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Copy-Number Variations of the Human Olfactory Receptor Gene Family in Patients with Macromastia and Prepubertal Gynecomastia

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Background: Aromatase excess syndrome (AEXS) (OMIM 139300) is a rare condition characterized by gynecomastia in boys and macromastia in girls. Estrogen excess in boys can lead to prepubertal and pubertal gynecomastia, bone age progression and short adult stature. While most girls are usually asymptomatic, there are few reported female patients with excessive breast growth, early puberty, menstrual irregularities, and short adult stature. Male and female children with AEXS have shown heterozygous structural mutations in *CYP19A1*, leading to increased activity of aromatase enzyme and consequently excessive estrogen production.

Aim: Investigation of Copy Number Variations (CNVs) in *CYP19A1* and other pathways related genes in patients with prepubertal gynecomastia and macromastia.

Subjects and Methods: 13 patients (6 males with prepubertal gynecomastia and 7 females with macromastia) followed with AEXS diagnosis constituted the study group. Clinical and hormonal findings of the patients at diagnosis and follow-up were reviewed retrospectively.

Oligonucleotide array comparative genomic hybridization (a-CGH) was performed (Agilent Technologies, Inc., Santa Clara, CA, USA) according to manufacturer protocol. Data were analyzed with the use of Agilent Genomics Workbench, with data aligned to the Human Genome release 19 (hg19). Predicted pathogenic CNVs was searched and group of genes (*CYP19A1, TNFAIP8L3, AP4E1, GLDN, DMXL2, TMOD3, SEMA6D, SCG3, CGNL1, ESR1,* and *PTEN)* were specifically investigated.

SPSS version 22 (Chicago, IL, USA) was used for statistical analyses.

Results: The clinical findings and laboratory results of the patients at presentation are given in **Table 1**, and at the time of the study in **Table 2.** The median age of the patients at presentation was 14.4 (range 12.3-22.1) years in female patients and 8.7 (range 6.3-12.7) years in male patients.

All female patients were pubetal. With the onset of puberty breast tissue grew very rapidly and macromastia occurred.

There was a history of consanguineous marriage in 42.9% of boys and 28.5% of girls. Among boys, 3 had gynecomastia, one had only macromastia in a paternal aunt and 2 had both gynecomastia and macromastia history in their families.

In 3 girls there was a history of macromastia in the family. Excessive growth of breasts in the pubertal period occurred in the mother of one girl, in the maternal grandmother, paternal grandmother and paternal aunts of another and in the maternal aunt of the third girl. All of these cases had surgical treatment and breast tissues were reduced in size.

During the study, none of the patients were obese, apart from two female patients. There was no medication intake.

In males both E2 and E1 levels were high. Bone age was advanced more clearly in males, as expected.

All of the male patients had normal beta HCG, alpha-fetoprotein and carcinoembryonic antigen levels.

One of the girls received aromatase inhibitor (tamoxifen) treatment. Breast reduction surgery could only be performed in 3 girls. One of the boys had mastectomy performed after no reduction in breast size after aromatase inhibitor (anastrazole) treatment.

No mutation was detected by a-CGH technique neither in *CYP19A1* nor investigated genes. However, interestingly, we detected a 65.172 kb deletion in olfactory receptor gene cluster *OR4P4, OR4S2, OR4C6* (CHR 11q11.1) in four females and in 2 males (46.2%). Whereas, in our a-CGH cohort we detected the same type of deletion in 34 out of 988 cases (3.4%). This deletion was significantly high in the patients with prepubertal gynecomastia and macromastia **[χ^2=61.02; p<0.0001 and odds ratio 24.05 (95% CI:7.67-75.41**)].

There was no correlation between the presence of CNV in the OR4P4, OR4S2, OR4C6 gene cluster and BMI SDS, sex, and hormonal values at presentation.

