

## For the Record

### Mitochondrial DNA HVI and HVII Variation in a North-East Spanish Population

B. Martinez-Jarreta, M.D., Ph.D.<sup>1</sup>, A. Prades, M.D., Ph.D.<sup>1</sup>,  
F. Calafell Ph.D.<sup>2</sup>, B. Budowle, Ph.D.<sup>3</sup>

**Number of People and Name of the Population:** 103 Caucasians from Zaragoza (often spelled *Saragossa* in English), Aragon (North Spain)

**Keywords:** forensic science, DNA typing, population genetics, short tandem repeat, HLA-DQA1, LDLR, GYPA, HBG, D7S8, GC, Zaragoza, Aragon

### Material and Methods

**DNA Extraction**—Using a standard phenol-chloroform procedure.

**DNA Quantification**—By fluorimetry (Hoefer DyNA Quant 200 Fluoremeter, Pharmacia, Uppsala Sweden).

**Mt DNA Amplifications and Sequencing**—HVI was amplified by means of a semi-nested PCR strategy. In the first round of amplification, primers L15996 (5' CACCATTAGCACCCAAAGCT3') and H408 (5' CTGTTAAAAGTGATACCGCCA 3') were used, and a fragment of 1021 bp (i.e., the whole control region) was obtained. In the second round of PCR, a fragment of 443 bp spanning the HVI segment was amplified by means of primers L15996 and H16401 (5' TGATTCACGGAGGATGGTG 3'). HVII was amplified in two overlapping fragments, namely HVII-A (278 bp) and HVII-B (277 bp). The former fragment was amplified with primers L047 (5'-CTCACGGGAGCTCTCCATGC-3') and H285 (5'GGGGTTTGGTGAAATTTT TTTG-3'), and the latter fragment with primers L172 (5'-ATTATTTATCGCACC-TACGT-3') and H408. The PCR products were purified using MICROSPIN HR S-300 (Pharmacia, Uppsala, Sweden) before cycle sequencing. The sequence reactions were carried out using the PCR Fentomol Sequencing Kit (Promega, Madison) and fluores-

cently labeled sequencing primers. The sequence products were denatured with deionized formamide and separated in a 6% PAGE gel and analyzed in an A.L.F. automatic sequencer (Pharmacia, Uppsala, Sweden). Both the L-strand and the H-strand were sequenced and the base calling was confirmed when the two strands showed clearly complementary peaks. The mtDNA haplotypes are recorded based on their differences from a standard mtDNA reference sequence (2).

**Statistical analysis**—The population data were analyzed using the program "Mitosearch" (developed at the FBI), which performs haplotype matching and calculates random match probability and number of nucleotide differences between haplotypes. Genetic diversity was calculated according to Tajima (3) and random match probability was calculated according to Stoneking et al. (4). A database containing more than 2000 mtDNA sequences of both hypervariable regions from European and U.S. Caucasian, Hispanic, African, and Asian individuals, and provided by one of the authors (B.B.) was used to perform the pairwise comparisons.

### Results and Discussion

Considering HVI and HVII, 99 different sequences were observed. Ninety-five haplotypes were seen only once in the database, and four other haplotypes were observed twice. Genetic diversity was 0.999, random match probability was 1.05% and the average number of nucleotide differences between pairs of individuals was  $8.090 \pm 3.266$ . The complete data will be made available to any interested researcher upon request.

### References

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<sup>1</sup> Department of Forensic Medicine. Faculty of Medicine. University of Zaragoza, Zaragoza, Spain.

<sup>2</sup> Evolutionary Biology Unit. Health and Life Sciences School, Universitat Pompeu Fabra, Doctor Aiguader 80, 08003 Barcelona, Spain.

<sup>3</sup> Forensic Science and Training Center, FBI Academy, Quantico, Virginia 22135.

TABLE 1—Sites at which nucleotides are different from that in the Cambridge Reference sequence (2) are the most frequent in the Zaragoza sample, compared with those from the other population groups of the database provided with Mitosearch (&gt;2000 profiles) and described by Budowie, et al. (7).

| Population         | 315.1, % | 263, % | 73, % | 16223, % | 309.1, % | 16126, % | 16069, % | 295, % | 195, % |
|--------------------|----------|--------|-------|----------|----------|----------|----------|--------|--------|
| African American   | 100      | 99.69  | 92.46 | 90.63    | 46.98    | *        | *        | *      | 60.45  |
| Afro Caribbean     | 99.13    | 97.41  | 88.79 | 80.17    | 37.93    | *        | *        | *      | 55.17  |
| Japanese           | 98.76    | 98.76  | 100   | 81.48    | 66.77    | *        | *        | *      | *      |
| Korean             | 98.88    | 98.88  | 100   | 74.44    | 61.66    | *        | *        | *      | *      |
| Hispanic           | 98.33    | 97.00  | 91.69 | 68.10    | 56.17    | *        | *        | *      | *      |
| U.S. Caucasian     | 98.88    | 98.63  | 55.22 | *        | 50.99    | 19.40    | 9.57     | 9.45   | 16.16  |
| English Caucasian  | 99.02    | 99.02  | 44.66 | *        | 58.25    | 18.44    | 11.65    | 11.65  | 18.44  |
| Austrian Caucasian | 99.00    | 99.00  | 52.47 | *        | 60.39    | 22.27    | 10.89    | 10.89  | 17.82  |
| French Caucasian   | 100      | 100    | 44.95 | *        | 52.29    | 16.51    | 7.33     | 8.25   | 11.00  |
| Zaragoza           | 100      | 100    | 56.31 | *        | 47.51    | 30.09    | 16.50    | 15.53  | 8.73   |

\* Observed frequency &lt;5%.

TABLE 2—Summary of the Pairwise Comparisons of mtDNA haplotypes in different populations and the average number of nucleotide differences between individuals from Zaragoza and other populations (7).

| Comparison                  | Matches/Comparisons | Frequency             | Average Number of Nucleotide Differences Between Individuals |
|-----------------------------|---------------------|-----------------------|--|
| Zaragoza*                   | 5/5253              | 0.00114 (1 in 876)    | 8.090 ± 3.226  |
| African American/Zaragoza   | 1/34196             | 0.00003 (1 in 34 196) | 13.818 ± 5.192   |
| Afro Caribbean/Zaragoza     | 3/11948             | 0.00025 (1 in 3983)   | 12.031 ± 4.606   |
| U.S. Caucasian/Zaragoza     | 122/82812           | 0.00147 (1 in 679)    | 7.985 ± 3.514  |
| English Caucasian/Zaragoza  | 19/10609            | 0.00179 (1 in 558)    | 8.098 ± 3.698  |
| Austrian Caucasian/Zaragoza | 13/10403            | 0.00125 (1 in 800)    | 8.186 ± 3.612  |
| French Caucasian/Zaragoza   | 14/11227            | 0.002125 (1 in 802)   | 7.664 ± 3.377  |
| Hispanic/Zaragoza           | 6/31003             | 0.00019 (1 in 5167)   | 11.272 ± 3.625   |
| Japanese/Zaragoza           | 0/16686             | 0                     | 9.827 ± 3.328  |
| Korean/Zaragoza             | 0/18540             | 0                     | 9.966 ± 3.101  |

\* = within-group comparison.