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Genomic Analyses Identify Rare Variants in Genes Associated with Age at Menarche in Patients Affected with Anorexia Nervosa and Support a Role for Puberty Timing in Anorexia Nervosa Risk

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Abstract

Puberty is a critical risk period for eating disorders, especially in girls. Previous reports showed that the association between puberty and eating disorders is mainly due to genetic factors, but the nature of these factors is unclear. To identify these genetic factors, we carried out exome analysis in 10 girls with anorexia nervosa compared to 10 unaffected women controls and identified low-frequency variants in genes among a list of 185 puberty-associated genes. After filtering data, 9 controls and 9 affected girls had at least one potential pathogenic rare (PPR) variant. In controls, 35 PPR variants were identified located (in 31 genes) whereas 52 PPR variants (in 38 genes) were identified in patients with AN. Among the genes identified only in the AN patients, we found a significant enrichment as compared to the general population and the control sample, in variants previously identified as associated with age at menarche (*TNRC6A*, *LAMB2* and *FAAH2*). Two AN patients presented at least one rare missense variant in these genes. Moreover, nine patients with AN carried out missense/frameshift variants in puberty-associated genes previously identified by GWAS studies. Our results suggest that rare variants in genes

involved in the timing of puberty such as *TNRC6A*, *LAMB2* and *FAAH2* may predispose to anorexia nervosa susceptibility during puberty.

Keywords: Anorexia nervosa; Puberty; Exome; TNRC6A; LAMB2; FAAH2

1. Introduction

Puberty is the maturational process of the reproductive endocrine system that results in adult height and body proportion, in addition to the capacity to reproduce. The time of human puberty is highly variable, and sexually dimorphic. Known influences on the timing of this event in mammals include the light/dark cycle, lept in levels, and the increased expression of neurokinin B, kisspeptin, and their receptors, NK3R and KISS1R [1-3]. Moreover, over 20 genes carrying rare pathogenic variants have been identified in pubertal disorders, many of them encode critical components of the hypothalamic-pituitary-gonadal (HPG) axis [4]. Recent genome-wide association studies (GWAS) have also identified more than 100 candidate genes at loci associated with age at menarche (AAM) or voice breaking in males [5, 6].

Disordered pubertal timing affects up to 5% of adolescents and is associated with adverse health and psychosocial outcomes. Interestingly, early pubertal timing increases risk for eating disorders. For example, adolescents reporting a younger age at menarche are more likely to report bulimic symptomatology, and women with bulimia nervosa (BN) report an earlier age of menarche compared to women with anorexia nervosa (AN) or healthy controls. A population based study of adolescents also indicated that the median ages of onset for AN, BN and subthreshold eating disorders ranged between 12.3 and 12.6 years-old, which are quite similar to the average age of menarche in the general population (12.4 years-old) [7]. However, the mechanism by which pubertal timing increases risk for eating disorders is unclear. One of the major hypothesis is that the association between pubertal timing and eating disorders could be linked to common biological phenomena. Twin studies showed that eating disorder symptomatology is heritable. Genetic effects for eating disorders are also almost not present in prepubertal girls and increase markedly to approximately 50%-60% in advancing-pubertal girls and young adults [8-12]. Taken together, these findings raise the possibility that the genes involved in age of menarche play a role in the genetic influence on eating disorders. Up to now, only few studies showed that genetic factors that predispose girls to early menarche also increase eating disorders [13].

To better identify these genetic factors predisposing to eating disorders, we carried out an exome analysis in 10 girls with anorexia nervosa and in 10 unaffected women as controls, and searched for low-frequency variants among a list of 185 puberty-associated genes.

2. Participants

Patients with AN were recruited at the Clinique des Maladies Mentales et de l'Encéphale (CMME) (Hôpital Sainte-Anne, Paris, France). Diagnosis was made according to Diagnostic and Statistical Manual of Mental Disorders 5 (DSM5) scores. Individuals that consented to participation in the study had blood drawn for the collection of DNA and received a diagnostic interview administered by a trained research team member. Consensus diagnosis was obtained by agreement of clinicians specializing in AN. Eight individuals were considered as having a restrictive-subtype anorexia nervosa and two with the bulimia subtype. Three of these eating disorders (ED) patients are prepubertal at the time of the examination. Exclusion criteria included other types of ED than AN, confounding psychiatric diagnoses, for example, psychotic disorders, medical or neurological conditions causing weight loss. This cohort of patients consisted of 10 female individuals (13 to 47 years-old). Control group consists of 10 unaffected women. These controls were used to determine the frequency of rare variant in the different candidate genes.

