

Prevalence of Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency, in a Jordanian sample pool

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Abstract

The main aim of the current study was to screen the Jordanian population for the 8 most common *CYP21A2* mutations and introduce a prenatal and a neonatal genetic test for CAH in Jordan. Similar mutational analyses studies have been performed in other Arab countries such as Tunisia and Iran, however, this is the first comprehensive study of its kind, performed on the Jordanian population. Our sample pool consisted of 46 clinically diagnosed index patients, including 22 males and 24 females. In most cases, DNA samples for family members were also available, bringing the total number of samples up to 170. Different experimental methodologies have been implemented in this study including ARMS, MLPA and sequencing. Preliminary results suggest a unique allelic spread of the most common *CYP21A2* mutations within a Jordanian sample pool.

Introduction

CAH represents a family of autosomal recessive disorders. About 95% of CAH cases are caused by 21-hydroxylase deficiency, resulting from a defect in the *cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2)* gene (1). Two different forms of CAH exist, the classic form, which is the severe form and is further subdivided into simple virilizing and salt wasting depending on whether the sodium levels in the patient are abnormal or not. 75% of classic CAH cases are salt-wasting. Non-classic CAH is a milder form and patients are either asymptomatic or show mild signs of androgen excess. It is important to note that *CYP21A2* has an inactive counterpart, the pseudogene *CYP21A2P*, with the two sharing high degree of sequence similarity. Recombination events occurring between *CYP21A2* and *CYP21A2P* are the main cause of mutations leading to CAH (2).

Methods

A mutational screen (P30L in exon1, In2cs in intron2, 8bp deletion in exon3, I172N in exon4, exon6 cluster mutation, V281L in exon7, Q318X and R356W in exon8) was conducted on 46 index patients clinically and biochemically diagnosed with CAH. The implemented methods included Amplification Refractory Mutational Screen (ARMS) (Figure 1) and Multiplex Ligation-dependant Probe Amplification (MLPA) (3) (Figure 2). Amplification of the *SRY* gene was performed for 3 patients.

