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Short communication

An homoplasmic large deletion in mtDNA Control Region: Case report

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ABSTRACT

We report a new case of a large, homoplasmic Control Region deletion in human mitochondrial DNA. A missing 154 bp fragment spanning positions 16154–16307 was found in an apparently healthy blood donor from Salta (NW Argentina) whose maternal lineage was attributable to Native American haplogroup D1. The same mutation, to the best of our knowledge, has been independently reported before only twice, in both homoplasmic and heteroplasmic states.

1. Introduction and aims

Human mtDNA sequence variation has been analyzed for more than 3 decades in research fields like Medical Genetics, Biological Anthropology, Forensic Genetics, etc. The non-coding Control Region (CR) has been the locus of choice in several of those studies, and >50,000 CR sequences have been published and/or are available in databases of public access, usually as partial HVSI or HVSI + II segments. Most of the observed variation in CR is accounted for by single nucleotide polymorphisms and small insertion–deletion events, while homoplasmic deletions longer than 6 bp have rarely been described in healthy individuals. We report here the finding of a deletion spanning 154 bp in Hypervariable Region I in a healthy blood donor from Salta (Northwestern Argentina) whose maternal lineage belongs to Native American haplogroup D1.

2. Materials and methods

Blood or saliva samples from admixed, cosmopolitan Argentinean populations were obtained at public hospitals in the cities of Mendoza ($N = 66$) and Lavalle ($N = 75$), province of Mendoza; Jáchal ($N = 13$), Calingasta ($N = 70$) and San Juan ($N = 102$), province of San Juan; Chepes ($N = 50$) and La Rioja ($N = 90$), province of La Rioja; Belén ($N = 63$), province of Catamarca; San Salvador ($N = 35$), province of Jujuy; Salta ($N = 25$) and Tartagal ($N = 174$), province of Salta; Reconquista ($N = 85$), province of Santa Fe; and La Paz ($N = 60$), province of Entre Ríos. Saliva samples from Pampa de Achala, a rural isolate in province of Córdoba, were obtained from 13 individuals while further 58 Native American members of the Cochinoca, Rinconada and Susques communities in the highlands of Jujuy donated blood samples. Samples from the city of Salta were amplified with primers F15905 (TAA TAC ACC AGT CTT GTA AAC C) and R599 (TTG AGG AGG TAA GCT ACA TA) and submitted to sequencing with a commercial provider (Macrogen Inc., Korea) with primers F15905, F16475 (TAG CTA AAG TGA ACT GTA TCC), R16498 (CCT GAA GTA GGA ACC AGA TG), R186 (GCC TGT AAT ATT GAA CGT AGG TG) and R599. For the remaining samples, PCR amplification and sequencing were performed following the procedures outlined in Refs. [1] and [2]. In brief, automated PCR amplification of the full Control Region was prepared on the Corbett Robotics CAS-1200 robotic platform (Concorde, NSW, Australia) using the primers F15971 (TTA ACT CCA CCA TTA GCA CC) and R599 or F15878 (AAA TGG GCC TGT CCT TGT AG) and R649 (TTT GTT TAT GGG GTG ATG TGA). Samples were amplified in an Applied Biosystems GeneAmp 9700 Thermal Cycler and post-amplification cleanup of unincorporated primers and dNTPs was carried out by adding 1.5 μl ExoSapIt (USB, Cleveland, OH, USA) plus 18.5 μl of dilution buffer to the PCR reaction and incubating in the thermal cycler at 37 °C for 20 min, followed by an inactivation of the reaction at 90 °C for 20 min. Cycle sequencing was prepared using the Big DyeTerminator version 1.1 sequencing kit (Applied Biosystems) utilizing the sequencing primers described in Ref. [1] with the updates listed in Ref. [2]. Post-sequencing cleanup of the cycle sequencing reaction was purified using the Performa V3 96-

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well short plate (EdgeBiosystems, Gaithersburg, MD, USA) following the manufacturer's guidelines. Samples were then dried down and resuspended in 10 μl of HiDiFormamide (AppliedBiosystems) and analyzed on either an Applied Biosystems 3130 or 3730 Genetic Analyzer. Sequences were aligned according to the revised Cambridge Reference Sequence [4] using Sequencher software version 4.7 (GeneCodes, AnnArbor, MI, USA).

Samples from Salta and Tartagal were typed for the Native American/Asian haplogroups A–D "classical" coding region markers by PCR-RFLP and PCR-AFLP as in Ref. [3]. Haplogroup D1 diagnostic mutation C2092T was analyzed through PCR-RFLP with primers F2069/mism (CTC TAA ATC CCC TTG TAA AGT TAA) and B2583 (GTT AGG GTA CCG CGG CCG TTA). Underlined G in F2069/mism is a T-to-G mismatched base that creates an artificial HincII recognizing site at 2087 in the presence of rCRS allele 2092*C.

3. Results and discussion

A homoplasmic, shorter than expected Control Region amplicon was obtained in one out of 25 apparently healthy blood donors from the city of Salta, Northwestern Argentina. "Complete" Control Region sequencing led us to the discovery of a 154 bp deletion involving positions 16165–16307 plus one of the 11 bp-long direct repeats (TAGTACATAAA) in the flanking positions 16154–16164 and 16308–16318. Native American haplogroup D1 memberhip for this lineage was inferred from the presence of T16325C, T16362C and T489C, and confirmed by PCR-RFLP typing of C5178A and C2092T. No further cases of similarly deleted haplotypes were found in newly obtained CR sequences from geographically close populations of NW Argentina ($N = 354$ individuals from Salta, Jujuy and Catamarca provinces) nor in the rest of the country ($N = 624$) (this report).

To the best of our knowledge, the deletion reported here has been described only twice before [5]. Bua et al. reported its presence in heteroplasmic state in one out of 48 muscle fibres – from 7 individuals – selected for the presence of electron-transport-system abnormalities [6] while Behar and collaborators identified an homoplasmic case in 1 out of over 120,000 mtDNA genomes tested by the Genographic Project's public participation program [7]. In this case, Behar et al. also demonstrated normal transmission from a Japanese mother to her two sons without apparent health consequences.

Bua et al. detected the deleted genome in one out 8 muscle fibers from an aged woman and stated that the deletion involved one of two 7 bp flanking perfect repeats at 16158–16164 and 16312–16318 while Behar et al. reported the repeats as being 12 bp long in the Japanese kindred, beginning at rCRS positions 16154 and 16308. This 1 bp increase in the repeats size respect that present in the rCRS is consequence of the presence of mutation G16319A, part of the ancestral motif for haplogroup A.

Independent phylogenetic origin for both homoplasmic deletion cases is warranted given its haplogroup status: while the Argentinean case belongs with Native American haplogroup D1, the complete mtDNA sequence available from the Japanese trio indicates membership within Asian haplogroup A5a2. Lack of enough sequence information precluded haplogroup assignation in the heteroplasmic case.

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Conflict of interest

None.

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