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BRIEF COMMUNICATION

Investigation into length heteroplasmy in the mitochondrial DNA control region after treatment with bisulfite

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We report on a method to analyze length heteroplasmy within the human mitochondrial genome in which there are polycytosine [poly(C)] stretches. These poly(C) tracts induce heteroplasmy with the resultant inherent problems of accurate sequence designations. In this study, 20 samples that exhibited length heteroplasmy due to variation in the C-tracts within hypervariable region I (HVI) were treated with bisulfite, and one or more cytosine bases in these C-tracts were converted randomly to uracil. This resulted in an accurate sequence designation for nearly all samples. The only exceptions in which the DNA sequence could still not be determined occurred when there was total conversion, or a lack of conversion, of the cytosine bases. Replicate tests on the same samples showed that individual cytosine bases were randomly converted to uracil. This simple method was useful for investigating length heteroplasmy due to 16189C and 310C transitions in the mitochondrial-DNA control region. It is valuable for medical and forensic investigations.

Length heteroplasmy in the human mitochondrial genome [i.e., mitochondrial DNA (mtDNA)] control region is encountered frequently in populations.[1] This phenomenon is mostly because of polycytosine stretches (i.e., C-tracts).[1–3] The C-tracts are classified by whether they are interrupted with a thymine or uninterrupted and occur as a

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string of cytosine bases. In instances in which the sequence is not interrupted by a thymine, such as 16189C or 310C, a subsequent phase shift pattern occurs in the downstream sequence following the C-tracts of one or more bases. This phase shift creates uncertainty in the number of cytosine bases and makes the subsequent DNA sequence difficult to interpret.

Instances of length heteroplasmy have been studied because of their relationship with a variety of diseases such as diabetes or other metabolic conditions, although several reports do not consistently reproduce an association of common mtDNA variants with the diseases.[4,5] Length variants because of heteroplasmy have been reported in forensic and anthropological studies in which a complex DNA sequence could not be easily resolved, and multiple amplicons from a single sample existed in nearly all such studies.[6,7] Subsequent cloning of amplicons results in length variants within a single clone.[6,8,9]

Bisulfite has been used to determine patterns of DNA methylation.[10] It will convert cytosine bases to uracil but leaves methylated cytosine bases unaffected and has been used in medically related studies.[11] The aim and outcome of previous reports was for the total conversion of the nonmethylated cytosine bases to uracil. This paper reports the application of bisulfite treatment to accurately decode the DNA sequence with known length heteroplasmic C-tracts (nucleotide positions 16184—16193) in the hypervariable region I (HVI) locus by random conversion of nonmethylated cytosine to uracil.

Buccal swabs were collected with informed consent from volunteers after the project had been approved by the Institutional Review Board of National Taiwan University Hospital (Taipei, Taiwan; approval code, 201312041RINB). DNA was extracted from the volunteers and quantified. Prior to the bisulfite treatment, length heteroplasmy within the HVI locus was confirmed by using the standard sequencing method to analyze accurately the DNA sequence from 20 unrelated individuals. These sequences served as a comparison to the post-treated DNA sequences.

Approximately 300 ng of genomic DNA was treated with sodium bisulfite using the MethylEasy Xceed Rapid DNA Bisulphite Modification Kit (Human Genetic Signatures Pty Ltd, North Ryde, NSW, Australia) in accordance with the manufacturer's recommendations.

Whole D-loop amplification of the prebisulfite-treated DNA (approximately 1 ng) was performed using the primer pair L15969 (5′GGACAAATCAGAGAAAAAGTC3′) and H638 (5′ACCAAACCTATTTGTTTATGG3 ′). Polymerase chain reaction (PCR) products were sequenced using the same sequencing primers as those used in the PCR.