Table 1. Clinical and laboratory findings of patients at presentation [median (range)]

	Prepubertal gynecomastia n=6 (males)	Macromastia n=7 (females)	p
Clinical Findings			
Age at diagnosis (year)	9.1 (6.3 to12.7)	13 (12 to18)	**0.008**
Age (year) at presentation	8.7 (6.3 to 12.7)	14.4 (12.3 to 22.1)	**0.005**
Birth weight SDS	-0.8 (-3.2 to 1.15)	-0.3 (-1.8 to 2.4)	0.792
Weight SDS	1.9 (-0.4 to 2.4)	2.0 (-0.8 to 4.8)	0.818
Height SDS	1.3 (-1.0 to 2.0)	-0.1 (-1.8 to 2.3)	0.53
BMI SDS	1.6 (0.2 to 2.4)	1.6 (-0.6 to 3.6)	0.818
Non-obese (BMI<2 SDS)	5	4	0.241
Obese (BMI>2 SDS)	1	3	
Pubertal stage (Tanner)			
1	6	0	0.001
5	0	7	
Consanguineous marriage n(%)	2 (33,3)	2 (28.5)	0.859
In the family			
Gynecomastia	3	0	>0.05
Macromastia	1	3	
Gynecomastia and macromastia	2	0	
Target height SDS	-1.3 (-1.6 to -0.5)	-1.3 (-2.3 to 1.7)	0.762
Laboratory			
LH (mIU/ml)	0.1 (0.01 to 0.3)	4.4 (1.6 to 14.2)	**0.001**
FSH (mIU/ml)	0.8 (0.02 to 2.0)	4.6 (1.8 to 9.5)	**0.01**
Testosterone (ng/ml)	0.2 (0.01 to 0.3)	0.36 (0.18 to 0.38)	0.250
Estradiol (pg/ml)	19.5 (11.4 to 64.3)	96.8 (25.8 to 357.0)	**0.035**
Estrone (pg/ml)	22.0 (10.8 to 30)		-
Prolactin (ng/ml)	9.9 (5.9 to 15.1)	11.8 (9.8 to 23.8)	0.24
Bone age (year)	11.5 (5 to 14)	15 (13 to 18)	0.190

Table 2. Clinical and laboratory findings of patients during the study [median

	Prepubertal gynecomastia n=6 (males)	Macromastia n=7 (females)	P
Clincal findings			
Age (years)	16.9 (13.3 to 19.3)	20.5 (16.3 to 21.6)	0.052
Weight SDS	0.5 (-0.6 to 1.6)	1.7 (-1.2 to 5.2)	0.662
Height SDS	0.2 (-1.0 to 1.0)	-0.5 (-2.2 to 2.5)	0.429
BMI SDS	0.86 (-0.4 to 1.3)	1.4 (-1.0 to 4.0)	0.329
Non-obese (BMI<2 SDS)	6	5	0.102
Obese (BMI>2 SDS)	0	2	
Treatment			
Treatment with AI	1	1	0.11
Surgical Treatment	0	3	
AI+Surgical Treatment	1	0	
Laboratory			
LH (mIU/ml)	6.4 (3.7 to 11.3)	5.2 (4.8 to 5.6)	0.53
FSH (mIU/ml)	3.5 (1.7 to 6.2)	6.5 (6.1 to 7.0)	0.2
Testosterone (ng/ml)	4.0 (2.4 to 9.2)	-	
Estradiol (pg/ml)	39.7 (11.3 to 55)	111.3 (38.9 to 183.7)	>0.05
OR4P4 /OR4S2/OR4C6 (for CNV)			
Wild	4	3	0.529
HT	2	3	
HM	0	1	

BMI: body mass index, SDS: standard deviation score, LH: luteinizing hormone, FSH: follicle stimulating hormone, AI; aromatase inhibitor, OR: olfactory receptor gene

Conclusion:

- ❑No association of *CYP19A1* CNVs revealed in our cohort.
- ❑Rare CNVs of OR4P4, OR4S2, OR4C6 gene cluster might have a role in development of gynecomastia and macromastia. This area requires further study.

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