Novel genotype in two siblings with 5-alpha-reductase 2 deficiency: different clinical course due to the time of diagnosis

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Introduction and objectives

Steroid 5-Alpha-Reductase-2 deficiency(5-ARD) is a result of mutations in the SRD5A2 gene (frequently in exon 1). It causes disorder of sexual differentiation (DSD) in 46XY individuals with a variable genital phenotype. Patients have been assigned to both genders with different success. Definite diagnosis requires genetic testing.

Objectives. To present two siblings with a female external genitalia at birth and bilateral inguinal testes, raised as females, to present novel genotype in the first molecularly sharacterized patients from the Republic of Macedonia with a different clinical course due to the time of the diagnosis. To point to the importance of the early genetic diagnosis.

Methods

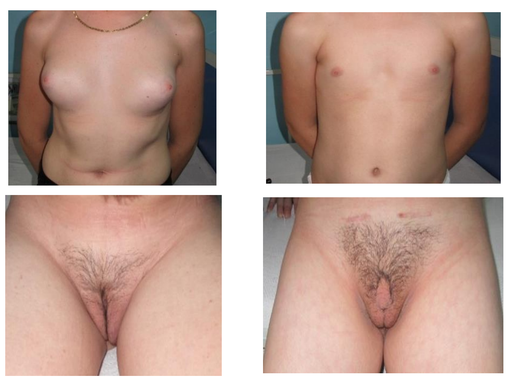
Clinical evaluation	Genetic analysis
Ultrasound	Saenger sequencing
Karyotype	MLPA analysis
Hormonal evaluation	Real time PCR

Clinical iinformation	Patient 1	Patient 2
Age at diagnosis	Newborn Inguinal hernia	7.5 years Virilization
Testosterone/DHTS Karyotype	22 46, XY	20 46, XY
Age at genetic analysis	18 years	12.5 years
Diagnosis	5 a reductase deficiency	5 a reductase deficiency
Age at the definitive gender ssingnment	Newborn	11.5 years
Intervention	Orchidectomy Breast implantation Vaginoplasty	Orchidectomy Hormonal therapy (Estrogene)

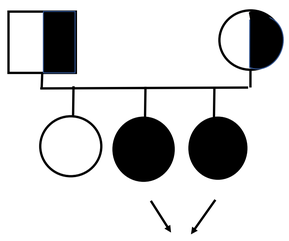
Figure 1.