To study bisulfite-treated DNA, the primer pair used to amplify the HVI region was L16023 (5′GTTYTTTYAT GGGGAAGYAGATTTGGGT3′) and H16493 (5′ATACARTTCAC TTTARCTACCC3′). The amplification conditions adhered to the manufacturer's recommendation (PyroMark PCR kit; QIAGEN, Turnberry Lane City, Valencia State, USA), except the annealing temperature was modified from 56°C to 50°C. The PCR products were sequenced with sequencing primers

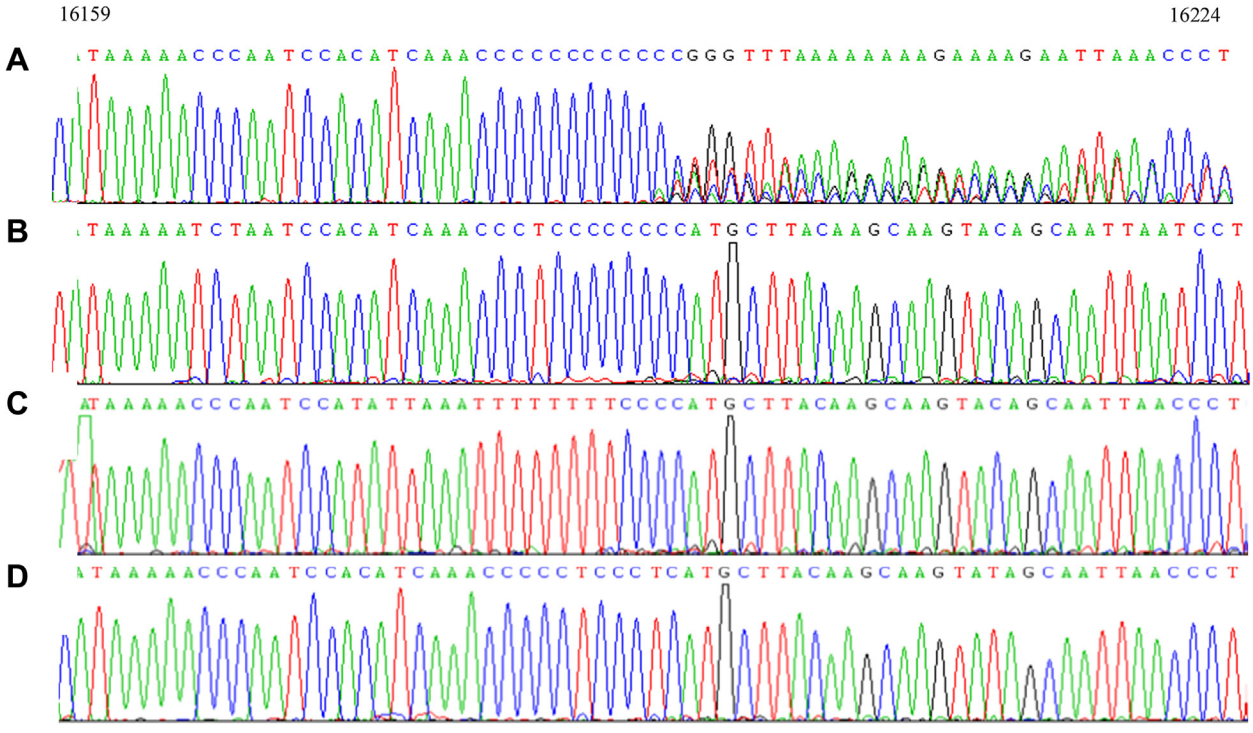

Figure 1 Sequences of (A) prebisulfite treatment and (B—D) postbisulfite treatment from nucleotide positions 16159—16224 in hypervariable region I (HVI) of mitochondrial DNA (mtDNA) control region in Sample 1. Sequences typical of homoplasmy occur when the C-tracts are interrupted by thymine bases. Therefore, the length of the C-tracts can be determined. Each electropherogram in B—D represents the result of one independent test. Sample 1b resulted in a sequence across the poly(C) tract of CCCTCCCCCCCC; 1c, the sequence of TTTTTTTTCCCC; and 1d, the sequence of CCCCCTCCCTC. These are tabulated in Table 1.