Study of the T16189C variant and mitochondrial lineages in Tunisian and overall Mediterranean region

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Abstract

The mitochondrial DNA (mtDNA) variant T16189C has been investigated in several metabolic diseases. In this study, we aimed to estimate the frequency of the T16189C variant in Tunisian and other Mediterranean populations and to evaluate the impact of this variant on the phylogeny of Mediterranean populations. Blood sample of 240 unrelated Tunisian subjects were recruited from several Tunisian localities. The hypervariable region 1 of the mtDNA were amplified and sequenced. Additional sequences (N = 4921) from Mediterranean populations were compiled from previous studies. The average frequency of T16189C variant in Tunisia (29%) is similar to that observed in North African and Near Eastern populations. Our findings showed positive correlation of the T16189C variant with Sub-Saharan and North African lineages, while a negative correlation was found with the Eurasian haplogroups, reaching its maximum with the Eurasian haplogroup H. The principal component analyses showed a high internal heterogeneity between Tunisian localities. At the Mediterranean scale, Tunisians are closer to North African (Algerian and Moroccan) and Near Eastern populations (Syrians and Palestinians) than to Europeans.

Introduction

Human mtDNA is a small circular double-stranded DNA molecule of 16,569 in length (Anderson et al., 1981). It contains 37 genes encoded for two ribosomal RNAs, 22 transfer RNAs and 13 proteins of the oxidative phosphorylation system. The mtDNA is maternally inherited and non-recombined molecule with higher mutation rate (10–12 times higher than that of nuclear DNA) (Ingman et al., 2000). Accumulation of mtDNA variants results in impairment of electron transport and the oxidative phosphorylation system (Schapira, 2006). MtDNA variants are implicated in impairment of electron transport and the oxidative phosphorylation system (Schapira, 2006). MtDNA variants are implicated in different pathologies. Among them, the T16189C variant was reported to be associated with increased risk of type 2 diabetes (Park et al., 2008, Poulton et al., 2002), metabolic syndrome (Weng et al., 2005), dilated cardiomyopathy (Khogali et al., 2001), hereditary haemochromatosis (Livesey et al., 2004) and in the reduced birth weight (Mohlke et al., 2005).

Most T16189C variant investigation have been performed mainly on European and Asian populations. We propose in the present study to estimate the frequency of the T16189C variant in Tunisian and other Mediterranean populations and to evaluate the interaction of T16189C variant with mitochondrial haplogroups.

Methods

Samples

Blood samples were collected from 240 unrelated Tunisian subjects recruited from several Tunisian localities: North (N = 113), Centre (N = 81) and South (N = 46).

Ethics statement

This study was conducted according to the principles of the Declaration of Helsinki. Participants gave their written informed consent to be part of scientific project without restriction.

Genetic analysis

DNA sequencing of the hypervariable segment 1 (HVS1) was performed on ABI 3130 (Applied Biosystems Life Technologies SAS, Saint Aubin, France). Sequences obtained were aligned using Blast 2 sequences and Seqscape software (V2.7) (Applied Biosystems, LifeTechnologies SAS, Saint Aubin, France) and were sorted into haplogroups following van Oven and Kayser (van Oven & Kayser, 2009). Generated sequences are available in GenBank under accession numbers (KF694756-KF694805; KF931344-KF931392; KJ187805-KJ187945).

Data analysis

We have analyzed 955 mtDNA sequences (HVS1) from 18 Tunisian populations collected from the literature and the present...
study (Table 1). We also extended analyses to 39 Mediterranean populations (total = 5161) (Table 1).

Statistical analysis

Principal component analyses (PCA) as well as Pearson’s chi-square or Fisher’s exact tests were performed using the statistical package for the social sciences (SPSS, version 13.0, Chicago, IL).