3. Methods

3.1 Whole exome analysis

All individuals provided a signed informed written consent. Peripheral blood samples were collected from patients and controls in EDTA tubes. Genomic DNA was extracted from blood samples by a standard procedure using the Maxwell system (Promega, Madison, WI, USA) and the LEV DNA BLOOD AS 1290 kit (Promega, Madison, WI, USA). Whole exome capture, next-generation sequencing and data analysis were carried out by Cochin Institute and Paris Descartes Platform, respectively. Briefly, libraries were prepared from 3 µg genomic DNA extracted from whole blood using an optimized SureSelect Human Exome kit (Agilent) following the manufacturer's instructions. Captured, purified and clonally amplified libraries targeting the exome were then sequenced on a Nextseq 500 instrument (Illumina) according to the manufacturer's recommendations. Obtained sequence reads were aligned to the human genome (hg19) using BWA software. Downstream processing was carried out with the Genome analysis toolkit (GATK), SAMtools and Picard Tools (http://picard.sourceforge.net). Single-nucleotide variants and indels were subsequently called by the SAMtools suite (mpileup, bcftools, vcfutil). All calls with a read coverage ≤20× and a Phred-scaled SNP quality of ≤20 were filtered out. We only searched for variants in a list of 185 puberty-associated genes (23 genes with known mutations in patients diagnosed with Kallmann syndrome, normosmic hypogonadotrophic hypogonadism or precocious puberty; 145 candidate genes near genetic variants identified in large-scale GWAS studies for age at menarche in women; 7 genes associated with age at menarche; and 10 literature-based genes) [14-17]. Variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67). Four consecutive filters were applied to narrow down the variants: 1- exonic or located in consensus splice junction (+/- 2 bp); 2- non-synonymous; 3- located in a gene identified by an NM_accession number (NM_); and 4- minor allele frequency (MAF)<1% (in gnomAD database https://gnomad.broadinstitute.org/). In all cases, sequences variant in a gene are numbered starting from the first

base of the ATG codon, numbering based on the reference sequence. Description of the sequence (Human Genome Variation Society, http://www.hgvs.org/mutnomen/recs.html) was done with the assistance of Alamut Visual software version 2.4.2 (Interactive Biosoftware, Rouen, France).

3.2 Sanger sequencing

DNA variants of interest were confirmed by PCR amplification and direct sequencing. DNA primer sequences and PCR conditions are available upon request to the corresponding author. Sequencing reactions were carried out with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Courtaboeuf, France) and loaded on the ABI 3130XL genetic analyser (Applied Biosystems, Courtaboeuf, France). Results were visualized using the Sequencher software (Gene codes, Ann Arbor, United States).

3.3 In silico analysis of missense variants

To find the influence of each missense variant on its protein structure that may have an important role in disease susceptibility, *in silico* analysis was performed. For each variant, bioinformatics tools (SIFT, MutationTaster, Polyphen-2) (https://www.interactive-biosoftware.com/alamut-visual/) were used to identify non-synonymous variant as possibly or probably damaging. These prediction tools are based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences.

4. Results

4.1 Rare variants identified in AN patients

We performed exome sequencing for ten affected girls and 10 unaffected women as controls. In total 122,380 to 140,758 single nucleotide variants and 16,162 to 19,483 small deletions/insertions (1-10 bp) were identified across the exomes [18]. To identify genetic factors involved in anorexia and age of menarche, we search for low-frequency variants in genes among a list of 185 puberty-associated genes (Table 1). After filtering our data, it resulted in 9 affected individuals (9/10) at least one variant (no variant in patient NR279) (Table 2). In these patients, the number of variants varied from 2 (NR227) to 11(NR224) for each affected AN individual. In total, 52 variants were identified including 11 variants not previously described in all genomic databases (Table 2). One variant was a frameshift variant (*VGLL3*), two were in frame deletions (*SCRIB, GALNT10*) and all other variants were missense variants (Table 2). In total, variants were identified in 38 of the tested genes.