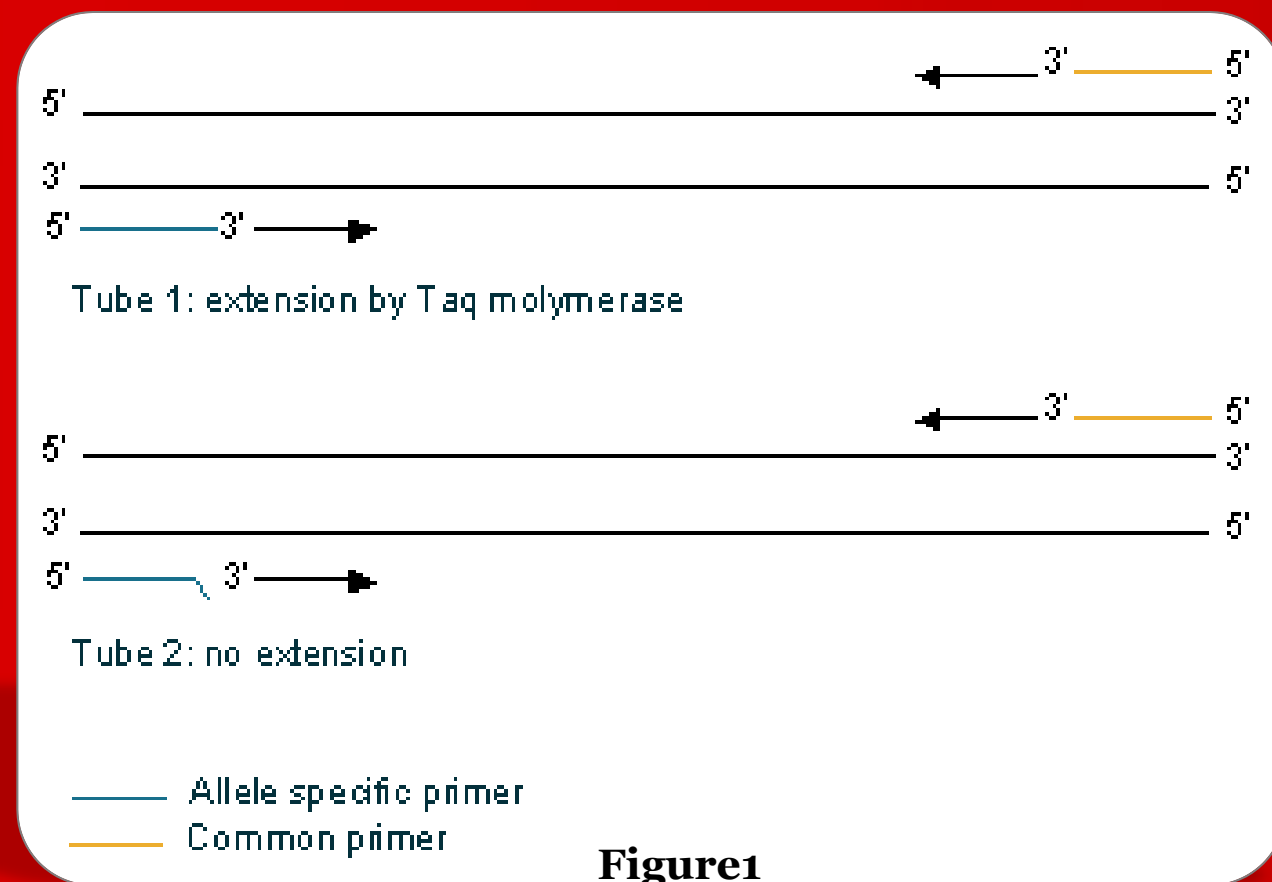


Figure1. ARMS method used for the detection of the 8 most common *CYP21A2* mutations (P30L in exon1, Incs2 in intron2, 8bp deletion in exon3, I172N in exon4, exon6 cluster mutation, V281L in exon7, Q318X and R356W in exon8).

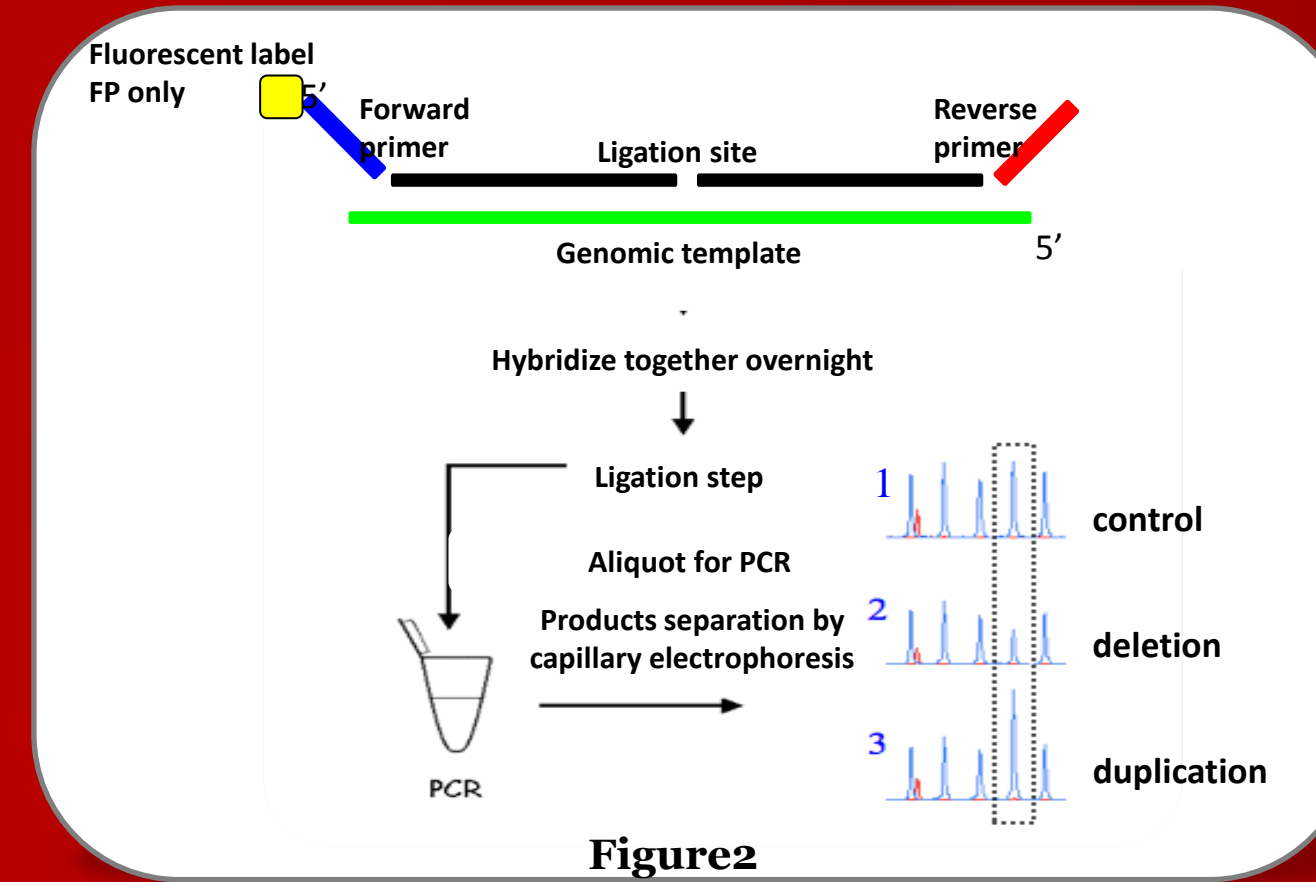


Figure2. MLPA method used for the detection of the 30kb deletion encompassing regions of the *CYP21A2* and *CYP21A2P* genes.

