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GENETIC EVALUATION OF IDIOPATHIC SHORT STATURE

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BACKGROUND

Short stature is a multifactorial condition caused by both genetic and environmental factors. Genetic causes include chromosomal disorders and diseases inherited by monogenic and multifactorial inheritance. The purpose of genetic evaluation in short stature is not only for diagnosis, but also to provide additional information to the patients and their families about prognosis of the disease, treatment approaches and genetic counseling.

AIM

This study aims to investigate genetic etiology by using cytogenetic, molecular cytogenetic and next generation sequencing methods in patients with idiopathic short stature.

PATIENTS AND METHODS

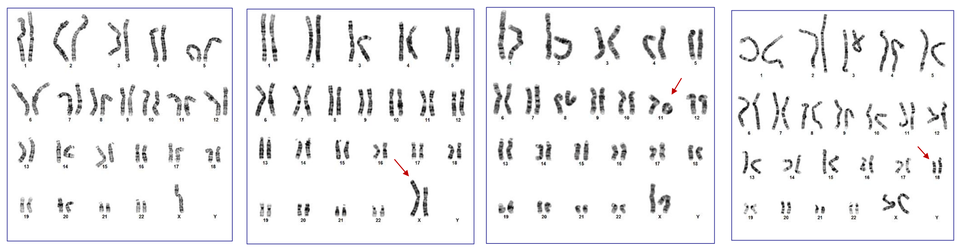
In this study, 189 patients, in whom chronic diseases, hormonal disorders and skeletal dysplasia were excluded, and diagnosed as idiopathic short stature were included in the study. We did an algorithmic approach for genetic screening. In the first phase cytogenetic investigations were done and chromosomal anomalies were excluded. Then SHOX gene deletions were investigated by fluorescent in situ hybridization and possible submicroscopic deletions and duplications by a-CGH technique. After this evaluation 41patients, found to have normal chromosomal segments, underwent to next generation sequencing (NGS) of the Ion Torrent platform with 25 gene-containing panel-gene tests. Gene panel consisted of 10 genes associated with short stature (*GH1, GHR, GHRH, GHSR, IGF1, IGF1R, IGFALS, IGFBP3, SHOX, STAT5B*) and 15 genes *(POU1F1, PROP1, HESX1, LHX3, LHX4, IGSF1, OTX2, BMP4, SHH, WDR11, FGFR1, FGF8, PROKR2, SOX3, HHIP)* associated with isolated or multiple pituitary hormone deficiency (MPHD).

RESULTS

Of the 189 patients with short stature, 16 (8.5%) had chromosomal anomaly,1 had microdeletion in the SHOX gene with FISH examination, and 1 patient had a deletion of 2.7MB in the 5q32 region with a-CGH assay **(Table1).** In five patients, 5 different variations were detected *(BMP4, GHR, IGSF1, LHX4* and *PROKR2)* (one in short stature genes, 4 in MPHD genes). One of this mutations was novel, one of them was previously defined and 3 of them were found in databases **(Table2)**. The changes that were thought to be of clinical importance were confirmed by Sanger sequencing method. It was shown that 4 heterozygous changes found in the segregation analysis were also found in the healthy individuals in the family and in one patient with homozygous change, the parents were shown to be heterozygous carriers.

Table 1. Some cytogenetic and molecular findings of the patients

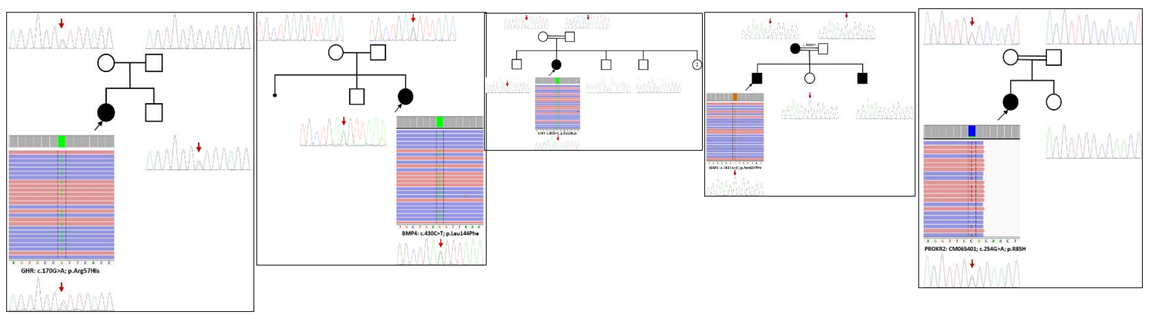
Methods	Σn	Normal n	Abnormal n	Abnormal %
Karyotype	189	173	16	8.5
SHOX deletion (FISH)	153	152	1	0.65
NGS Panel	41	36	5	12.2



