NON-CLASSIC CONGENITAL ADRENAL HYPERPLASIA CAUSING ALLELES AMONG ADOLESCENT GIRLS WITH PCOS – GENETICAL STUDY

Lāsma Līdaka MD[1]; Gunta Lazdāne MD, PhD[2]; Linda Gailīte MD, PhD[3]; Iveta Dzīvīte – Krišāne MD, PhD[1]

1Children's Clinical University Hospital, Latvia
3 Riga Stradins university, Department of Obstetrics and Gynaecology, Latvia
2 Riga Stradins university, Scientific laboratory of molecular genetics, Latvia

Introduction and objective

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in adult women. Syndrome is characterised by hiperandrogenism, oligo/amenorrhoea and polycystic ovary morphology (PCOM) in ultrasound. Clinical signs of the syndrome usually start already during adolescent years. Non-classic congenital adrenal hyperplasia (NCAH), caused by several mutations in *CYP21A2* (6p21.3) gene is the most common differential diagnosis for girls presented with symptoms of PCOS. It is diagnosed in 1/1000 women in reproductive age. NCAH is characterised with the same symptoms as PCOS, no specific clinical signs can usually be observed. Level of 17 – OH progesterone and AKTH stimulation test is the main diagnostic tool. Nevertheless, genetic testing provides more accurate diagnosis.

Objective: To asess prevalence of NCAH causing alleles among adolescent patients with PCOS.

Methods

40 adolescent patients at least two years after *menarche* attending paediatric gyneacologist with PCOS according to Rotterdam criteria were included in the study. Hyperandrogenism was defined as Ferriman – Gallway score more than seven or elevated total testosterone (Total T), androstendione or DHEASO4 levels. DNA was extracted from venous blood using fenole – chloroform methode. Genetic variations in the *CYP21A2* gene were tested by using standart Multiplex Ligation-dependent Probe Amplification test (SALSA MLPA probemix P050-C1 CAH, MRC Holland), according to methodology established by producers. Following CYP21A2 mutations were tested: Single nucleotide polymorphism (SNP)at 113 bp before the start codon; I2G sequence at 13 bp before exon 3; 8 bp deletion in exon 3 (del8bp; 706_713del8); I172N mutation in exon 4; V237E mutation in exon 6; M239K mutation in exon 6; F306+T mutation in exon 7. Research was approved by Central Medical Ethics Committee of Latvia.

Results

Median age of the study group was 16 (SD 1.4) years. Average score in Ferriman-Gallway (F-G) scale was 10.8 (SD 6.3). We detected pathogenic variants in *CYP21A2* for four patients, that constitutes 10% of the tested alleles. All discovered variants were in heterozygous state, that does not establish definitive diagnosis of NCAH. Two patients had -c.-113 A>G, I172N (rs6475) and other two I2G (rs6467). Characteristics of patients with established mutations are presented in Table1. MLPA testing results are showed in Figure 1. – 4.

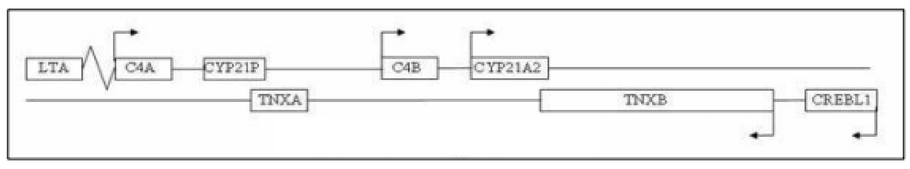

Figure 1: A schematic representation of the chromosome 6p21.3 region (SALSA MLPA probemix P050-B3 CAH, Description version 28; 21-12-2012)

Patient	F-G score	Total T level (ng/ml)	PCOM (+/-)	Lenght of menstrual cycle (days)	Mutation detected
A	9	0.634	+	20 - 50	I2G (rs6467)
B	5	0.64	+	28	CYP21A2, c.-113 A>G
C	28	0.590	+	30-96	I2G (rs6467)
D	8	0.78	-	>90	CYP21A1, I172N

Table 2: Characteristics of patients with detected mutations.

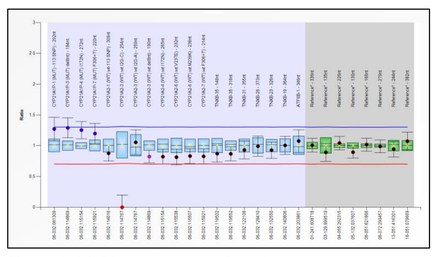

Figure 1. MLPA results for patient A.
Mutation: I2G (rs6467)

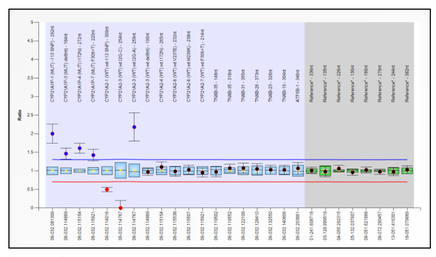

Figure 2. MLPA results for patient B.
Mutation: CYP21A2, c.-113 A>G

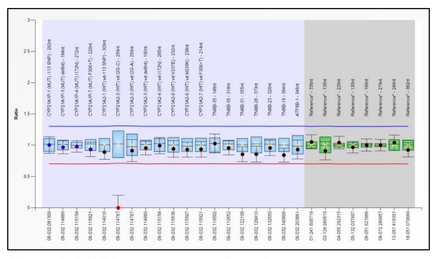

Figure 3. MLPA results for patient C.
Mutation: I2G (rs6467)

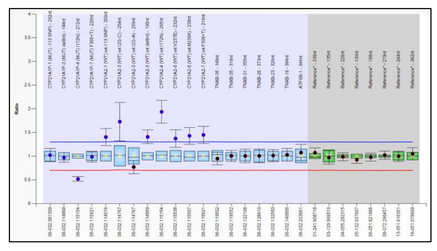

Figure 4. MLPA results for patient D.
Mutation: CYP21A1, I172N

Conclusions

As NCAH is one of the reasons for hiperandrogenism masked by clinical appearance of PCOS, genetical testing is an important tool to distinguish between these two conditions.

Prevalence of NCAH causing alleles among PCOS patients in our study was lower than in literature (usually around 10%). It could be because the test we used is designed to assess rather large rearrangements of DNA, further studies in this area are required in order to test minor DNA changes and less prevalent mutations.

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RĪGAS STRADIŅA UNIVERSITĀTE

Contactinformation:
LASMA.LIDAKA@BKUS.LV
+371 25547935
Riga, Vienibas gatve 45, LV-1004

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Adrenals and HPA Axis
Lasma Lidaka
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