Results

Out of 46 index patients, 4 carried a heterozygote mutation, 13 were homozygous and 11 patients were compound heterozygotes/homozygotes for the *CYP21A2* mutations (Table 1.). The Incs2 mutation had the highest allelic frequency, at 35.7% followed by Q318X at 23.2%, P30L at 17.8%, 8bp deletion and I172N mutation both at 16%, R356W at 3.5% and exon6 cluster mutation at 1.7% (Figure 3). The V281L mutation was not detected in any of the index patients screened in this study. The high allelic frequency observed for the In2cs mutation in the Jordanian population was also observed in Tunisia, Turkey, Iran and average world studies (4) (Figure 4). Allelic spread for Q318X mutation was relatively high in both the Tunisian and Jordanian population. Surprisingly, P30L mutation showed a very low rate of occurrence (if any) in the countries presented in Figure 1b, with the exception of the Jordanian population. The 30kb deletion on the other hand, was not detected in any of the 18 index patients tested by MLPA. Interestingly, in 3 patients analyzed by MLPA the genotype did not match the phenotype. 2 phenotypic males showed absence of the Y chromosome (Figure 5a,b) while 1 phenotypic female showed the presence of the Y chromosome (Figure 5c). PCR analysis showed the presence of the *SRY* gene in patient 1, indicating a translocation from the Y onto the X chromosome. In patient 2 *SRY* was not present, suggesting the patient was a normal female affected with CAH whereas in patient 3 *SRY* could be detected, indicating that the patient was a normal male affected with CAH.

Table 1. Summary of the data obtained for the 46 CAH patients

Index Patients					
Total nos.	Male	Females	Heterozygous	Homozygous	Compound
46	22	24	2 males 2 females	3 males 8 females	5 males 6 females

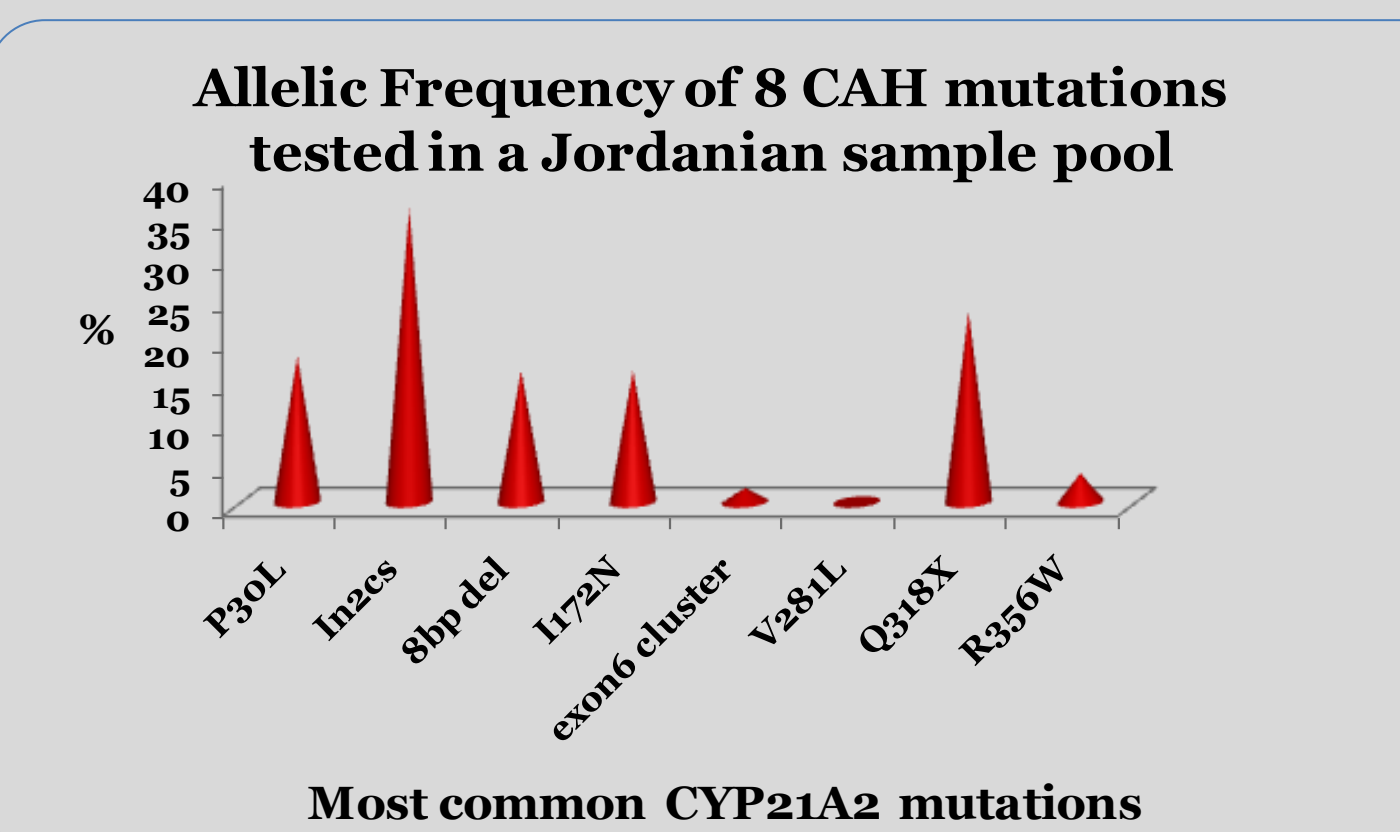


Figure 3. Allelic frequency distribution for the 8 most common *CYP21A2* mutations in the Jordanian sample pool.

