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Study of Different Mutation Types of HVI Region and TSPY1 Copy Number in Different Nationalism of Kirkuk City

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Abstract: Geographical barriers have been recognized as significant impediments to population mobility, and these barriers may have varying effects on various genetic markers, including mitochondrial DNA (mtDNA) and the Y chromosome. Therefore, the current study aimed to detect a different types of mutations of HVI and TSPY1 copy number in different Nationalism of Kirkuk city. The present investigation was carried out at the University of Kirkuk's Department of Life Sciences, College of Science, molecular laboratory. Blood samples were taken in November 2022 from 500 people in the Kirkuk governorate, 300 of whom were male and 250 of whom were female. This included unrelated people. Data and samples are collected in accordance with environmental ethics. The results were found that 16 mutations were of the Transition type, with C\T being the highest, accounting for 16.8% of the total mutations. While the number of Transversion mutations was 33, with A\T being the highest, accounting for 8.7% of the total mutations. Deletion and Insertion mutations were also found, especially T addition, accounting for 20.1%, and C deletion, accounting for 5.4%. The median TSPY1 copy number of original Arabs of Kirkuk was 1717.224+450.30, Kakai was 4220.5+187.57, Original Kurds of Kirkuk was 3278.8+755.62, Turkmen of Kirkuk was 2359.25+756.71, Christian was 4885.8+76.57 and also the median TSPY1copy number of other nationalism was appaered in table (2). the median TSPY1copy number of all kirkuk nationalism did not differ significantly Kruskal-Wallis test (p = 4.05). its concluded that more variations of the TSPY1 copy number was observed in case of Kakai and Christian compared to other nationalism.

Keywords: mtDNA, TSPY1, gene copy number, HVI region.

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Introduction

Almost every species of plant or animal has isolated communities. The fossil genetic record, for instance, offers well-established proof of a lengthy history of interbreeding, subpopulation formation, and isolation among humans [1]. A given region's general populace may identify with a specific nationality, yet the population's actual heritage and historical roots might frequently diverge greatly. A genetic isolate is formed gradually and is impacted by a number of factors. Geographical separation, sometimes known as isolation by distance, is a typical component of this process, and the distance between locales greatly influences the genetic variations among populations [2,3]. There are 75–80% Arabs and 20–25% other people in Iraq. The frequency of a specific sequence within a population determines the genetic structure of each population. Regarding genetic diversity, human anthropology, and forensic applications, the frequency of variation in mtDNA D-loop regions can be a helpful tool [4]. The male-specific Y chromosome in mammals contains genes necessary for spermatogenesis and is crucial in determining sex. Nevertheless, many of the genes required to form a testis or germ cells are known to be found on the X chromosome or the autosomes (chromosomes other than the X and Y). Not all of these genes must be found on the Y chromosome. Human males have a single X and a single Y chromosome (XY), while females have two X chromosomes (XX), similar to other mammals [5,6]. A large portion of our cells' energy requirements are met by the mitochondria. OXPHOS, which produces mitochondrial energy, is the process by which the oxygen we breathe oxidizes hydrogen from the lipids and carbohydrates in our diets to produce water [7]. According to Brown et al. [8], the mtDNA has a very high sequence evolution rate, roughly 10–17 times quicker than nuclear DNA genes with comparable functions [9]. This may be a frequent source of mutations that cause mitochondrial illness, but it has also led to the accumulation of a wide range of mtDNA sequence polymorphisms in human cultures. Therefore, the current study aimed to detect a different types of mutations of HVI and TSPY1 copy number in different Nationalism of Kirkuk city.

Materials and Methods

Study Design, Sample, and Data Collection Time

The present investigation was carried out at the University of Kirkuk's Department of Life Sciences, College of Science, molecular laboratory. Blood samples were taken in November 2022 from 500 people in the Kirkuk governorate, 300 of whom were male and 250 of whom were female. This included unrelated people. Data and samples are collected in accordance with environmental ethics.

Blood collection

In the study a samples of venous blood in size three milliliters were collected from each person. Into (EDTA) tubes the blood was collected for DNA extraction and stored at - 20˚C (deep freeze) [10,11].