Chromosomal abnormalities	Σn	Our study %	General population %
45,X	4	25	2,1
45,X/46,XX[26/4]	1	6.25	0.5
Peripheric blood 45,X/47,XXX[30/55], buccal smear: nuc ish (DXZ1x1/DXZ1x2/DXZ1x3/DYZ3x0) [16/19/65]	1	6.25	0.5
46,X,i(X)(q10)	1	6.25	0.5
45,X/46,X,i(X)(q10) [15/35]	1	6.25	0.5
45,X/46,X,idic(X)(p11.22)	1	6.25	0.5
45,X/46,X,Xq-?. ish der(X)(pter->q13.1::p11.4->pter)	1	6.25	0.5
46,X,Xp-.ish del(X)(p11.1->pter)	1	6.25	0.5
46,X,Xq-.arrXq21.1q28(82809860_155208244)x1	1	6.25	0.5
46,X,idic(Y)(p11.31),(SHOX -)	1	6.25	0.5
46,XX,del (X)(p22.3)(SHOX-)	1	6.25	0.5
47,XX,+mar.ish +mar(SHOXx2/DXZ1x2).arr(1-22,X)x2	1	6.25	0.5
46,XX,del(18)(p10)dn	1	6,25	0.5
46,XX,r(11)(pterq24,2?)/47,XX,r(11)(pterq24.2?),+8[32/4] ish (D11S2071+/VIJyRM2072-,D8Z2x2/D8Z2x3)[49/32]	1	6.25	0.5

Table 2. Some clinical and laboratory findings of the five patients

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
CLINICAL FINDINGS AT PRESENTATION						
Age (years)		13.3	8.6	11.6	15.8	11.1
Birth weight / height SDS		0.2 / 0.9	-0.5 / 0.0	-0.1 / -0.6	0.1 / -0.3	0.7 / -
Weight SDS / Height SDS		-1.1 / -2.9	-1.1 / -1.9	-1.4 / -2.9	-1.9 / -3.5	-1.1 / -2.6
Head Circumference SDS		0.13	-0.9	-0.9	-2.2	
Sitting height /Height ratio		-	0.54	0.55	0.53	0.53
Bone age (years)		-	6.8	10	11	8
Target height SDS		-0.9	0.2	-0.4	-2.1	-0.6
Consanguinity		-	-	1st degree	1st degree	1st degree
IGF1 SDS		-0.6	-1.2	2.0	0.8	-1.6
Clonidiine / L-Dopa test Peak GH response (ng/ml)		- / -	7.9 / 8.6	12.1 / 5.6	6.2 / 6.2	8.7 / 1.9
MOLECULAR FINDINGS						
Karyotype		46,XX	46,XX	46,XX	46,XY	46,XX
Gene		*GHR*	*BMP4*	*LHX4*	*IGSF1*	*PROKR2*
Chromosomal location		5p13.1.p12	14q22.2	1q25.2	Xq26.1	20p12.3
Trancript id		NM_000163.4	NM_001202.5	NM_033343.3	NM_001170961.1	NM_144773.2
Exon		4	4	3	12	1
Zygosity		Heterozygous	Heterozygous	Homozygous	Homozygous	Heterozygous
Nucleotide		c.170G>A	c.430C>T	c.385G>A	c.1811A>C	c.254G>A
Protein		p.Arg57His	p.Leu144Phe	p.Glu129Lys	p.Asn604Thr	p.Arg85HisVriant
Variant id		rs373412197	rs199698258	rs150875319	rs146462069	rs74315418 / CM065401
Minor allele frequency		A=0.00006/7 (ExAC) A=0.0002/2 (GO-ESP) A=0.0002/5 (TOPMED)	-	A=0.0005/57 (ExAC) A=0.0020/10 (1000 Genomes) A=0.0015/20 (GO-ESP) A=0.0021/61 (TOPMED)	G=0.0057/485 (ExAC) G=0.0056/21 (1000 Genomes) G=0.0100/106 (GO-ESP)	T=0.0007/90 (ExAC) T=0.0012/16 (GO-ESP) T=0.0008/104 (TOPMED)
Reference		This study	This study	This study	This study	Known
In silico analysis	Mutation Taster	disease causing	disease causing	disease causing	polymorphism	disease causing
	Polyphen2 (Hum Var)	probably damaging	probably damaging	probably damaging	-	Probable damaging
	Provean	neutral	deleterious	deleterious	neutral	deleterious
	SIFT	damaging	damaging	damaging	damaging	damaging
	InterVar	uncertain significance	likely pathogenic	likely benign	uncertain significance	uncertain significance



CONCLUSIONS

- We recommend cytogenetic examination before molecular analysis to exclude chromosomal anomalies and microdeletions. Because short stature has a wide genetic spectrum, we think that the targeted panels are not sufficient.
- We propose whole exom or whole genome sequencing analysis with a healthy control group and the index patients and parents.

References

1. Baron J, Sävendahl L, De Luca F, Dauber A, Phillip M, Wit JM, Nilsson O. Short and tall stature: a new paradigm emerges. Nat Rev Endocrinol. 2015 Dec;11(12):735-46.
2. Wit JM, Oostdijk W, Losekoot M, van Duyvenvoorde HA, Ruivenkamp CA, Kant SG. MECHANISMS IN ENDOCRINOLOGY: Novel genetic causes of short stature. Eur J Endocrinol. 2016 Apr;174(4):R145-73.
3. Guo MH, Hirschhorn JN, Dauber A. Insights and Implications of Genome-Wide Association Studies of Height.J Clin Endocrinol Metab. 2018 Sep 1;103(9):3155-3168.
4. Huang Z, Sun Y, Fan Y, Wang L, Liu H, Gong Z, Wang J, Yan H, Wang Y, Hu G, Wang R, Ye J, Han L, Qiu W, Zhang H, Liang L, Yang Y, Dauber A, Yu Y, Gu XF. Genetic Evaluation of 114 Chinese Short Stature Children in the Next Generation Era: a Single Center Study. Cell Physiol Biochem. 2018;49(1):295-305.