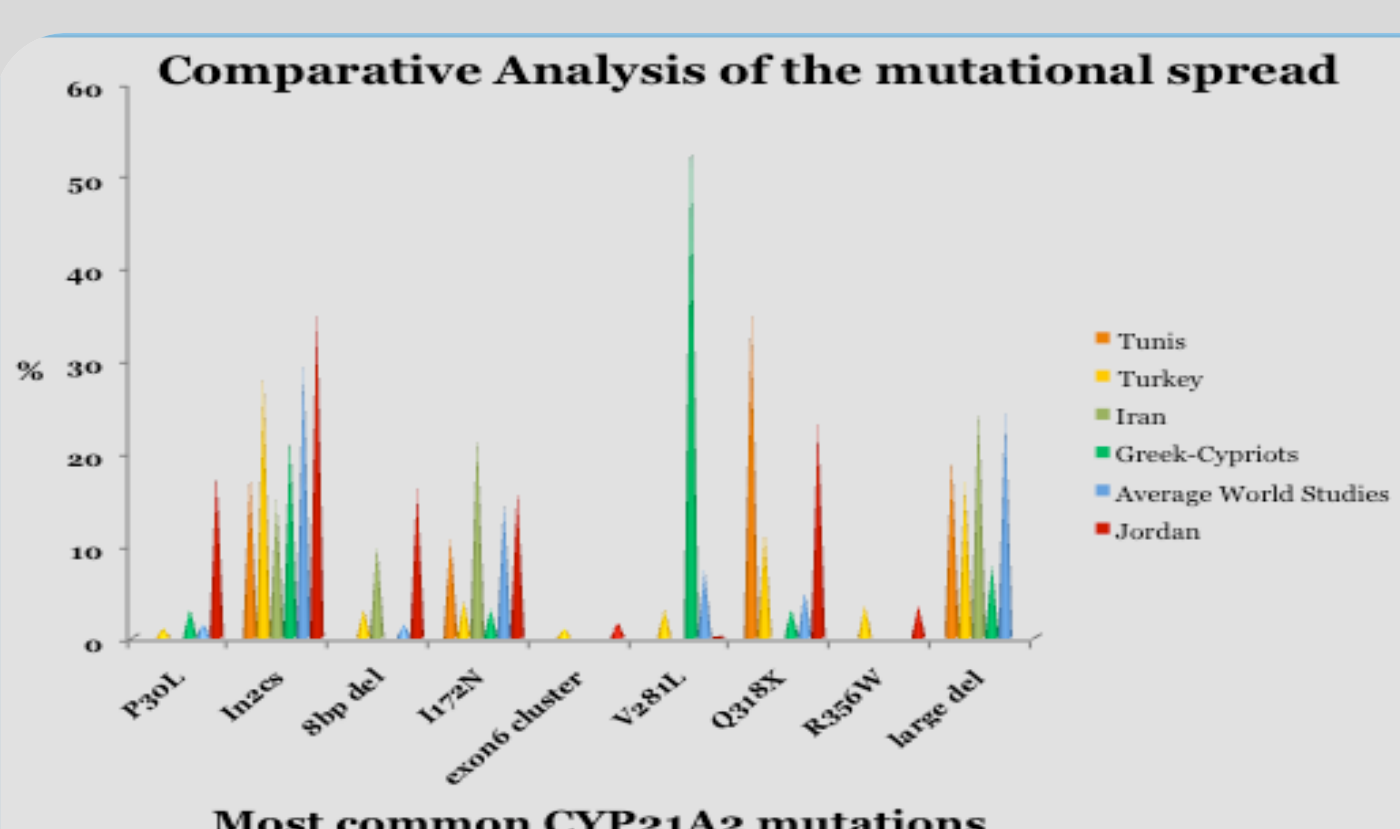


Figure 4. Comparative analysis of the *CYP21A2* mutational spread in different countries, including Jordan.

