# Molecular and Cytogenetical Studies in Iraq

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## Introduction

The Iraqi population contain several ethnic groups that need to be genetically characterized and evaluated for possible substructures. Previous studies on the Iraqi population based on Y-STR markers were limited by a restricted number of markers. A larger database for Iraqi Arab population needed to be developed to help study and compare the population with other Middle Eastern populations. Prediction indicated predominance (36.6%) of haplogroup J1 in Iraqi Arabs. The migration rate between other populations and the Iraqis was inferred using coalescence theory in the Migrate-n program. Y-STR data were used to test different out-of-Africa migration models as well as more recent migrations within the Arabian Peninsula. The migration models demonstrated that gene flow to Iraq began from East Africa, with the Levantine corridor the most probable passageway out of Africa (Lazim et al., 2020).

β-thalassemia is a significant problem in the north-eastern part of Iraq, and has imposed a huge burden on the health authorities.

Objective: To identify the molecular characterization and morbidity prevalence in transfusion-dependent thalassemia (TDT) and non-transfusion dependent thalassemia (NTDT) phenotypes in north-eastern Iraq. They conclude that the overall complications rate was 78.9%, with a growing probability of complications with advanced age, with evidently higher rates in patients with $\beta^0\beta^0$ and $\beta^0\beta^+$ genotypes that explain the role of underlying genetic defects in the pathophysiology of disease complications (Amin et al., 2020)

From another genetic aspect common variants among genes coding for enzymes in sex steroid biosynthetic pathways may influence the risk of endometriosis in Iraqi women patients in the last years. Cytochrome P450c17a1 (*CYP17*), a gene that codes for a key enzyme (cytochrome P450c17a1) in a rate-limiting step of estrogen biosynthesis has attracted considerable attention as an important gene for endometriosis. To evaluate the relationship between common genetic variations in *CYP17* and endometriosis risk and determine the main effects of those variations on the gene expression. A women-based case control study of Iraqi women aged range (23–46), the associations between selected single-nucleotide polymorphisms (SNPs) in the *CYP17* gene and endometriosis diagnosis in fifty women and thirty disease-free controls were evaluated. The study found a significant association ($P \leq 0.01$)between endometriosis and selected SNPs

of *CYP17* gene, with the homozygous genotype conferring decreased risk. A highly significant difference ($P \leq 0.01$) in *CYP17* gene expression from women with versus without endometriosis and increased by 1.56-fold in women with endometriosis. These findings suggest that variation in or around *CYP17* may be associated with endometriosis development in the Iraqi women (Al-Rubae'i et al., 2016)

Basrah, the southern most province in Iraq in attempt to investigate the origin of Basrah, two researcher examined the mitochondrial DNA(mt-DNA) variations by hypervariable segment 1(HVS1) Sequencing and determination of specific site haplogroups. The results in Basrah show no significant differences diversity among Iraqis' HVS1 compared with other countries. The values were within the range of gene diversity across the Middle East and exhibited the unimodal pattern of differences in the pairwise sequence. Given the small genetic differences between people living in this area, phylogenetic analysis showed a large variability of the communities of Basrah; they didn't cluster on the phylogenetic tree (Bassim and Adnan 2020). Forty-nine of the 52 autosomal single nucleotide polymorphisms (SNPs) in the SNP for ID 52plex were typed in 101 unrelated Iraqis living in Denmark. No significant deviation from HTHEYwas found in all but one of the 49 SNP systems and no significant pairwise linkage disequilibrium was observed for any SNP pair. When 18 worldwide populations were compared (including populations in Iraq, Turkey, Israel, Pakistan, India, China, Taiwan, Japan, Siberia, Algeria, Somalia, Uganda, Mozambique, Angola, Nigeria, Denmark, Portugal, Spain), a significant global FST value was obtained. All but six FST values were statistically significant when pairwise comparisons were performed between the 18 populations. The Iraqi population did not show significant difference from the population in Turkey and it grouped together with other Middle-Eastern populations when a multidimensional scaling plot was drawn based on the pairwise FST values. The combined mean match probability and the typical paternity index for trios were 8.3 1020 and 259,000, respectively, for the Iraqi population (Carmen et al., 2013). A lot of genetics and molecular data has been reported in the last 10 years and a huge results gained from these observations. One of the most interesting researches field is the researches about relation between molecular and behavioral disorders to assess the prevalence, symptom severity, functional impairment, and treatment of major depressive episode (MDE) in the Iraqi general population. So the Iraq Mental Health Survey is a nationally representative face-to-face survey of 4,332 non-institutionalized adults aged 18+ interviewed in 2006–2007 as part of the WHO World Mental Health Surveys. Prevalence and correlates of DSM-IV MDE were determined with the WHO Composite International Diagnostic Interview (CIDI). Which concludes that MDE is a commonly occurring disorder in the Iraqi general population and is associated with considerable disability and low treatment. Efforts are needed to decrease the barriers to treatment and to educate general medical providers in Iraq about the recognition and treatment of depression (Al-Hamzawi et al., 2015).

