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Exploring of rare differences in mtGenomes between MZ twins using massively parallel sequencing

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ABSTRACT

Compared with nuclear DNA, fewer DNA repair mechanisms in mitochondria and lack of proofreading capabilities in the mtDNA polymerase help introduce more variability between MZ twins. In our previous study, we used ultra-deep mtGenome sequencing to characterize point heteroplasmy and nucleotide variant in blood samples of MZ twins. In the present study, we characterize minor differences of mtGenomes in saliva and hair shaft samples from six sets of MZ twins using the Precision ID mtDNA Whole Genome Panel, Ion S5 XL system, and Converge Software. Additionally, the effectiveness of different tissue samples for differentiating between MZ twins was evaluated. Point heteroplasmies were observed in all sets of MZ twins regardless of sample type. Overall, more variants were observed in the mtGenome from hair shaft samples than that from blood and saliva samples. The results of this study further support that the mtGenome analysis could be used to distinguish MZ twins from each other.

1. Introduction

Monozygotic (MZ) twins, considered to be genetically identical, cannot be distinguished one from another by standard forensic DNA testing, i.e. short tandem repeat (STR) genotyping [1]. Weber-Lehmann et al. [2] employed whole genome sequencing to identify rare de novo mutations between MZ twins, and reported that five single nucleotide polymorphisms (SNPs) exist in the twin A but not in the twin B, which can be used for distinguishing MZ twins. Compared with nuclear DNA, mitochondrial DNA (mtDNA), with 10-fold higher mutation rate relative to nuclear DNA, helps introduce more variability in mitochondrial genome (mtGenome). In our previous study [3], we used long-range PCR and massive parallel sequencing (MPS, the Illumina HiSeq 2000 Sequencing System) to characterize nucleotide difference in blood samples of MZ twins. Point heteroplasmies were observed in eight sets of MZ twins and a single nucleotide variant (nt15301) was detected in five sets of MZ twins. The results demonstrated that mtGenome sequencing has the potential to differentiate between MZ twins. Recently, Thermo Fisher Scientific released the whole mtGenome panel for forensic applications based on the Ion Torrent sequencing platform, the Precision ID mtDNA Whole Genome Panel. This panel was constructed using a small amplicon design (163 bp average amplicon length), optimized with the inclusion of degenerate primers, which could help to obtain balanced coverage and increases success with degraded and low template DNA samples. In this study, we employed the Precision ID mtDNA Whole Genome Panel and the Ion S5 XL system to further characterize minor differences of mtGenomes between MZ twins using not only blood samples but also saliva and hair shaft samples.

2. Materials and methods

2.1. Sample preparation

Human biological samples were collected upon approval of the Ethics Committee of Sichuan University. Venous blood, saliva and hair shaft samples were collected from six sets of MZ twins (20–33 years old) after receiving written informed consents. The monozygosity of the twins was confirmed using standard forensic STR typing with the Huaxia Platinum PCR Amplification Kit (Thermo Fisher Scientific) [4]. The QIAamp DNA Investigator Kit (QIAGEN) was utilized to isolate total (genomic and mitochondrial) DNA according to the manufacturer's protocol. The quantity of the DNA was measured with the Quantifiler Human DNA Quantification Kit (Thermo Fisher Scientific) on an Applied Biosystem 7500 Real-time PCR System according to the manufacturer's recommendations. The DNA samples were normalized to 0.1 ng/μL and stored at −20 °C until mtDNA enrichment.

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2.2. mtDNA amplification, template preparation and sequencing

The mtDNA amplification reactions were performed with the Precision ID Library Kit and the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) using the "conservative" method according to the manufacturer's recommendations. The libraries were purified with AMPure XP reagent (Beckman Coulter) and quantified using the Ion Library TaqMan Quantitation kit (Thermo Fisher Scientific). The purified libraries were normalized to an equal concentration of 30 pM and pooled in equal volume for template preparation. Automated template preparation was performed using 25 μL pooled library with the Ion 520 & Ion 530 Kit on the Ion Chef System (Thermo Fisher Scientific), and sequencing was implemented on the Ion S5 XL Sequencer (Thermo Fisher Scientific) with the loaded Ion 530 chip as per the manufacturer's protocol.