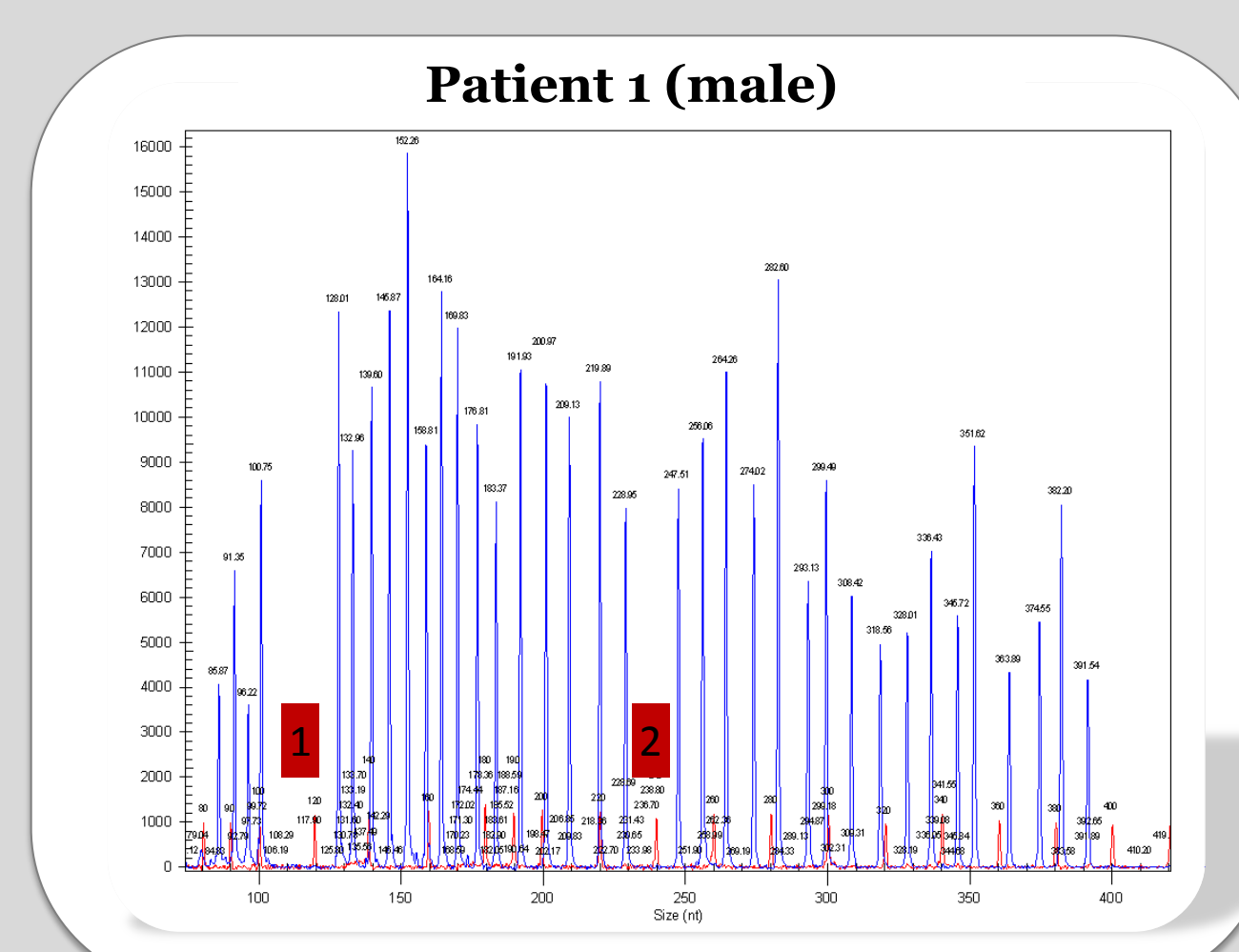


Figure 5a. MLPA analysis for CAH male patient. Peak at 105nt and 238nt is absent (Y-fragment and UTY probe).