Amplification of mtDNA

Extracted genomic DNA using Geneaid Company's gSYNCTM DNA extraction kit's rapid methodology. Since each cell contains many copies of mt-DNA, HV1 was amplified using a straightforward technique for extracting and measuring DNA. We independently intensified these polymorphic areas using the PCR preliminaries that are included. Tsutsumi et al. [12] amplified 15,997 nt to 16,401 nt of HV1 by synthesizing forward primer 5′-CTCCACCATAGCACCCAAAGC-3′ and reverse primer 5′-CCTGAAGTAGGAACCAGATG-3′. These primers were evaluated in silico against the reference human genome using the Basic search tool with local alignment and a release number of 2.2.27. The PCR amplification condition was denaturation at 94 °C for one minute. After 35 cycles of denaturation at 94

°C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute, a final extension at 72 °C for 10 minutes was carried out. The PCR product was sent to Macrogen, a South Korean company that may be accessed at httb://dna.macrogen.com, for sequencing..

Estimation of the TSPY1 copy number

The TaqMan probe-based qPCR assay was used to determine the TSPY1 copy numbers. Internal controls for tests I and II of the analysis were SRY and TERT, respectively. To carry out two separate co-amplifications of TSPY1/SRY and TSPY1/TERT, their primers and probes were created using AlleleID 6.0 (PREMIER Biosoft, Palo Alto, CA, USA) (Supplementary Material, Table S2). The NCBI database (http://blast.ncbi.nlm.nih.gov/) was searched using BLAST to verify the PCR primer sets' specificity. These amplicon sequences have not been found or reported to include SNVs (http://www.ncbi.nlm.nih.gov/snp/).

Statistical analyses

Using the (exhaustive) CHAID analysis in the Decision Tree add-on module of the statistical software SPSS 17.0 (SPSS Inc.), the TSPY1 copy number ranges were identified. The analytic software SPSS 17.0 was used to conduct additional statistical analysis. The frequencies of the individual TSPY1 copy number ranges and the distributions of the Y haplogroups were compared between the groups using the $\chi 2$ test [14,15].

Results & Discussion

Table 1 shows the different types of mutations detected in the HVI region of Kirkuk Governorate population. It was found that 16 mutations were of the Transition type, with C\T being the highest, accounting for 16.8% of the total mutations. While the number of Transversion mutations was 33, with A\T being the highest, accounting for 8.7% of the total mutations. Deletion and Insertion mutations were also found, especially T addition, accounting for 20.1%, and C deletion, accounting for 5.4%.

Table 1: different types of mutations of HVI found in Kirkuk population

Type of mutation		HVI
Transition	A\G	7(4.7%)
	T\C	15(10.1%)
	C\T	25(16.8%)
	G\A	10(6.7%)
	A\G	4(2.7%)
Transvertion	A\C	6(4.0%)
	A\T	13(8.7%)
	G\C	1(0.8%)
	C\A	13(8.7%)
Insertion	-\A	11(7.4%)
	-\T	30(20.1%)
	-\C	3(2.0%)
Deletion	C\-	8(5.4%)
	A\-	3(2.0%)
Total	-	149(100.0%)

Information on 500 people's HVI mtDNA sequences from ten population subgroups is given. The data show that mtDNA sequencing can be instructive in forensic identity cases, as all groups had a significant degree of mtDNA polymorphism (i.e., low RMPs and high genetic diversity in all sample populations investigated). The frequency with which a specific sequence appears in a database or databases is counted for forensic reasons in order to indicate how uncommon a given mtDNA type is among unrelated people. However, when determining haplotype frequencies, one should consider the size of a database. For the

majority of mtDNA sequences that have never been seen before or have only been seen once in a data set, overestimates are probably possible. Additionally, the number of observed mtDNA types might be subjected to confidence intervals if desired [15]; however, this would only represent the uncertainty in the database sample and not the population as a whole. This work complies with Lincoln and Carracedo's [16] recommendations for mitochondrial DNA type and population data publishing. This location contains the insertion or deletion (indels) of CA or AC bases [17,18]. Notably, CA/AC residues were indicative of length heteroplasmy or autosomal short tandem repeat (STR)-like factors [19].

The median TSPY1 copy number of original Arabs of Kirkuk was 1717.224+450.30, Kakai was 4220.5+187.57, Original Kurds of Kirkuk was 3278.8+755.62, Turkmen of Kirkuk was 2359.25+756.71, Christian was 4885.8+76.57 and also the median TSPY1copy number of other nationalism was appaered in table (2). the median TSPY1copy number of all kirkuk nationalism did not differ significantly Kruskal-Wallis test (p = 4.05). More variations of the TSPY1 copy number was observed in case of Kakai and Christian compared to other nationalism.