Results

Comparisons frequencies of the T16189C variant among Tunisian regions

We proceeded by classifying our Tunisian sample into three regions according to their geographic origin: the North, the Centre and the South of Tunisia. Then, we investigated the T16189C frequency among these groups. Statistical analysis showed similar frequencies of the 16189C variant between the three regions ($p < 0.05$) (Supplementary Appendix A). Similar results were obtained when we included all previous studied Tunisian populations in the analysis, which we classified into the North, the Centre and the South of Tunisia (Supplementary Appendix B).

Comparisons of the T16189C variant frequencies between Tunisian localities

The T16189C variant was widely represented in different Tunisian regions and its frequency ranges from 0% in Berbers from Jerba ($N = 30$) to 62% in the population of Bou Saâd ($N = 40$) (Supplementary Appendices C and D (A)) with an average frequency of 29%. Statistical analyses comparing the frequency of T16189C variant among Tunisians revealed that both Berber Berbers and Bou Saâd had significant differences with the rest of the studied Tunisian localities with the exception of Arabs from Zriba who were not differentiated from Bou Saâd (Supplementary Appendix C).

Comparisons of the T16189C variant frequencies between Tunisians and 39 Mediterranean populations

We proceeded by grouping all Tunisian populations into one group (TUN) and comparing them to 39 Mediterranean populations for the frequencies of the T16189C variant. Results from this study showed that the frequency of the T16189C variant in Tunisia is similar to that found in North African (Except for: Upper Egypt (EGY), Algerians Mozabites (MOZ), Moroccans (MBS and MBN)) and Near Eastern populations, whereas it was different from that observed in Europeans (Except for Andalusians (AGP) and Corsicans (COB)) (Supplementary Appendices D, E, and F).

Principal component analyses of the T16189C variant and haplogroup distribution in 18 studied Tunisian localities

PCA of the mitochondrial T16189C variant and haplogroup frequencies in Tunisian population revealed 14.2%, 12.3% and 12.2% of the variance in PCA1, PCA2 and PCA3, respectively (Figure 1, Supplementary Appendices G and H). Overall, eight components (T16189C, L0, L1, L2, L3, M1, N and R) of the PCA explained 86.7% of the total genetic variance. The T16189C variant was mainly correlated with Sub-Saharan haplogroups, North African lineages and with the Eurasian haplogroup H. It is noteworthy that the T16189C variant correlated negatively with most Eurasian haplogroups, reaching its maximum with the Eurasian haplogroup H (Supplementary Appendices E and G). Accordingly, the five Tunisian localities from Chenini-Douiret (CHO), Bou Saâd (SAB), Bou Omrane (OMB), Kesra (Kesra) and Skira (SKI) appeared dispersed in the plot. PCA showed that Tunisian locality from SAB was most correlated with axis 1. Besides, CHO and KES correlated positively with axis 2, while OMB and SKI correlated negatively with axis 2. Whereas, the remaining Tunisian populations were clustered together, occupying the first and the second axes (Figure 1).

Principal component analyses of the mitochondrial T16189C variant and haplogroup distribution in Tunisians (TUN) and 39 Mediterranean populations

We proceeded by grouping all Tunisian populations into one group (TUN). PCA analyses for the T16189C variant and haplogroup frequencies in Tunisians (TUN) and 39 Mediterranean populations revealed 14.8%, 14.3% and 11% of the variance in PCA1, PCA2 and PCA3, respectively (Figure 2, Supplementary Appendix I). Overall, seven components (T16189C, L0, L1, L3, M1, N and I) of the PCA explained 76.4% of the total genetic variance. PCA analysis showed that most North African populations segregated positively with at least one axis, while the majority of the European populations showed a negative segregation with two axes with intermittent position for Near Eastern populations (SYR, PAL). Accordingly, Tunisians were most segregated with axis 2. Tunisians were most related to North Africans from Algeria (ALG) and Morocco (ASB, FIB, and MOA) and were differentiated from Egyptians (GUR, EGY, and ALX) (Figure 2).