Gene stable ID	Chromosome	Gene start (bp)	Gene end (bp)	Karyotype band	Gene name	RefSeq match transcript	RefSeq mRNA ID	References
ENSG00000240303	3	132558138	132960082	q22.1	ACAD11	NM_032169.5	NM_032169	Hou et al. 2017
ENSG00000185736	10	1177313	1737525	p15.3	ADARB2	NM_018702.4	NM_018702	Hou et al. 2017
ENSG00000116127	2	73385758	73625166	q13.1	ALMS1		NM_015120	Lunetta et al. 2015
ENSG00000179593	17	8036034	8046134	p13.1	ALOX15B	NM_001141.3	NM_001141	Hou et al. 2017
ENSG00000133794	11	1327665	1338726	p15.3	ARNTL		NM_001178	Hou et al. 2017
ENSG00000112249	6	100508194	100881372	q16.3	ASCC3	NM_006828.4	NM_006828	Hou et al. 2017
ENSG00000186868	12	40836043	49843106	q13.12	BCDIN3D	NM_181708.3	NM_181708	Hou et al. 2017
ENSG00000119866	2	60455520	60554487	p16.1	BCL11A	NM_022893.4	NM_022893	Hou et al. 2017
ENSG00000176697	11	27654893	27720358	p14.1	BDNF	NM_001709.5	NM_001709	Hou et al. 2017
ENSG00000183922	14	100537147	100587413	q32.2	BEGAIN		NM_020836	Hou et al. 2017
ENSG00000115917	6	56905107	57027346	q12.1	BEND6	NM_152731.3	NM_152731	Hou et al. 2017
ENSG00000112963	5	138139766	138178866	q31.2	BRD8		NM_139199	Hou et al. 2017
ENSG00000185658	21	39184176	39321559	q22.2	BRWD1		NM_033656	Hou et al. 2017
ENSG00000188909	11	122977570	122981834	q24.1	BSX	NM_001098169.2	NM_001098169	Hou et al. 2017
ENSG00000112578	6	41921499	41933046	p21.1	BYSL	NM_004053.4	NM_004053	Hou et al. 2017
ENSG00000154975	17	61630313	52189017	q22	CA10	NM_020178.5	NM_020178	Hou et al. 2017
ENSG00000182985	11	115169218	115504957	q23.3	CADM1		NM_014333	Hou et al. 2017
ENSG00000081803	7	122318411	122886759	q31.32	CADPS2	NM_017954.11	NM_017954	Hou et al. 2017
ENSG00000100397	22	39120167	39152680	q13.1	CBX7	NM_175709.5	NM_175709	Hou et al. 2017
ENSG00000095813	2	56183990	56386172	p16.1	CCDC85A	NM_001080433.2	NM_001080433	Hou et al. 2017
ENSG00000163660	3	157146508	157160760	q25.31	CCNL1		NM_020307	Hou et al. 2017
ENSG00000203760	6	126340174	126348875	q22.32	CENPW		NM_001012507	Hou et al. 2017
ENSG00000171316	8	60676740	60868028	q12.2	CHD7	NM_017780.4	NM_017780	Hou et al. 2017 (disease genes)
ENSG00000103951	16	70480568	70523558	q22.1	CDH4	NM_015386.3	NM_015386	Hou et al. 2017
ENSG00000133103	13	39656527	39791665	q14.11	COG6	NM_020751.3	NM_020751	Hou et al. 2017
ENSG00000105662	19	18683678	18782333	q13.11	CRTC1	NM_015321.3	NM_015321	Hou et al. 2017
ENSG00000183117	8	2935353	4994972	q23.2	CSMD1	NM_033225.6	NM_033225	Hou et al. 2017
ENSG00000175029	10	124984317	125161170	q26.13	CTBP2	NM_022802.3	NM_022802	Hou et al. 2017
ENSG00000145543	15	89494440	89546675	q26.1	DET1		NM_017996	Hou et al. 2017
ENSG00000170579	18	3496032	4455307	p11.31	DLGAP1	NM_004746.4	NM_004746	Hou et al. 2017
ENSG00000185650	14	100725705	100738224	q32.2	DLK1		NM_003836	Hou et al. 2017
ENSG00000188641	1	97077743	97095000	q21.3	DPYD	NM_000110.4	NM_000110	Hou et al. 2017
ENSG00000215051	6	56457987	56958449	q12.1	DST		NM_183380	Hou et al. 2017
ENSG00000124205	20	59300443	59325592	q13.32	EDN3	NM_207034.3	NM_207034	Tang et al. 2018 (methylation)
ENSG00000132394	3	128153481	128408646	q21.3	EEFSEC	NM_021937.5	NM_021937	Hou et al. 2017
ENSG00000114867	3	184314495	184335358	q27.1	EIF4G1		NM_198244	Hou et al. 2017
ENSG00000091831	6	151656691	152129619	q25.1	ESR1		NM_000125	Hou et al. 2017