Table (2): TSPY1 copy number in different Nationalism of Kirkuk city

Nationalism	Mean gene copy number	Sd gene copy number	Min gene copy number	Max gene copy number	Kruskal-Wallis Test (p value)
Original Arabs of Kirkuk	1717.224	450.3	1005	3560	4.05
Christian	4885.8	76.57	4740	4970	
Kakai	4220.5	187.57	3760	4390	
Original Kurds of Kirkuk	3278.8	755.62	1215	3995	
Kurds of Erbil	3357	649.99	1700	3880	
Kurds of Sulaymaniyah	3269.1	793.08	1398	3987	
Middle Arabs	1981.333	663.97	1519	3702	
Southern Arabs	1396.818	295.69	1003	1817	
Turkmen of Kirkuk	2359.25	756.71	110	3220	
Turkmen outside of Kirkuk	2490.65	335.41	1660	2960	

Sd: standard deviation.

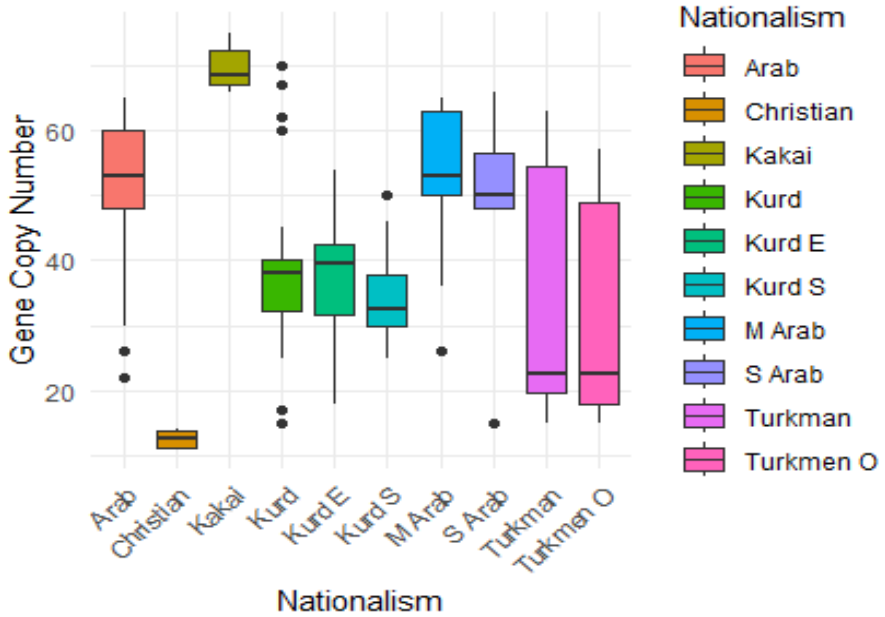

Figure (1): TSPY1 copy number in different Nationalism of Kirkuk city

We first looked into whether the various Y hgs that are widespread in the Kirkuk communities showed varied TSPY1 copy numbers because the Y chromosome exhibits noticeable population stratification and there is a dearth of information in the literature about TSPY1 copy number variation in distinct Y lineages. While an earlier analysis that was restricted to the few hgs that could be discovered in 1994 found higher variance within hgs, Repping et al. [20] had previously found little change in TSPY1 copy number within and between Y hgs. Within the para-groups that comprise our study population, we now report significant variance in the number of TSPY1 copies. The TSPY1 copy numbers of Arabic blood from southern and central Iraq and the TSPY1 copy numbers of original Arabic blood were shown to be possibly correlated; the same was true for Kurdish and Turkmen blood. This offers new information on how TSPY1 copy numbers are inherited and implies a genetic link between people of the same nationality from various geographical areas. Nevertheless, at $p = 0.8.189$, this correlation fell short of the Kruskal-Wallis significance level. Therefore, additional research is required to validate this discovery in the future. Only one form of mutation affects most polymorphic locations [21,22]. This was noted in the present investigation since no multiple mutations occurred at a single location.

Conclusions

It is concluded from the current study that more variations of the TSPY1 copy number was observed in case of Kakai and Christian compared to other nationalism, this is due to the lack of mixing of the lineages of the Kakai and Christian nationalities with the rest of the nationalities of the city of Kirkuk.

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