2.3. Data processing

All sequencing data were analyzed using the Torrent Suite Software v5.10.0 and then mapped to the revised Cambridge Reference Sequence. The CoverageAnalysis v5.10.0.3 plugin was applied to conduct coverage analysis. The Converge platform (Thermo Fisher Scientific) was used for further analysis. All variants were directly identified when the read depth ≥ 100X as well as the quality score ≥ 20, and the heteroplasmy detection threshold was set to 10% as suggested in updated guidelines for mtDNA [5]. Variants or variants designated as "Heterozygous" were verified using the Integrative Genomics Viewer (IGV) [6].

3. Results and discussion

The average mapped reads were > 572,000 per individual after filtering the unmapped reads and each sample would be expected to have more than 4700X coverage at each base position of the mtGenome (assuming equal coverage across), suggesting the depth level in this study is sufficient for a reliable detection of heteroplasmy. Point heteroplasmy (PHP) refers to the presence of more than one base call at a specific position and the minor component detection threshold was set at 10.0% in this study. With this threshold, we identified a total of 14 bases presenting varying degrees of heterogeneity (17 PHPs) in 6 sets of MZ twins (Table 1). Among these nucleotide positions, nt16108, nt16209, nt16222 and nt26223 are located in hypervariable region I (HV I) and other 10 positions come from the coding region. What's more, PHPs were not dispersed evenly among three types of samples. PHPs could be detected in mtGenome from hair shaft sample of all MZ twins, however, no PHPs was observed in the blood and saliva samples of the majority of MZ twins. One particular set of twins, MZ 6, showed a remarkably large difference: PHP was observed at four positions and furthermore different base (or major component) was presented in nt16209 (Table 1). This polymorphic site is located in the hypervariable region II (HV2) and in Human Mitochondrial Genome Database (http://www.mtdb.igp.uu.se), 78 C nucleotide (2.88%) was observed in a database of 2704 sequences.

4. Conclusion

In this study, we used the Ion S5 XL System to sequence whole mtGenome of three types of samples from six sets MZ twins. 17 PHPs and one SNP in mtGenomes between MZ twins were observed. By comparing three sample types, mtGenome of hair shaft generally exists more heterogeneity than that of the other two. 72.22% variants (12 PHPs and one SNP) of mtGenomes were presented in hair shaft samples, indicating that hair shaft samples are more suitable for mtGenome sequencing to distinguish MZ twins. Although there is considerably more work to be conducted before mtGenome sequencing could be used in forensic casework, the results of this study further support that the mtGenome analysis could be used to differentiate between MZ twins.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Table 1
The detailed PHPs and sequence variant of MZ twins' mtGenomes.

		MZ 1	MZ 2	MZ 3	MZ 4	MZ 5	MZ 6
PHP	Blood		**T13246** 2A: C 2B: C (88%)				
	Saliva		**A750** 2A: G (90%) 2B: G (83%) **C756** 2A: C (84%) 2B: C			**A750** 5A: G (84%) 5B: G **C6531** 5A: T (75%) 5B: T (65%)	
	Hair shaft	**C4086** 1A: T 1B: T (82%) **G12406** 1A: A 1B: A (88%) **C16108** 1A: T (86%) 1B: T (77%)	**A8860** 2A: G 2B: G (71%)	**T16209** 3A: T (86%) 3B: T (74%)	**T8183** 4A: T 4B: T (80%)	**C6531** 5A: T (64%) 5B: T (75%) **T16209** 5A: T (66%) 5B: T (80%)	**G4294** 6A: G 6B: G (70%) **A12033** 6A: A 6B: A (79%) **C16222** 6A: C (63%) 6B: C **C16223** 6A: T (58%) 6B: T
Variant	Hair shaft						**T16209** 6A: C (62%) 6B: T (80%)

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