Similar position of Tunisians was obtained when we excluded outliers from Chenini-Douiret and Bou Saâd mentioned in Kefi et al. (2014).

Discussion

The present study provides an overview on the distribution of the variant T16189C in Tunisia and in the Mediterranean. T16189C variant is frequent in Tunisia (29%) (Supplementary Appendix D). This value is similar to that reported in a case-control study performed on Tunisians with type 2 diabetes (Hsouna et al., 2013).

The distribution of the T16189C variant is not significantly different between the North, the Centre and the South of Tunisia (Supplementary Appendices A and B). This finding is shown by AMOVA analyses (Kefi et al., 2014).

It is noteworthy that the maximum of genetic variance between Tunisian populations was explained by the interaction between T16189C variant and mitochondrial haplogroups. T16189C variant correlates positively with Sub-Saharan and North African lineages, and negatively with the Eurasian haplogroup H.

Our investigations contribute to the stratification of the Tunisian population. Five Tunisian populations (Chenini-Douiret (CHO), Bou Saâd (SAB), Bou Omrane (OMB), Kesra (KES) and Skira (SKI)) differ from the rest of Tunisians. The position of SAB at the extreme one end of axis 1 is mainly due to the most frequent T16189C variant and haplogroup L0 in Tunisia. Similarly, the positive correlation of CHO and KES with axis 2 is linked to the high values of the Eurasian haplogroups K, T and HV for CHO and to the frequent haplogroup K for KES. While the position of OMB and SKI at the extreme other end of axis 2 is related to the higher frequencies of haplogroups H and L3 for OMB and of the haplogroup H for SKI (Figure 1).

At the Mediterranean scale, the present study showed that the frequency of the T16189C variant in Tunisia is similar to most North Africans and Near Eastern populations and higher than that encountered in Mauritians (~12.5%) (Meiloud et al., 2013) and Europeans (Supplementary Appendices D–F). Position of
Table 1. Estimated Frequencies of the T16189C variant in 57 Mediterranean populations.

