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CLINICAL AND MUTATIONAL SPECTRUM IN SLOVENIAN PATIENTS WITH HYPOGONADOTROPIC HYPOGONADISM

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Background

Congenital hypogonadotropic hypogonadism (HH) without or with anosmia (Kallmann syndrome (KS)) is

• clinically and genetically heterogeneous disease with

• X-linked, recessive, oligogenic or dominant inheritance with variable penetrance

• Molecular genetic testing may prompt the treatment in adolescence

Aim: To identify causative variants in genes associated with HH in a cohort of 14 Slovenian patients.

Patients and methods

• 14 subjects (13 males, 1 female) with HH (Table 1)
• Targeted next generation sequencing of of genomic DNA isolated from peripheral blood
• TruSight One sequencing Panel Kit on miSeq (Illumina) apparatus
• 24 genes analysed:
• *ANOS1, FGFR1, FGF8, PROK2, PROKR2, WDR11, SOX10, GNRHR, GNRH1, TAC3, TACR3, KISS1R, CHD7, HS6ST1, NSMF, KISS1, LEP, LEPR, NROB1, SEMA3A, HESX1, SOX2, AXL, SOX10*
• Analysis of results with Illumina Variant Studio programme
• Identified mutations confirmed by Sanger sequencing
• Coverage of genes checked with Galaxy web tool
• In case of coverage <10x - Sanger sequencing

Results (Table 1)

• 9 mutations in 6 genes identified in 9 out of 14 patients (64%), each of them carrying a single heterozygous mutation in a single gene.
• 3 variants were novel.
• Of the remaining 5 patients 4 were part of the pedigrees with multiple affected members, which suggests an unidentified genetic cause.

Conclusions

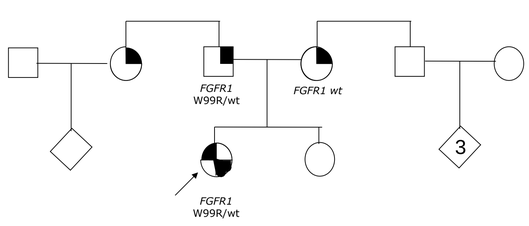
1. NGS enables fast and reliable identification of causal mutations in several genes related to HH simultaneously.
2. Presented subject group with HH was genetically very diverse and the results expand the spectrum of mutations implicated in HH.
3. By examining known genes oligogenicity was not identified and variable penetrance demonstrated in some pedigrees remained unexplained.

Table 1: Clinical & genetic characteristics of the cohort

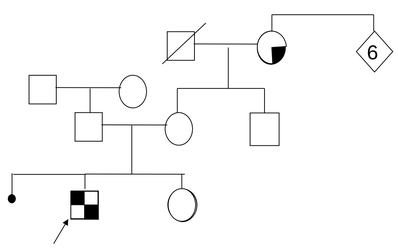
	ID #	Sex	Age (y)	HH	Cryptorchid.	Additional phenotype	Family history	Gene	Mutation	dbSNP	MAF	Sift	PolyPhen
	1	F	18	KS	NA	/	Fath DP	*FGFR1*	**c.295T>C, p.Trp99Arg**	/		Delet.	Prob. damaging
	2	M	33	nHH	Bil	color blindness, miopia	/	*GNRHR*	c.317A>G, p.Gln106Arg	rs104893836	0,01	Delet.	Prob. damaging
	3	M	58	nHH	NA	/	/	*GNRHR*	c.416G>A,p.Arg139H	rs104893842	/	Delet.	Prob. damaging
	4	M	16	nHH	/	short stature	Parents DP	*FGF8*	c.77C>T, p.Pro26Leu	rs137852660	0,0019	Delet.	Benign
	5	M	24	nHH	Bil	schizophrenia	Moth DP	*PROKR2*	c.254G>T, p.Arg85Leu	rs74315418	0,0006	Delet.	Prob. damaging
	6	M	21	KS	R	aortic coarctation	Fath DP	*PROKR2*	c.518T>G, p.Leu173Arg	rs74315416	0,0022	Delet.	Possib. damaging
	7	M	68	KS	NA	/	/	*PROK2*	**c.171_172 delTT, p.Ile57MetfsTer17**	/		Frameshift, premature STOP	
	8	M	22	KS	Bil	ASD prim, mitral valve cleft, kifoscoliosis, GERD, develop. delay, short stature, dysmorphic signs	/	*CHD7*	**5050+1G>T**	/		Splice site mutation	
	9	M	19	KS	Bil	TGA, kifoscoliosis, short stature, dysmorhic signs	/	*CHD7*	c.7879C>T, p.Arg2627*	/		STOP gain	
	10	M	39	KS	R	unilateral sensorineural deafness	Fath hearing loss	/					
	11	M	35	KS	L	impaired glucose tolerance	Cousins, aunt KS	/					
	12	M	22	KS	Bil	equinovarus, depression, feed. disorder	Moth hyposmia, fath DP	/					
	13	M	37	KS	Bil	/	Grandmoth hyposmia	/					
	14	M	25	KS	/	hypocalciuric hypercalcemia	Fath anosmia & hypercalcemia	*CASR*	c.2383C>T, p.Arg795Trp	rs121909258	/	Loss-of-function in vitro	

Table legend: Green letters mark novel mutations, F-female, M-male, HH-hypogonadotropic hypogonadism, nHH-normosmicHH, KS-Kallmann syndrome, NA-not applicabale, Bil – bilateral, R-right side, L-left side, ASD-atrium septum defect, GERB-gastroesophageal reflux disease, TGA-transposition of the great arteries, DP-delayed puberty

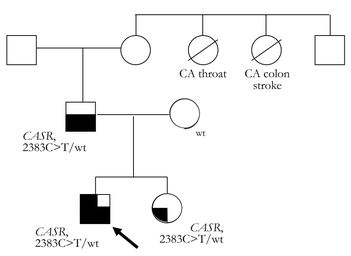
Pedigree #1



Pedigree #13



Pedigree #14

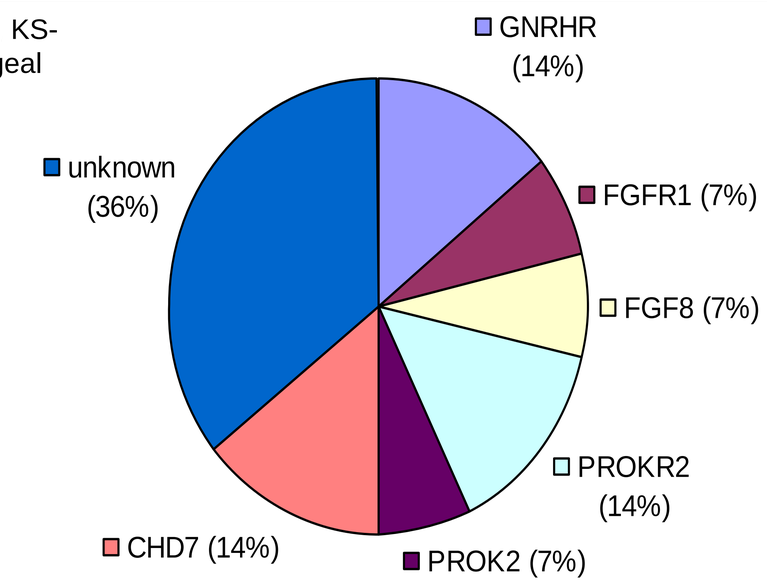


Hypogonadotropic hypogonadism

Delayed puberty

Anosmia

Familial hypocalciuric hypercalcemia



Distrubution of genetic causes in the cohort

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