ENSG00000244405	3	186040314	186110218	q27.2	ETV5	NM_004454.3	NM_004454	Hou et al. 2017
ENSG00000142459	19	7830233	7884976	q13.2	EVSL		NM_145245	Hou et al. 2017
ENSG00000165591	X	57288706	57486196	p11.21	FAAH2		NM_174912	Lunetta et al. 2015
ENSG00000145284	4	119317250	119322138	q26	FABP2	NM_000134.4	NM_000134	Yermachenko et al. 2016 (Monarche timing)
ENSG00000168143	6	54846771	54945099	q12.1	FAM83B	NM_001010872.3	NM_001010872	Hou et al. 2017
ENSG00000107831	10	101770130	101780369	q24.32	FGFR2	NM_033164.4	NM_033164	Hou et al. 2017 (disease genes)
ENSG00000077782	8	38400215	38468834	q11.23	FGFR1		NM_023110	Hou et al. 2017; Yermachenko et al. 2016
ENSG00000185070	14	85530144	85854428	q31.3	FLRT2	NM_013231.6	NM_013231	Demerath et al. 2013
ENSG00000137218	6	4177016	4178542	p21.1	FRS3	NM_006653.5	NM_006653	Hou et al. 2017
ENSG00000131808	11	30231016	30235261	q14.1	FSHB		NM_000510	Hou et al. 2017
ENSG00000140718	16	53701692	54158512	q12.2	FTO	NM_001080432.3	NM_001080432	Hou et al. 2017
ENSG00000033170	14	65410592	65744121	q23.3	FUT8	NM_001371533.1	NM_001371533	Hou et al. 2017
ENSG00000033327	11	78215293	78418348	q14.1	GAB2	NM_080491.3	NM_080491	Hou et al. 2017 (disease genes)
ENSG00000164574	5	154139730	154430884	q33.2	GALNT10	NM_198321.4	NM_198321	Hou et al. 2017
ENSG00000112964	5	42423439	42721878	q13.1	GHR	NM_000163.5	NM_000163	Hou et al. 2017
ENSG00000106128	7	30938669	30991254	q14.3	GHRHR	NM_000823.4	NM_000823	Cohen et al. 2019 (sex-related disorders).
ENSG00000183281	4	44682200	44726588	q12	GNPDA2	NM_138335.3	NM_138335	Hou et al. 2017
ENSG00000147437	8	25410258	25434654	q21.2	GNRH1	NM_001083111.2	NM_001083111	Hou et al. 2017 (disease genes)
ENSG00000109183	4	67733928	67754366	q13.2	GNRHR		NM_000406	Hou et al. 2017 (disease genes)
ENSG00000129973	2	105241743	105243467	q12.1	GPR45	NM_007227.3	NM_007227	Hou et al. 2017
ENSG00000167191	16	19856991	19889167	q12.3	GPRC5B	NM_016235.3	NM_016235	Hou et al. 2017; Yermachenko et al. 2016
ENSG00000182771	10	95599652	86396795	q23.2	GRID1	NM_017551.3	NM_017551	Tang et al. 2018 (methylation)
ENSG00000263001	7	74650231	74765692	q11.23	GTF2I		NM_033001	Hou et al. 2017
ENSG00000137252	6	55106460	55282620	q12.1	HCRTR2		NM_001526	Hou et al. 2017
ENSG00000163666	3	57197838	57227606	q14.3	HESX1	NM_003865.3	NM_003865	Hou et al. 2017 (disease genes)
ENSG00000206503	6	29941260	29945894	q22.1	HLA-A	NM_002116.8	NM_002116	Hou et al. 2017
ENSG00000136720	2	128236716	128318868	q14.3	HS6ST1	NM_004807.3	NM_004807	Hou et al. 2017 (disease genes)
ENSG00000179997	3	87990696	87993835	q11.1	HTR1F		NM_000866	Hou et al. 2017
ENSG00000073792	3	185643739	185825058	q27.2	IGF2BP2		NM_006548	Hou et al. 2017
ENSG00000197081	6	15996099	16011507	q25.3	IGF2R		NM_000876	Ryun et al. 2014
ENSG00000147457	X	13127506	13178899	q26.2	IGSF1	NM_001555.5	NM_001555	Lunetta et al. 2015
ENSG00000144947	3	118900557	119146068	q13.32	IGSF11	NM_001015887.3	NM_001015887	Hou et al. 2017
ENSG00000174564	3	136946230	137011085	q22.3	IL20RB	NM_144717.4	NM_144717	Hou et al. 2017
ENSG00000112706	6	75921114	76072678	q14.1	IMPG1	NM_001563.4	NM_001563	Hou et al. 2017
ENSG00000122641	7	41667168	41705834	q14.1	INHBA	NM_002192.4	NM_002192	Hou et al. 2017
ENSG00000177508	16	54283304	54288787	q12.2	IRX5	NM_024336.3	NM_024336	Hou et al. 2017