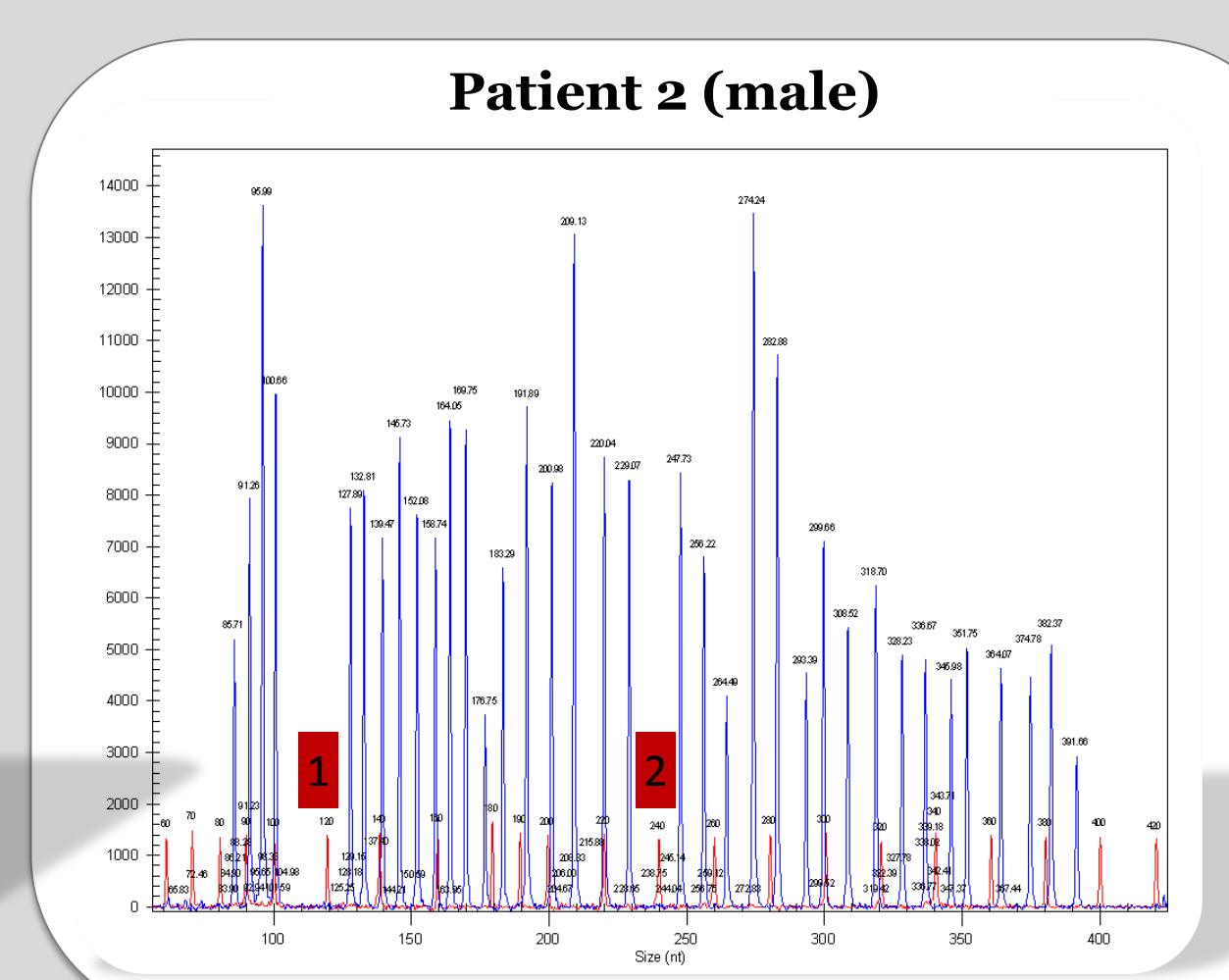


Figure 5b. MLPA analysis for CAH male patient. Peak at 105nt and 238nt is absent (Y-fragment and UTY probe).

