefore, family specific DNA methylation sites showed lower level of specify in senior person (>60 years-old).

Further analysis showed that these age-specific methylation sites were mostly located in non-CpG Islands (57%) and gene body (35%) regions (Fig. S6b, c, Online). Through functional annotation analysis, we found that these age-specific associated genes were enriched in neuroactive ligand-receptor interaction and vascular smooth muscle contraction pathway, which would be associated with the gerontal disease (Fig. S6d, Online). Our results indicated that with the growth of age, the relationship between DNA methylation profile and genetic relationship became gradually weakened, which could also resulted from the accumulation epigenetic variations in senior male samples.

Overall, through study the DNA methylation pattern of 72 healthy male blood samples we found that there was a high correlation between the DNA methylation profile and the blood relationship. In specific, the DNA methylation pattern of paternal samples (e.g. grandfather, father and son) was more similar and shared 115 family-specific methylation sites. Further analysis confirmed that all *trans*-general families employed in our study exhibited this kind of DNA methylation "fingerprint" for male family members.

Our results indicated that DNA methylation could be used for paternity test in the future. Our results also showed that the DNA methylation pattern of the same haplogroup has a higher degree of similarity than that from different haplogroups.

Our work provides a very interesting way to identify the relationship between male members using the DNA methylation profile in the future. This novel finding about DNA methylation could be of many potential applications. For example, DNA methylation comparison could be performed for paternity test when the biological father refused to cooperate. It can also help a family to find the lost member when parent's DNA is not available. By detecting DNA methylation, multiple relative's DNA could be used as alternative way when parent's DNA is absent.

Several studies showed that the genome-wide DNA methylation would undergo methylation reprogramming during early embryonic development [6,7]. However, in our study, we found some family or haplogroup specific methylation sites, which stably inherited between generations. Some studies also found that there were some small molecules *in vivo*, such as miRNA, tRNA and prions, play an important role in regulating the epigenetic modifications inheritance between generations [2,8–10]. The mechanism underline above phenomenon requires further investigation in the future.

In summary, we found that the similarity degree of a man's, but not a woman's DNA methylation profile with others is positively correlated with their blood relationship (father > uncle > other men in family > men from the same sub-haplogroup > men from the different sub-haplogroup but same haplogroup > men from different haplogroup).

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.scib.2017.12.007.

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