Patient 1 after breast implant and vaginoplasty

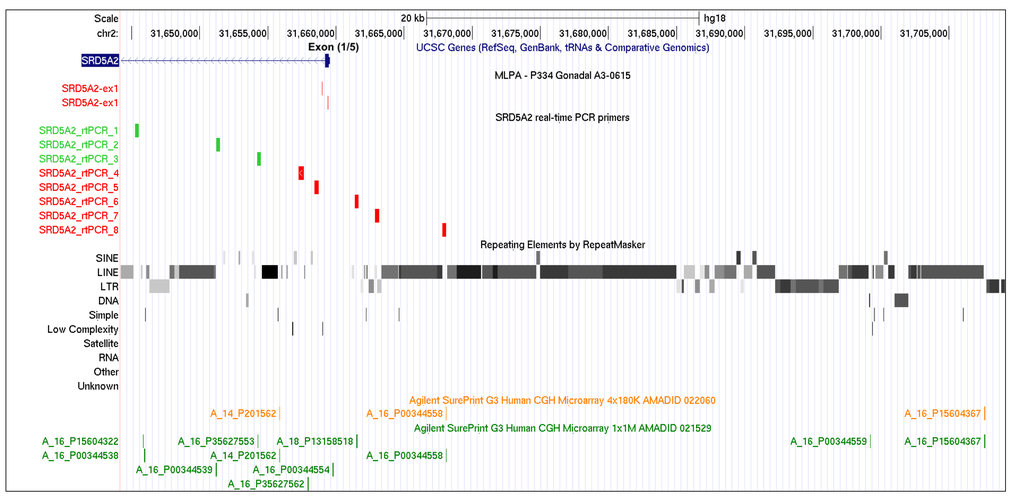
Patient 2 at the age of 11years



Del. Ex 1/c.145 G > A c.146 C > A



c.145 G > A Del. Ex 1/c.146 C > A



Graphical presentation of the targeted sequences by MLPA and real-time PCR analysis with the use of the UCSC genome browser (Kent, W. J et al [2002] *Genome research*, *12*(6), 996-1006.). Besides custom tracks ("MLPA –P334 Gonadal" and "SRD5A2 real-time PCR primers"), the "UCSC Genes", "RepeatMasker" and Agilent Arrays" tracks are shown. In the custom tracks, the sequences colored red are those deleted (only one copy) in the father and the two affected siblings, while sequences colored green in the "SRD5A2 real-time PCR primers" track are non-deleted (present in two copies). The deletion breakpoint from the 3' side (intron 1) is between SRD5A2_rtPCR_3 and SRD5A2_rtPCR_4 primers, while from the 5' side (before SRD5A2 gene) is undetermined. There is increased complexity of the region before the SRD5A2 gene as shown by sequences annotated with "RepeatMasker". The distance between A_16_P00244558 and the A_16_P15604367 probes from Agilent 4x180K is large (approximately 40 kilobases) which indicates the difficulty of creating the primers in this region. As comparison, the probes from the densest Agilent chip with the 1 milion probes are shown with the distance between A_16_P00244558 and the next closest probe A_16_P0344559 of approximately 30 kilobases.