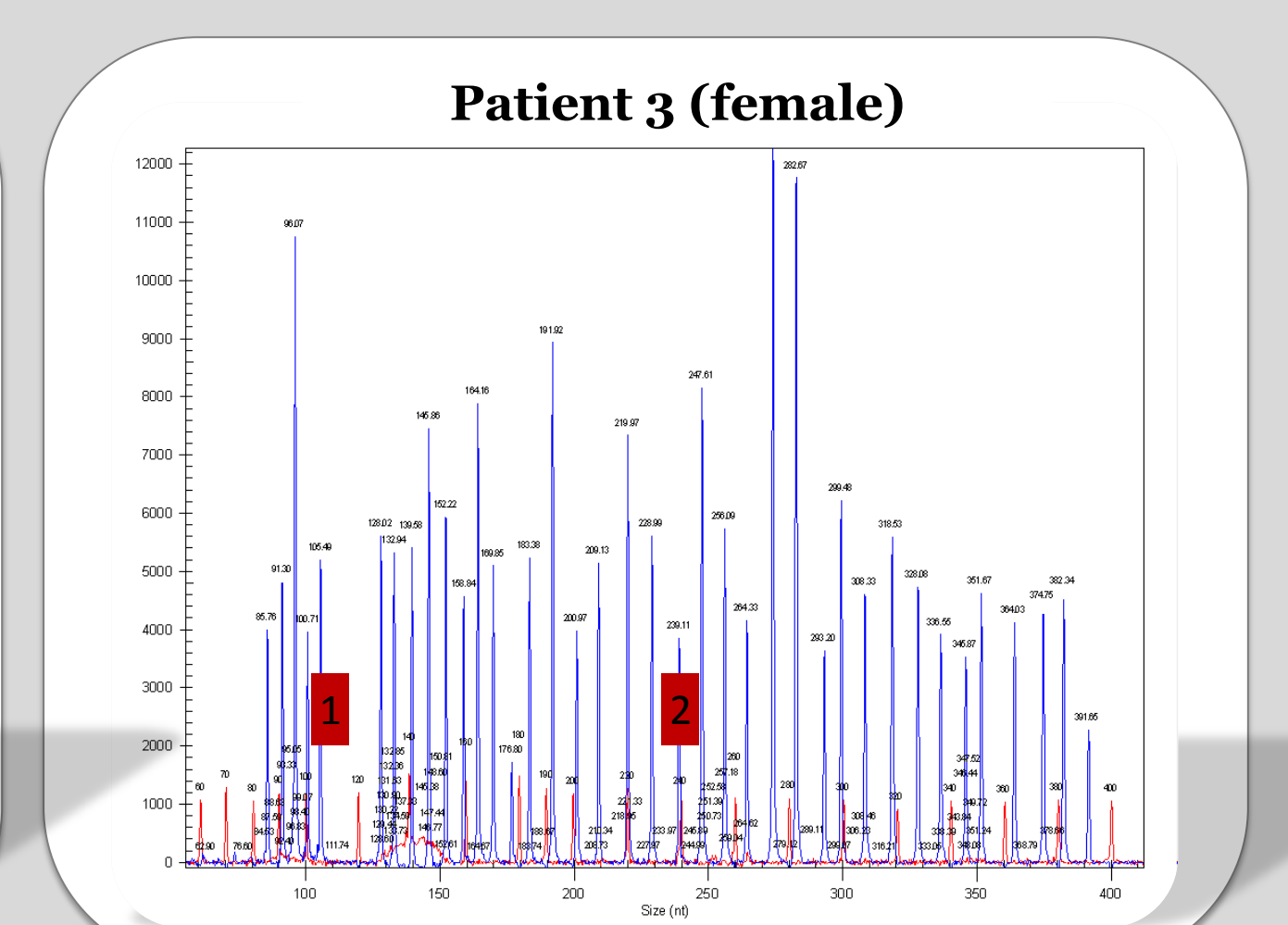
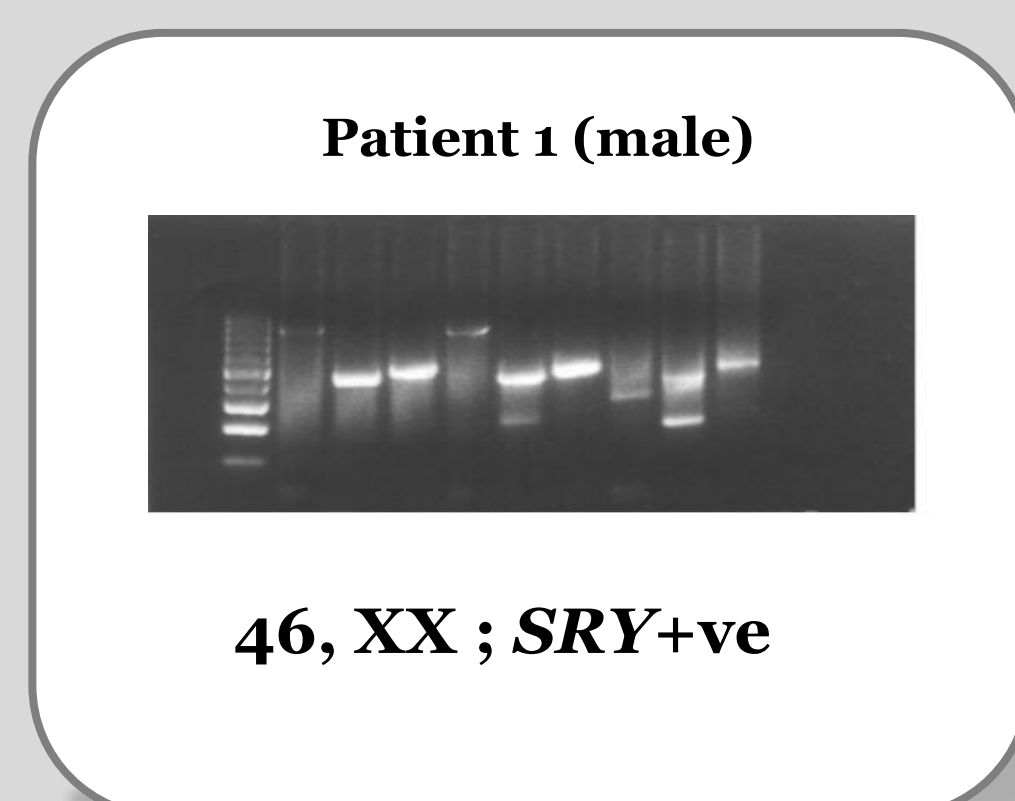
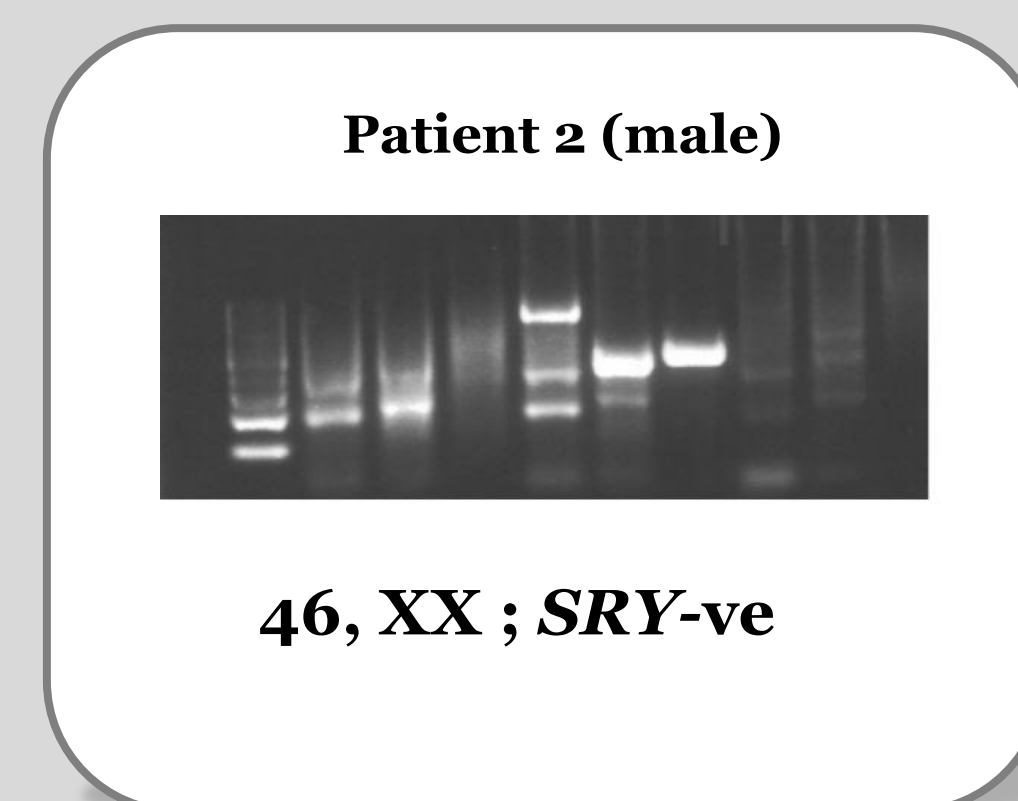