ENSG00000043143	5	134524312	134583230	q31.1	JADE2		NM_015288	Hou et al. 2017
ENSG00000169427	8	139600838	139704109	q24.3	KCNK9	NM_001282534.2	NM_001282534	Hou et al. 2017
ENSG00000174943	16	29905012	29926236	q11.2	KCTD13	NM_178863.5	NM_178863	Hou et al. 2017
ENSG00000120733	5	13835265	138437018	q31.2	KDM3B	NM_016604.4	NM_016604	Hou et al. 2017
ENSG00000068135	1	43650149	43709518	q34.2	KDM4A	NM_014663.3	NM_014663	Hou et al. 2017
ENSG00000107077	9	8720863	7175048	p24.1	KDM4C		NM_015061	Hou et al. 2017
ENSG00000170498	1	204190341	204196491	q32.1	KIS51	NM_002256.4	NM_002256	Hou et al. 2017 (disease genes)
ENSG00000116014	19	917287	921005	q13.3	KISS1R	NM_032551.5	NM_032551	Hou et al. 2017 (disease genes)
ENSG00000118922	13	73686089	73695156	q22.1	KLF12		NM_007249	Hou et al. 2017
ENSG00000185909	3	49171598	49176486	q21.31	KLHDC8B	NM_173546.3	NM_173546	Hou et al. 2017
ENSG00000172037	3	49121114	49133118	q21.31	LAMB2	NM_002292.4	NM_002292	Lunetta et al. 2015
ENSG00000197080	3	156825481	157046129	q25.31	LEKR1		NM_001004316	Hou et al. 2017
ENSG00000174697	7	128241278	128257929	q32.1	LEP	NM_000230.3	NM_000230	Hou et al. 2017 (disease genes)
ENSG00000116678	1	85420652	85641559	q31.3	LEPR		NM_002303	Hou et al. 2017 (disease genes)
ENSG00000205213	11	27365861	27472730	q14.1	LGR4	NM_018490.5	NM_018490	Hou et al. 2017
ENSG00000187772	6	104916616	105083332	q16.3	LIN28B	NM_001004317.4	NM_001004317	Hou et al. 2017; Elks et al. 2010
ENSG00000168702	2	140231423	142131016	q22.2	LRP1B	NM_018557.3	NM_018557	Hou et al. 2017
ENSG00000254585	15	23643549	23647867	q11.2	MAGEL2	NM_019066.5	NM_019066	Hou et al. 2017
ENSG00000137764	15	67547703	67807117	q23	MAP2K5	NM_145160.3	NM_145160	Hou et al. 2017
ENSG00000152034	6	99918519	99994247	q16.2	MCHR2	NM_001040178.2	NM_001040179	Hou et al. 2017
ENSG00000179455	15	23568674	23630075	q11.2	MKRN3	NM_005664.4	NM_005664	Hou et al. 2017 (disease genes)
ENSG00000111912	6	125781161	125910334	q22.31	NCOA7	NM_181782.5	NM_181782	Hou et al. 2017 (disease genes)
ENSG00000162636	15	23685400	23687305	q11.2	NDN	NM_002487.3	NM_002487	Hou et al. 2017 (disease genes)
ENSG00000177269	1	7159943	7235539	p31.1	NEGR1	NM_173808.3	NM_173808	Hou et al. 2017
ENSG00000102608	16	69585094	69704666	q22.1	NFAT5	NM_138713.4	NM_138713	Hou et al. 2017
ENSG00000183259	8	52938431	53941117	q11.23	NPBWR1		NM_005285	Hou et al. 2017
ENSG00000113971	3	132680609	132722414	q22.1	NPHP3	NM_153240.5	NM_153240	Hou et al. 2017
ENSG00000221890	22	38814852	38844028	q13.1	NPTXR		NM_014293	Hou et al. 2017
ENSG00000169057	X	30304206	30309390	q21.2	NR0B1	NM_000475.5	NM_000475	Hou et al. 2017 (disease genes)
ENSG00000153234	2	156324437	156342348	q24.1	NR4A2	NM_006186.4	NM_006186	Hou et al. 2017
ENSG00000116833	1	200027814	200177420	q32.1	NRSA2	NM_205860.3	NM_205860	Hou et al. 2017
ENSG00000165802	9	137447573	137459334	q34.3	NSMF		NM_015537	Hou et al. 2017 (disease genes)
ENSG00000148053	9	94648851	8927070	q21.33	NTRK2		NM_006180	Hou et al. 2017
ENSG00000065275	1	20571822	20575218	q32.1	NUCKS1	NM_022731.5	NM_022731	Hou et al. 2017
ENSG00000105088	19	9853718	9939552	q13.2	OLFM2		NM_058164	Hou et al. 2017
ENSG00000118733	1	101902574	101997030	q21.1	OLFM3		NM_058170	Hou et al. 2017
ENSG00000171133	9	11132483	11133018	q33.3	ORS2K2		NM_205859	Hou et al. 2017
