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Congenital Hypogonadotropic hypogonadism in a large french cohort: New genetic findings

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INTRODUCTION

Congenital hypogonadotropic hypogonadism (CHH) is a rare condition caused by a dysfunction of the GnRH Axis. The clinical variability of the disease is accompanied by genetic heterogeneity. Indeed, more than 40 genes are implicated in the pathogenesis of this condition. (1,2)

Classified for a long time as a monogenic disorder, CHH does not show regular Mendelian segregation patterns and synergistic effects between CHH genes have been suspected in CHH individuals with rare variants in more than 1 candidate gene (3)

AIM

The main goal of this present study was to characterize genetic defects in a large cohort of French CHH patients using a targeted NGS panel.

METHOD

Patients

121 patients (77 males/44 females) with CHH diagnosis (94 normosmic CHH, 24 Kallmann syndrome, 1 Woodhouse Sakatti Syndrome, 1 4H syndrome and 1 Gordon Holmes Syndrome) were enrolled for this study. Patients were followed in French pediatric and adult endocrinology units. Informed consent was obtained from all patients.

Variant Analysis

DNA was extracted from peripheral-blood leukocytes using the kit QIAamp DNA Mini (QIAGEN). A custom SureSelect QXT DNA target enrichment panel (Agilent Technologies Inc) was designed to capture 54 CHH candidate genes.

Alignment, and variant calling were performed with a homemade Pipeline designed with CLC Genomics Workbench (CLC GWP), then variants were annotated and filtered with IVA (Ingenuity Variant Analysis). Copy number variations (CNVs) were screened using CLC GWP.

RESULTS

Variants calling were limited to rare variants of the coding sequence or within acceptor or donor splice sites with a frequency less than 0,5 % in the general population. Variants were annotated in pathogenic, likely pathogenic or "of unknown significance" (based on the ACMG recommendations).

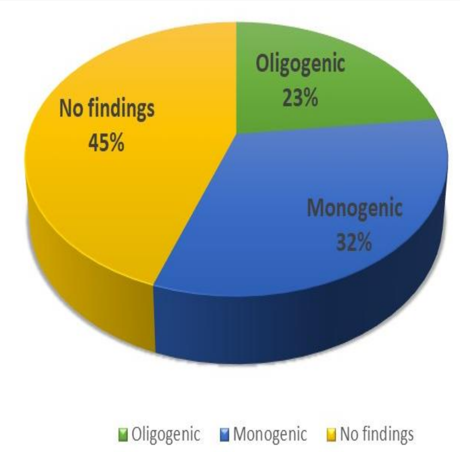
121 unrelated CHH patients were analyzed. A total of 101 rare variants were detected in 28 genes.

32% (39/121) patients were identified with a monogenic variant whereas 23% of patients (28/121) presented at least 2 rare variants in 2 different candidate genes (see Graph1). 54 patients (45%) did not display any rare variant in the 54 candidate genes.

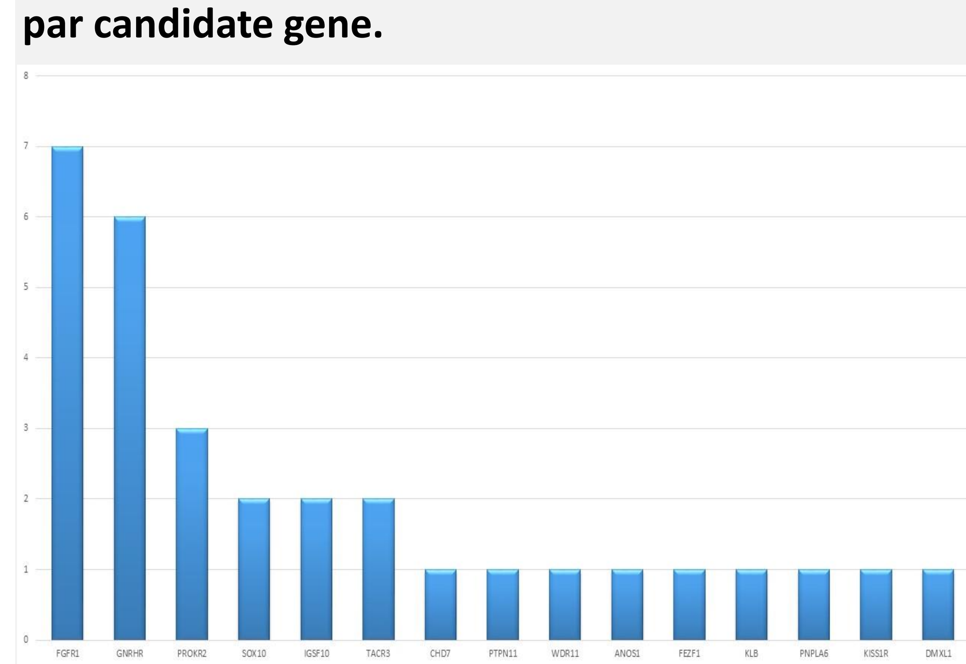
In 31 patients, a molecular diagnosis was reached because of the presence of pathogenic or likely pathogenic variants (classe 5 or 4). In 14 of those patients, an additional rare variants were found in at least one additional candidate gene.