Figure 5c. MLPA analysis for CAH female patient. Peak at 105nt and 238nt is present (Y-fragment and UTY probe).



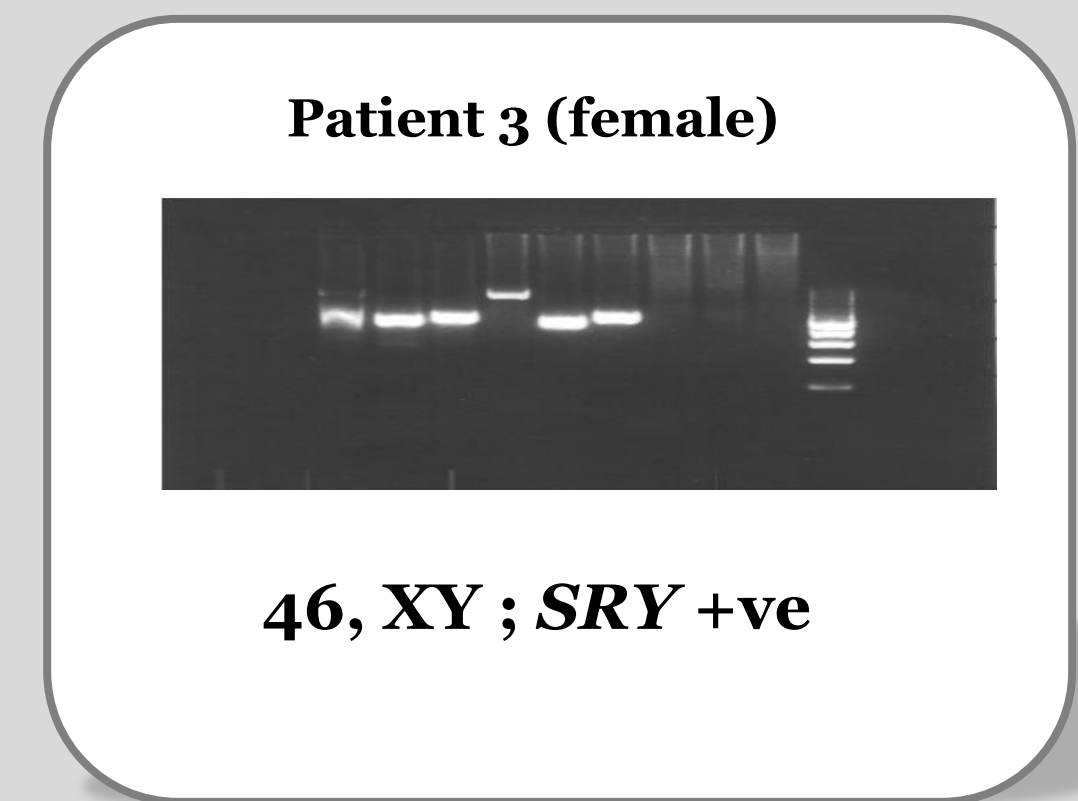
- *SRY* translocation from Y to X
- Enlarged penis
- High 17-OH progesterone
- Classic salt wasting

Figure 6a. Amplification of the *SRY* gene. The patient carried the *SRY* gene.



- Ambiguous genitalia
- Abnormal menstrual cycle
- High 17-OH progesterone
- Heterozygote for Q318X

Figure 6b. Amplification of the *SRY* gene. The patient did not carry the *SRY* gene.



- Ambiguous genitalia
- Enlarged clitoris
- High 17-OH progesterone
- Simple virilization

Figure 6c. Amplification of the *SRY* gene. The patient carried the *SRY* gene.

Discussion

CYP21A2 mutations were identified in 28 patients out of 46 examined, representing 60% of the total sample pool. 63% of those came from consanguineous marriages. Absence of the most common mutations warrants *CYP21A2* gene sequencing in the remaining 40% of the patients. Based on the obtained results, it can be argued that the Jordanian population shows a unique allelic spread for the *CYP21A2* mutations. Although, it has to be taken into consideration that a similar study has not been conducted in neighboring countries, where it could show that the identified allelic frequencies are similar for the whole Middle Eastern region. Also, based on our experience, it is clear that in order to avoid wrong diagnosis and incorrect rearing of the child, all patients suspected to have CAH should be karyotyped and *SRY* analysis needs to take place. As the incidence of CAH is relatively high in the Arab population (5), we hope that we will be able to introduce prenatal and neonatal testing in Jordan for CAH in the near future.

References

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