ENSG00000178685	8	143977153	144012772	q24.3	PARP10	NM_032789.5	NM_032789	Hou et al. 2017
ENSG00000169851	4	3072415	3144855	p15.1	PCDH7		NM_032457	Hou et al. 2017
ENSG00000175426	5	96390333	96434143	q15	PCSK1	NM_000439.5	NM_000439	Hou et al. 2017 (disease genes)
ENSG00000125851	20	17230107	17484578	q12.1	PCSK2	NM_002594.5	NM_002594	Hou et al. 2017
ENSG00000164751	8	76880258	77001044	q21.13	PEX2	NM_000318.3	NM_000318	Hou et al. 2017
ENSG00000092175	11	101029624	101129813	q22.1	PGR	NM_000926.4	NM_000926	Hou et al. 2017
ENSG00000135365	11	45929323	46121178	q13.2	PHF21A		NM_016621	Hou et al. 2017
ENSG00000145675	5	68215756	68301821	q13.1	PIK3R1	NM_181523.3	NM_181523	Demerath et al. 2013
ENSG00000115966	2	197804593	198577581	q33.1	PLCL1	NM_006226.4	NM_006226	Hou et al. 2017
ENSG00000115138	2	25146853	25168903	q23.3	POMC	NM_000939.4	NM_000939	Yermachenko et al. 2016 (Monarche timing)
ENSG00000162435	3	87295404	87378584	q11.2	POU1F1	NM_000306.4	NM_000306	Hou et al. 2017
ENSG00000112238	6	99606730	99615578	q16.2	PROM13		NM_021620	Hou et al. 2017
ENSG00000181929	12	49002274	49018807	q13.12	PRKAG1	NM_002733.5	NM_002733	Lunetta et al. 2015
ENSG00000183421	3	71771855	71785206	q13	PROK2	NM_001126128.2	NM_001126128	Hou et al. 2017 (disease genes)
ENSG00000101292	20	5302040	5314369	q12.3	PROKR2		NM_144773	Hou et al. 2017 (disease genes)
ENSG00000152256	11	13492054	13496181	q15.3	PTH	NM_000315.4	NM_000315	Hou et al. 2017
ENSG00000153707	9	8314246	10617723	p23	PTPRD		NM_130393	Hou et al. 2017
ENSG00000142949	1	43525187	43623688	q34.2	PTPRF	NM_002840.5	NM_002840	Hou et al. 2017
ENSG00000152894	6	127868779	128520674	q22.33	PTPRK		NM_002844	Hou et al. 2017
ENSG00000155093	7	157539056	158587923	q36.3	PTPRN2	NM_002847.5	NM_002847	Tang et al. 2018 (methylation)
ENSG00000204534	3	45940007	52100045	p21.31	RBM6	NM_005777.3	NM_005777	Hou et al. 2017
ENSG00000080511	19	10013249	10022279	q13.2	RDH8	NM_015725.4	NM_015725	Hou et al. 2017
ENSG00000104918	19	7669349	7670455	q13.2	RETN	NM_020415.4	NM_020415	Hou et al. 2017
ENSG00000178868	9	83980711	84004074	q21.32	RMI1		NM_024945	Hou et al. 2017
ENSG00000069667	15	60488284	61229302	q22.2	RORA	NM_134261.3	NM_134261	Hou et al. 2017
ENSG00000143171	1	165400922	165445265	q23.3	RXRG	NM_006917.5	NM_006917	Hou et al. 2017
ENSG00000119042	2	169269500	169471266	q33.1	SATB2	NM_001172509.2	NM_001172509	Hou et al. 2017
ENSG00000180660	8	143760920	143815773	q24.3	SCRIB	NM_182706.5	NM_182706	Hou et al. 2017
ENSG00000129341	1	177923656	165445265	q25.3	SEC16B		NM_031217	Hou et al. 2017
ENSG00000107651	10	119892730	119944657	q26.11	SEC23IP	NM_007190.4	NM_007190	Hou et al. 2017
ENSG00000075213	7	83955777	84492724	q21.11	SEMA3A	NM_006080.3	NM_006080	Hou et al. 2017
ENSG00000112246	6	100385015	100464929	q16.3	SIM1		NM_005068	Hou et al. 2017
ENSG00000143067	11	215099	236921	p15.5	SIRT3	NM_012239.6	NM_012239	Hou et al. 2017
ENSG00000184302	14	60505146	60513850	q23.1	SIX6	NM_007374.3	NM_007374	Hou et al. 2017 (disease genes)
ENSG00000215474	18	47206322	47251603	q21.1	SKOR2		NM_001037802	Hou et al. 2017
ENSG00000184347	5	16866173	16930129	q35.1	SLIT3		NM_003062	Hou et al. 2017
ENSG00000163104	4	94207611	94291292	q22.3	SMARCAD1		NM_020159	Hou et al. 2017