| Populations | Code | Sample size | Number of individuals with T16189C | Frequency of T16189C variant (%) | References |
|-------------|------|-------------|-----------------------------------|---------------------------------|------------|
| NORTH AFRICA |       |             |                                   |                                 |            |
| TUNISIA      |       |             |                                   |                                 |            |
| NORTH        |       |             |                                   |                                 |            |
| Northern Tunisia | NOT | 177         | 60                                | 34                              | (Turchi et al., 2009; Kefi et al., 2014), Present Study |
| Qalaat El Andalous | QAL | 29          | 9                                 | 31                              | (Cherni et al., 2009) |
| Capital Tunis | CTU | 98          | 33                                | 34                              | (Cherni et al., 2009, Plaza et al., 2003) |
| El Alia      | ELA  | 48          | 8                                 | 17                              | (Cherni et al., 2009) |
| Zriba        | ZRI  | 35          | 7                                 | 20                              | (Cherni et al., 2009) |
| Slouguiia    | SLO  | 28          | 4                                 | 14                              | (Cherni et al., 2009) |
| Testour      | TES  | 50          | 13                                | 26                              | (Cherni et al., 2009) |
| Kesra        | KES  | 43          | 16                                | 37                              | (Cherni et al., 2009) |
| CENTRE       |       |             |                                   |                                 |            |
| Central Tunisia | CET | 81          | 22                                | 27                              | Present Study |
| SOUTH        |       |             |                                   |                                 |            |
| Southern Tunisia | SOT | 46          | 13                                | 28                              | Present Study |
| Skira        | SKI  | 20          | 4                                 | 20                              | (Cherni et al., 2009) |
| Jerba Arabs  | JEA  | 29          | 8                                 | 28                              | (Loueslati et al., 2006) |
| Jerba Berbers | JEB | 30          | –                                 | –                               | (Loueslati et al., 2006) |
| Chenini-Douiret | CHO | 53          | 19                                | 36                              | (Fadhlaoui-Zid et al., 2004) |
| Senned       | SEN  | 55          | 23                                | 42                              | (Fadhlaoui-Zid et al., 2004) |
| Bou Omrane   | OMB  | 40          | 7                                 | 18                              | (Ennafaa et al., 2011) |
| Bou Sâid     | SAB  | 40          | 25                                | 62                              | (Ennafaa et al., 2011) |
| Matmata      | MAT  | 53          | 8                                 | 15                              | (Fadhlaoui-Zid et al., 2004) |
| TOTAL TUNISIANS | TUN | 955        | 279                               | 29                              |            |
| LIBYA        |       |             |                                   |                                 |            |
| Fezzan       | FAL  | 129         | 36                                | 28                              | (Ottoni et al., 2009) |
| EGYPT        |       |             |                                   |                                 |            |
| Upper Egypt  | EGY  | 102         | 41                                | 40                              | (Stevanovitch et al., 2004) |
| Gurna        | GUR  | 34          | 13                                | 38                              | (Stevanovitch et al., 2004) |
| Siwa Berbers | SIB  | 78          | 24                                | 31                              | (Coudray et al., 2009) |
| Alexandria   | ALX  | 277         | 75                                | 50                              | (Saunier et al., 2009) |
| ALGERIA      |       |             |                                   |                                 |            |
| Algerians    | ALG  | 47          | 14                                | 30                              | (Plaza et al., 2003) |
| Algerian Mozabites | MOZ | 85          | 42                                | 49                              | (Corte-Real et al., 1996) |
| MOROCCO      |       |             |                                   |                                 |            |
| NORTH AFRICA |       |             |                                   |                                 |            |
| MOROCCO      |       |             |                                   |                                 |            |
| Southern Moroccan (Berbers) | MBS | 50          | 5                                 | 10                              | (Brakez et al., 2001) |
| Northern Moroccan (Berbers) | MBN | 60          | 6                                 | 10                              | (Plaza et al., 2003) |
| Moroccan Arabs | MOA | 50          | 17                                | 34                              | (Plaza et al., 2003) |
| Saharawi     | SAH  | 56          | 16                                | 29                              | (Plaza et al., 2003) |
| Marrakech    | MAR  | 52          | 15                                | 29                              | (Falchi et al., 2006) |
| Asni Berbers | ASB  | 53          | 15                                | 28                              | (Coudray et al., 2009) |
| Bouhria Berbers | BOB | 70          | 21                                | 30                              | (Coudray et al., 2009) |
| Figuig Berbers | FIB | 94          | 23                                | 24                              | (Coudray et al., 2009) |
| Total North African sequences |       |             |                                   | 2192                             |            |
| NEAR EAST    |       |             |                                   |                                 |            |
| PALESTINE-ISRAEL |   |             |                                   |                                 |            |
| Palestinian-Israeli | PAL | 117         | 25                                | 21                              | (Richards et al., 2000) |
| Druze        | DRU  | 45          | 19                                | 42                              | (Macaulay et al., 1999) |
| SYRIA        |       |             |                                   |                                 |            |
| Syrian       | SYR  | 69          | 18                                | 26                              | (Richards et al., 2000) |
| Total Near Eastern populations |       |             |                                   | 231                             |            |
| EUROPE       |       |             |                                   |                                 |            |
| GREECE       |       |             |                                   |                                 |            |
| Greeks       | GRE  | 184         | 30                                | 16                              | (Villens, 2011) |
| Northern Greeks | GNG | 319         | 53                                | 16                              | (Irwin et al., 2008) |
| CYPRUS       |       |             |                                   |                                 |            |
| Cypriots     | GRC  | 91          | 10                                | 11                              | (Irwin et al., 2008) |
| TURKEY       |       |             |                                   |                                 |            |
| Turks        | TUR  | 213         | 45                                | 21                              | (Richards et al., 2000) |
| SPAIN        |       |             |                                   |                                 |            |
| Andalusian   | AND  | 158         | 31                                | 20                              | (Plaza et al., 2003) |
| Andalusia (Granada Province) | AGP | 66          | 12                                | 19                              | (Falchi et al., 2006) |
| Catalan      | CAT  | 162         | 12                                | 20                              | (Plaza et al., 2003) |