Structural variation (CNV) including deletion, duplication, displacement and reflection of chromosomes was identified in some individuals with autism spectrum disorder (ASD), but the full moral role is unknown. This study evaluated the genome extensively for structural abnormalities in 427 unrelated ASD cases through monoclonal monoclonal arrays and a nuclear pattern. With precise matrices, the researchers discovered 277 of unbalanced CNVs in 44% of ASD families not present in 500 controls (and re-examined in 1152 other controls). The nuclear pattern revealed additional balanced changes. Although most variables were inherited, they found a total of 27 cases with de novo modifications, and in three (11%) of these individuals, two or more new variables were observed. De Novo CNVs were found in about 7% and about 2% of idiopathic families had one child, or two or more ASD siblings, respectively. They also discovered 13 sites with repeated / overlapping CNVs in unrelated cases. In these sites, deletions and repetitions affecting the same gene were found in different individuals and sometimes in symptom-free vectors. Despite complications, their results include more SHANK3-NLGN4-NRXN1 involvement in post-clamp density genes as well as new positions in DPP6-DPP10-PCDH9 (compound clamp), ANKRD11, DPYD, PTCHD1, 15q24, among others, for the role In ASD sensitivity. Their most convincing results revealed CNV at 16p11.2 ($p = 0.002$) (with characteristics of genetic disturbance) at a frequency of about 1%. Some areas of ASD were also common in places of mental retardation. Structural variables were found in the high-frequency effect sufficiently ASD suggests that cellular genetic analyses and microarray are considered in the clinical workup routine (Hadi, 2019).

Another study aimed to detect the consistent chromosomal abnormalities of each type of common bone tumours . Thirty bone tumours specimens were processed for direct cytogenetic preparation , only (9 cases) showed success results for chromosomal preparation by banding technique , the results of cytogenetic study were 1- Osteosarcoma (4) cases revealed multiple numerical and structural changes with complex karyotype and pronounced cell to cell variation . chromosome 17 was the most frequent involved in these chromosomal alteration . Also loss or structural changes of chromosome 13 was found in (2) cases of osteosarcoma loss or gain of sex chromosome were detected in these cases , loss of Y chromosome in (2) cases ; loss of X chromosome in (1) case and gain of X chromosome in another case .2- Osteochondroma : revealed the simple numerical change with no structural change in one case . 3- Chondroblastoma : in this tumour structural and numerical abnormalities of chromosome 5 . 4- Chondrosarcoma : showed chromosomal aberration with multiple numerical and structural changes in chromosome (1) was of interest and monosomy 18 was reported in one case . 5- Giant cell tumours : It showed a complex changes and the range of chromosomal number was 50 – 58 with the characteristic telomeric fusion in malignant cases while simple numerical change only in benign giant cell tumour .Conclusion Cytogenetic study of

both benign and malignant bone tumours have revealed abnormalities in the number and / or structures of chromosomes X, Y , 1,5,6,11 , 13,17,18 and complex chromosomal changes in malignant types of bone tumours (Nahi et al., 2017).

Another group of researchers studied thyroid gland that requires a sequence of particular conditions to produce its hormones which are affected by a large number of factors. The disturbances of these factors lead to cause thyroid disorders. The study included 100 samples collected from patients who suffer from thyroid disorders in the Department of Radiation Nuclear Medicine Hospital and Yarmok hospital in Baghdad and 25 sample as quality control during the period from July to October 2009. Ranged in age from patients and healthy individuals (17-79) years. Blood samples were drown from all patients and control in order to be used in hormonal, cytogenetic and molecular studies. The results have showed that the most frequent thyroid disorders among patients are thyroid non-toxic goiter 32% and thyroid toxic goiter 31% while hypothyroidism 20% and thyroid cancer 17%. The results also showed that these disorders distributed highly among the age group 30-50 years old with high prevalence in females. Thyroid hormones (T3, T4 and TSH) levels were determined in all unrelated Iraqi cases by enzyme linked fluorescent assay, a different degree of hormones levels with thyroid disorder groups were shown. TSH level was significantly increased (18.76±6.44 µ IU/ml) in hypothyroidism group. In the thyroid cancer group, TSH and T3 showed to have a low levels (1.49±0.75 µ IU/ml and 1.32±0.13 nmol/L) respectively, combined with a significant increase in T4 level (104.71±9.72 nmol/L). Using new Locked Nucleic Acid primer (LNA) primers - PCR mutations screening technology, DNA from patients and control was screened to detect the existence of 8 TG and TPO selected mutations. Eight mutations were detected in thyroid disorder patients six of TG and two TPO genes mutations. These mutations include g.IVS5+G>A, c.886C >T, c.986A>C, c.2610G>T, g.IVS10-1G>A and g.IVS34-1G>C location in exons 5, 7, 8, 10 and 34 in TG While 1708C>T and 1978C>G location in exons 10 and 11, of TPO mutations, respectively. Also, the results showed a sort of relationship between some of the detected mutations and the level of hormones. The mean values of the hormones levels showed to be slightly varied in terms of age and gender groups. Using new LNA primer- PCR mutations screening technology, DNA from patients and control was screened to detect the existence of 8 TG and TPO selected mutations. Using first designed primers, the results revealed that seventy mutations have been identified to the first time in human TG and TPO genes in thyroid disorders of Iraqi. 53(75.7%) of them were detected in TG gene and 17 (24.3%) in TPO genes. Among 53 TG gene mutations, 26(49.053%) were detected as guanine to adenine transition IVS5 +1 G>A most of them are identified among thyroid toxic goiter and thyroid cancer groups. Other TG mutations such as exon – intron splice mutations and exon mutations were also detected in all tested groups. Seventeen TPO gene mutations including transversion cytosine either by thymine or guanine at the position 1708 of the exon 10