ENSG00000130148	22	17970686	17987422	q13.1	SOX10	NM_006941.4	NM_006941	Hou et al. 2017
ENSG00000181449	3	181711925	181714439	q26.33	SOX2	NM_003106.4	NM_003106	Hou et al. 2017
ENSG00000184211	5	111499033	111512690	q22.1	STARD4	NM_139164.3	NM_001306956	Hou et al. 2017
ENSG00000166263	17	54968727	55178632	q22	STXBP4	NM_178509.6	NM_178509	Hou et al. 2017
ENSG00000166863	12	57010000	57028883	q13.3	TAC3	NM_013251.4	NM_013251	Hou et al. 2017 (disease genes)
ENSG00000169836	4	103584031	103719685	q24	TACR3	NM_001059.3	NM_001059	Hou et al. 2017 (disease genes); Lunetta et al.
ENSG00000149922	16	30085793	30091887	q11.2	TBX6		NM_004608	Hou et al. 2017
ENSG00000081059	5	134114681	134151855	q31.1	TCF7	NM_003202.5	NM_003202	Hou et al. 2017
ENSG00000145604	5	167284799	168264157	q34	TENM2		NM_001080428	Hou et al. 2017
ENSG00000153495	13	111316184	111344249	q34	TEX29	NM_152324.3	NM_152324	Hou et al. 2017
ENSG00000151090	3	24117153	24405756	q24.2	THRB		NM_000461	Hou et al. 2017
ENSG00000151365	11	78063861	78068351	q14.1	THRSP	NM_003251.4	NM_003251	Hou et al. 2017
ENSG00000144869	3	133593593	133597702	q22.1	TMEM159		NM_019343	Hou et al. 2017
ENSG00000151353	2	663877	677409	p25.3	TMEM18	NM_152834.4	NM_152834	Hou et al. 2017
ENSG00000106771	9	109015135	109119947	q31.3	TMEM245	NM_032012.4	NM_032012	Hou et al. 2017
ENSG00000095209	9	105694541	105776629	q31.2	TMEM38B	NM_018112.3	NM_018112	Hou et al. 2017
ENSG00000116783	1	74235387	74544428	q31.1	TNN3K	NM_015978.3	NM_015978	Hou et al. 2017
ENSG00000106665	16	24610209	24627632	q12.1	TNRC6A	NM_014494.4	NM_014494	Lunetta et al. 2015
ENSG00000139527	3	18591459	18593810	q27.2	TRA2B	NM_004593.3	NM_004593	Hou et al. 2017
ENSG00000166436	11	8612037	8682694	p15.4	TRIM66		NM_014818	Hou et al. 2017
ENSG00000066651	6	125989479	126203817	q22.32	TRMT11	NM_001031712.3	NM_001031712	Hou et al. 2017
ENSG00000137672	11	101451564	101872562	q22.1	TRPC6	NM_004621.6	NM_004621	Hou et al. 2017
ENSG00000162923	1	74710152	74786678	q31.1	TYW3	NM_138467.3	NM_138467	Hou et al. 2017
ENSG00000182179	3	49805209	49813053	q21.31	UBA7	NM_003335.3	NM_003335	Hou et al. 2017
ENSG00000159099	4	69480165	69526014	q13.3	UGT2B4	NM_021139.3	NM_021139	Yermachenko et al. 2016 (Monarche timing)
ENSG00000111424	12	47841537	47945048	q13.11	VDR		NM_000376	Hou et al. 2017
ENSG00000206538	3	86876388	86991149	q12.1	VGLL3	NM_016206.4	NM_016206	Hou et al. 2017
ENSG00000120009	10	120851305	120936524	q26.12	WDR11	NM_018117.12	NM_018117	Hou et al. 2017 (disease genes)
ENSG00000174473	14	100376418	100530303	q32.2	WDR25		NM_024515	Hou et al. 2017
ENSG00000178252	3	49007062	49015053	q21.31	WDR6	NM_018031.6	NM_018031	Hou et al. 2017
ENSG00000179314	17	6057807	6124427	p13.2	WSCD1		NM_015253	Hou et al. 2017
ENSG00000169373	16	69762306	69941741	q22.1	WWP2		NM_199424	Hou et al. 2017
ENSG00000172262	5	43065178	43180021	q12	ZNF131		NM_003432	Hou et al. 2017
ENSG00000173258	9	111525159	111577844	q31.3	ZNF483	NM_133464.5	NM_133464	Hou et al. 2017
ENSG00000175105	3	88059255	88144664	q21.1	ZNF654	NM_001350134.2	NM_001350134	Hou et al. 2017
ENSG00000117280	1	207679386	208775482	q32.1	RAB29	NM_003929.3		Hou et al. 2017
ENSG00000188511	22	49414524	49657542	q13.33	C22orf34	NM_001289922		Hou et al. 2017
ENSG00000130758	19	40191426	40215575	q13.2	MKL2/MAP3K3	NM_002446.3		Hou et al. 2017