(continued)
Table 1. Continued

| Populations      | Code | Sample size | Number of individuals with T16189C | Frequency of T16189C variant (%) | References                              |
|------------------|------|-------------|-----------------------------------|----------------------------------|----------------------------------------|
| Galician         | GAL  | 374         | 34                                | 9                                | (Alvarez-Iglesias et al., 2009, Salas et al., 1998) |
| Basque           | BAS  | 45          | 4                                 | 9                                | (Bertranpetit et al., 1995)            |
| Majorcan         | MAJ  | 112         | 14                                | 13                               | (Falchi et al., 2006, Picornell et al., 2005) |
| Minorcan         | MIN  | 46          | 4                                 | 9                                | (Picornell et al., 2005)              |
| **EUROPE**       |      |             |                                   |                                  |                                        |
| **SPAIN**        |      |             |                                   |                                  |                                        |
| Valencian        | VAL  | 42          | 2                                 | 5                                | (Picornell et al., 2005)              |
| Ibizan           | IBI  | 50          | –                                 | –                                | (Picornell et al., 2005)              |
| Chuetas          | CHU  | 48          | 6                                 | 12                               | (Picornell et al., 2005)              |
| **FRANCE**       |      |             |                                   |                                  |                                        |
| French           | FRE  | 109         | 14                                | 13                               | (Dubut et al., 2004)                  |
| Centre Corsica   | COR  | 47          | 2                                 | 4                                | (Varesi et al., 2000)                 |
| Southern Corsica | COB  | 53          | 12                                | 23                               | (Falchi et al., 2006)                 |
| **ITALIA**       |      |             |                                   |                                  |                                        |
| South Italy      | ITS  | 86          | 9                                 | 10                               | (Francalacci et al., 1996, Richards et al., 2000) |
| Sardinian        | SAR  | 303         | 46                                | 15                               | (Di Rienzo and Wilson, 1991, Falchi et al., 2006) |
| Sicilian         | SIC  | 169         | 23                                | 14                               | (Cali et al., 2001, Richards et al., 2000) |
| Tuscan           | TUE  | 61          | 8                                 | 13                               | (Falchi et al., 2006)                 |
| Total Eurasiun sequences | 2738 |             |                                   |                                  |                                        |
| Total            |      |             |                                   |                                  | 5161                                   |

Figure 1. PCA of 18 Tunisian populations based on frequencies of the mitochondrial T16189C variant and haplogroups. See Table 1 for more details on codes and reference information. (TIF).
Tunisians is mainly attributed to frequencies of the Sub-Saharan haplogroups L2, L3 and of the North African haplogroup U6 (Figure 2).

These findings were confirmed by our phylogenetic study (Kefi et al., 2014) and were in accordance with a previous study using ancestry informative marker SNPs (Khodjet-el-Khil et al., 2011).

On the other hand, we revealed that Tunisians were genetically distant from Egyptian populations. These differences were previously reported in studies using uniparental markers (Coudray et al., 2009; Fadhlaoui-Zid et al., 2011) and in anthropological studies (Kefi, 2011).

Conclusions

Our results suggested that T16189C is common in Tunisia (29%), in North African and in Near Eastern populations belonging to the Mediterranean region. It correlated positively with Sub-Saharan and North African lineages and negatively with the Eurasian haplogroups H. Tunisians are genetically closer to North Africans (Algerian and Moroccans) and Near Eastern populations than to Europeans.

Findings from this study contribute to the better knowledge of the matrilineal background of Tunisia within Mediterranean populations. This genetic stratification will be useful for the design of future studies.

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Declaration of interest

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