A CNV analysis revealed 2 large duplications of ANOS1 gene in 2 patients without rare variant. One large deletion of GnRHR was found in one patient with a missense variant of GnRHR on the other allele.

Genetic findings in a French cohort of 121 patients



Cases with pathogenic or likely pathogenic variants par candidate gene.



Cases with at least two rare variants in candidate genes (KS=Kallmann Syndrome, *: New variant)

ID	Sex	gene	Variant	Gnomad MAF%	Class	Phenotype
4	M	CHD7	c.2830C>T;p.Arg944Cys	0,003	3	Cryptorchidism,gynecomastia
		DCC	c.2105A>G;p.Asn702Ser	0,282	3	
6	M	CHD7	c.3908A>C; p.Lys1303Thr	Absent	3	KS
		RNF216	c.1442G>C;p. Gly481Ala	Absent	3	
7	M	CHD7	c.2230G>A;p.Gly744Ser	0,144	3	CHH
		DUSP6	c.279C>G;p.Asp93Glu	0,01	3	
10	M	CHD7	c.1565G>T;p.Gly522Tyr	0,257	3	CHH
		KLB	c.3004T>G;p.Cys1002Gly	0,023	3	
11	M	CHD7	c.8416C>G;p.Leu2806Val	Absent	3	CHH
		DCC	c.2456-5C>T;p.?	Absent	3	
		PROKR2	c.802C>T;p.Arg268Cys	Absent	3	
13	M	CHD7	c.2209_2211delCCT;p.Pro737del	0,09	3	CHH, gynecomastia
		IGSF10	c.5983G>A,p.Val1995Ileu	0,24	3	
		Map3k1	c.14_16dupCGG, p.A5dup	0,39	3	
15	M	FGFR1	c.92-1G>A;p.	Absent	5	CHH
		KLB	c.1914_1917delinsTATCCG; p.Met638Ilefs*13	Absent	5*	
17	F	FGFR1	c.54_55del;p.Cys19Hisfs*3	Absent	5*	CHH
		DCC	c.200G>A; p.Arg67Gln	0.001	3	
20	F	FGFR1	c.797C>G;p.Thr266Arg	Absent	3	CHH
		DCC	c.1409G>A;p.Gly470Asp	0,288	3	
23	M	FGFR1	c.760C>T;p.Arg254Trp	Absent	4	CHH, Oligodentia, syndactyly
		KISS1	c.-7C>T;p.?	0.043	4	
28	M	GNRHR	c.317A>G;p.Gln106Arg	0,252	5	KS
		WDR11	c.644T>C;p,Leu215Pro	0,012	3	
29	F	GNRHR	c.317A>G;p.Gln106Arg	0,252	5	CHH
		POLR3A	c.3382A>G;p.Ile1128Val	Absent	3	
30	M	GNRHR	c.317A>G;p.Gln106Arg/ deletion exon 3 of GNRHR	0.252/Absent	5*	CHH
		DCC	c.3362G>C;p.Cys1121Ser	Absent	3*	
32	F	GNRHR	c.30_31delTCinsAA;p.Asn10_Gln11delins	Absent	4	KS
		KLB	c.2788G>A;p.Ala930Thr	Absent	3*	
40	M	ANOS1	c.67_92dup; p.Ala32Trpfs*32	Absent	5*	CHH, micropenis, Epilepsy, cryptorchidism
		PROKR2	c.797G>A;p.Arg266Gln	0.005	3	
		PROKR2	c.802C>T;p.Arg268Cys	0.494	3	

Genes most commonly identified in association with other rare variants were *CHD7* (6/13 patients) followed by *GNRHR* (4/6 patients) then *FGFR1* (4/10 patients)(table 1). In some cases, one variant alone would be sufficient to explain the phenotype but we cannot exclude a synergistic effect with the associated variant. One syndromic CHH case (patient 29) revealed an association of an heterozygous missense variant of GNRHR (classe 5) with a missense variant of *POLR3A* at heterozygous state. This association is singular and must be investigated before to affirm an oligogenic model of transmission.

Another astonishing association was a pathogenic variant in *ANOS1* with two rare missense variants of *PROKR2* in a patient (40) with CHH and an epilepsy. This association might explain the severe phenotype of the patient. Functional analysis must be done in order to explain how this association could modulate the severity of the phenotype.

CONCLUSIONS

CHH is a rare condition with an uncovered complex model of genetics (monogeniticy and oligogenicity).

Targeted sequencing using selected panel is effective for a molecular diagnostic (clinical diagnostic confirmation or shifting) but still insufficient to explain all cases.

Exome and whole genome sequencing (WGS) will should to overcome the limitation of selected panel.

Reporting more variants with CHH cases will help to make substantial progress in our understanding of CHH.

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