(c.1708C>T) and the position 1978 of the exon 11 (1978C>G) were also detected. Three TG homozygous mutations were detected among thyroid toxic goiter and thyroid cancer which reflect a high DNA instability and 12 compound mutations. Most of the detected mutations in this study were among thyroid toxic goiter (27.38%) and thyroid cancer (21.30%) groups. DNA instability was also identified in Toxic goiter and thyroid cancer groups. The cytogenetic study results indicated that significantly increased BNMN frequency (37.58±3.07) in thyroid cancer group versus other thyroid disorder groups but with significant increase in other thyroid disorder groups compared with healthy control group. On the other hand, NDI of micronucleus was found with a significant decrease (0.009±0.001) in hypothyroidism versus other groups (AL-Faisal et al., 2011).

Congenital adrenal hyperplasia is a group of autosomal recessive disorders. The most frequent one is 21-hydroxylase deficiency. Analyzing CYP21A2 gene mutations was so far not reported in Iraq. This work aims to analyze the spectrum and frequency of CYP21A2 mutations among Iraqi CAH patients. Sixty-two children were recruited from the Pediatric Endocrine Consultation Clinic, Children Welfare Teaching Hospital, Baghdad, Iraq, from September 2014 till June 2015. Their ages ranged between one day and 15 years. They presented with salt wasting, simple virilization, or pseudoprecocious puberty. Cytogenetic study was performed for cases with ambiguous genitalia. Molecular analysis of CYP21A2 gene was done using the CAH StripAssay (ViennaLab Diagnostics) for detection of 11 point mutations and >50% of large gene deletions/conversions. Mutations were found in 42 (67.7%) patients; 31 (50%) patients were homozygotes, 9 (14.5%) were heterozygotes, and 2 (3.2%) were compound heterozygotes with 3 mutations, while 20 (32.3%) patients had none of the tested mutations. The most frequently detected mutations were large gene deletions/conversions found in 12 (19.4%) patients, followed by I2Splice and Q318X in 8 (12.9%) patients each, I172N in 5 (8.1%) patients, and V281L in 4 (6.5%) patients. Del 8 bp, P453S, and R483P were each found in one (1.6%) and complex alleles were found in 2 (3.2%). Four point mutations (P30L, Cluster E6, L307 frameshift, and R356W) were not identified in any patient. In conclusion, gene deletions/conversions and 7 point mutations were recorded in varying proportions, the former being the commonest, generally similar to what was reported in regional countries **(**Ruqayah et al., 2016). Leishmaniasis is a group of parasitic diseases caused by Leishmania spp., an endemic infectious agent in developing countries, including Iraq. Diagnosis of cutaneous lesion by stained smears, serology or histopathology are inaccurate and unable to detect the species of Leishmania. Here, two molecular typing methods were examined to identify the promastigotes of suspected cutaneous leishmaniasis samples, on a species level. The first was species-specific B6-PCR and the second was ITS1-PCR followed by restriction fragment length polymorphism (RFLP) using restriction enzyme HaeIII. DNA was extracted from in vitro promastigote culture followed by amplification of kDNA by B6 or amplification and digestion of LITSR/

L5.8S. PCR produced bands of ~359 bp and ~450 bp for B6 and ITS1, respectively. Digestion of ITS1 by RFLP revealed two distinct bands of ~150 bp and ~300 bp size. The results reviled that the two isolates belong to cutaneous Leishmaniasis, specifically Leishmania tropica. In conclusion, the confirmation of the studied methods will improve rapid and accurate diagnosis of Leishmania species of the most prevalent Iraqi strain of cutaneous leishmaniasis, L. tropica (Bayram & Ali 2021).

In conclusion: each country or geographical zone has its molecular and genetic specifications which may reflects on the prevalence and dominance of some disorders rather than others and that what the previous researches had mention. Iraq is like many other countries that exposed to many pollutants that affects the molecular expression and suppression of some genes and DNA sequences that gave the chance to variable diseases to appear and respond poorly to the well known treatment protocols followed by another countries or another patients which raise the world -nowadays -distributted question of what is the molecular reason behind variable response to the same treatment of the same disease.

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