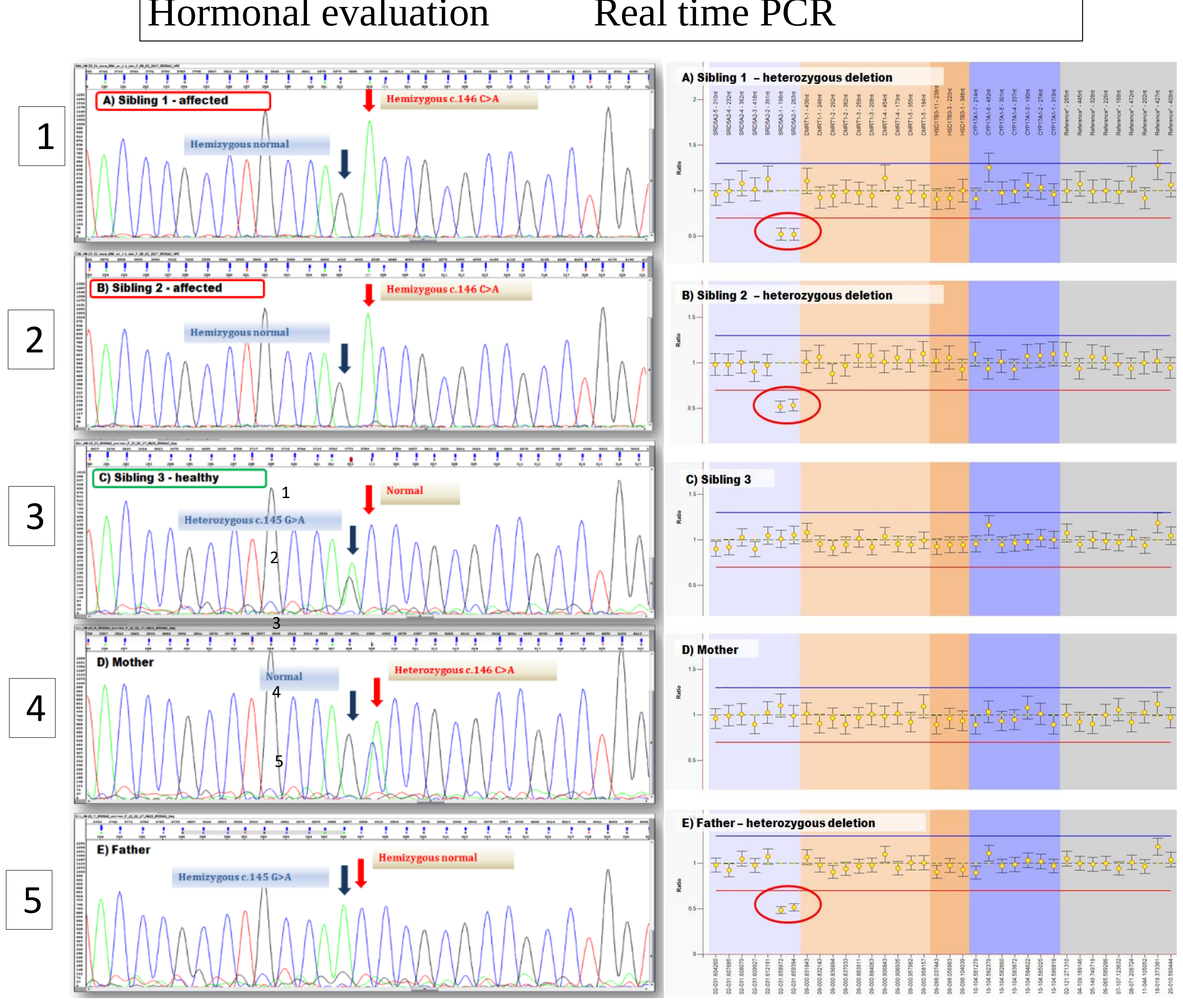


Figure 2. A. Saenger sequencing. Analysis of the exon 1 in the SRD5A2 gene**: 1 & 2** = Patient 1 and Patient 2 **-** hemizygous for the pathogenic c.146 C>A mutation. **3.** Healthy sibling is heterozygous for the benign c.145 G>A mutation inherited from the father. **4.** Mother is a carrier of the pathogenic c.146 C>A mutation. **5.** Father is hemizygous for the benign c.145 G>A.

B. Results from the MPLA analysis using P334-A3 Gonadal Development Disorder kit presented in the same order. Two affected siblings and the father are heterozygous for deletion in exon-1 of the SRD5A2

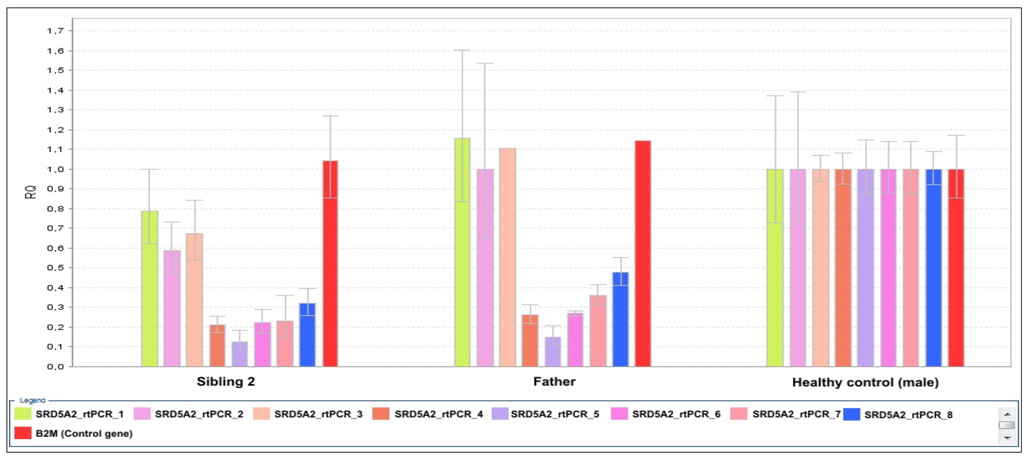


Figure 3. Real-time PCR analysis for the approximate determination of the deletion breakpoints. The first three primers (from rtPCR_1 to rtPCR_3) showed diploid state (two copies of the targeted sequence), while the remaining five primers (from rtPCR_4 to rtPCR_8) showed haploid state (one copy of the amplified sequence) in the affected sibling and the father as compared to normal healthy male sample used as reference.

Conclusions

- Both patients present identical genotype causing ASD deficiency.
- Timing of diagnosis is different causing delay in the therapeutic approach towards the Patient 2.
- Novel genotype causing 5ARD has been described
- This is the first family with 5ARD genetically analyzed from RNM
- Genetic analysis might be a necessary early test in 46, XY DSD

1.Mendonca BB, Domenice S, Arnhold IJ, Costa EM. 46,XY disorders of sex development (DSD). Review. Clin Endocrinol (Oxf).2009;70(2):173-87,

2.Alswailem MM, Alzahrani OS, Alghofaili L, Quasem E et al..Molecular genetics and phenotype/ genotype correlation of 5-α reductase deficiency in a highly consanguineous population. Endocrine. 2018;doi:10.1007/s12020-018-1767-1

3. Byers HM, Mohnach LH, Fechner PY, Chen M et al. Unexpected ethical dilemmas in sex assignment in 46 XY DSD due to 5-alpha reductase type 2 deficiency. Am J Med Genet C Semin Med Genet. 2017;175 (2):260-7