Table 1: List of the puberty-associated genes selected in the analysis of exome.

Family	Gene	Substitution	Variant	SIFT	Polyphen	gnomAD
NR147	*CSMD1*	c.2396C>G	T799S	Tolerated	Possibly damaging	Unknown
NR147	*ESR1*	c.16C>T	H6Y	Tolerated	Possibly damaging	915/279476
NR147	*ALOX15B*	c.769C>T	L257F	Deleterious	Probably damaging	958/2828487
NR147	*GHR*	c.469C>T	R157C	Deleterious	Benign	1107/282250
NR147	*DST*	c.3336G>C	E1112D	Tolerated	Probably damaging	952/281446
NR147	*MAGEL2*	c.260C>T	P87L	Tolerated	Benign	Unknown
NR147	*GALNT10*	c.145_153dup	p.Pro49_Ala51dup			0.014
NR15	*TNRC6A*	c.4804G>A	G1602S	Tolerated	Probably damaging	60/282866
NR15	*FAAH2*	c.820C>T	R274C	Deleterious	Probably damaging	11/204712
NR15	*NPTXR*	c.1423T>C	W475R	Deleterious	Probably damaging	Unknown
NR15	*GPRC5B*	c.793G>T	D265Y	Deleterious	Probably damaging	192/282294
NR15	*PROKR2*	c.518T>G	L173R	Deleterious	Possibly damaging	621/282842
NR15	*LAMB2*	c.3674G>A	G1225D	Deleterious	Probably damaging	52/282050
NR15	*PARP10*	c.2170C>G	R724G	Tolerated		Unknown
NR180	*PTPRD*	c.2721G>T	M907I	Tolerated	Benign	Unknown
NR180	*SIX6*	c.385G>A	E129K	Deleterious	Probably damaging	1144/282412
NR180	*BEGAIN*	c.526G>A	D176N	Deleterious	Probably damaging	1240/253934
NR180	*IGF2R*	c.1063G>A	D355N	Tolerated	Benign	374/281644
NR191	*LRP1B*	c.7420G>A	G2474S	Deleterious	Possibly damaging	1548/281550
NR191	*DST*	c.5095G>A	E1699K	Tolerated	Probably damaging	103/282440
NR191	*NPHP3*	c.1714T>G	S572A	Tolerated	Benign	8/282696
NR191	*PTPRN2*	c.1969G>A	D657N	Tolerated	Possibly damaging	6/282600
NR191	*CSMD1*	c.9925G>T	A3309S	Deleterious	Benign	1/249044
NR195	*NPHP3*	c.3904G>T	p.A1302S	Tolerated	Probably damaging	Unknown
NR195	*IMPG1*	c.1228C>A	P410T	Tolerated	Possibly damaging	24/282378
NR195	*WDR11*	c.2962G>A	E988K	Deleterious	Possibly damaging	501/282436
NR195	*LEKR1*	c.822G>C	M274I	Tolerated	Benign	327/172706
NR195	*PTPRN2*	c.590C>G	A197G	Tolerated	Benign	987/277654
NR195	*FLRT2*	c.1174A>G	S392G	Tolerated	Benign	437/282752
NR224	*VGLL3*	c.357del	Glu119Aspfs*11			Unknown
NR224	*VDR*	c.1182T>A	N394K	Deleterious	Possibly damaging	Unknown
NR224	*PTPRN2*	c.2099A>C	H700P	Deleterious	Probably damaging	Unknown
NR224	*TENM2*	c.20G>A	R7Q	Deleterious	Possibly damaging	807/188400
NR224	*TNRC6A*	c.5042A>G	Y1681C	Deleterious	Benign	39/282846
NR224	*TNRC6A*	c.5779A>G	S1927G	Tolerated	Benign	22/272242
NR224	*DST*	c.3336G>C	E1112D	Tolerated	Probably damaging	952/281446
NR224	*GALNT10*	c.899C>T	P300L	Tolerated	Probably damaging	Unknown
NR224	*NPHP3*	c.2197A>G	K733E	Tolerated	Benign	1/251326
NR224	*WWP2*	c.1202A>G	H401R	Deleterious		1/111230
NR224	*IGF2R*	c.4471G>A	V1491M	Deleterious	Possibly damaging	4/251462
NR227	*MKRN3*	c.1457C>G	P486R	Tolerated	Benign	315/282892
NR227	*PEX2*	c.748T>C	W250R	Deleterious	Probably damaging	1487/282714
NR227	*KDM3B*	c.4739G>A	G1580D	Tolerated	Benign	11/282624
NR227	*BRWD1*	c.5893C>T	L1965F	Tolerated	Benign	542/282274
NR227	*SCRIB*	c.2076_2078del	p.Glu692del			0.0087
NR243	*GHRHR*	c.512C>G	T171S	Deleterious	Probably damaging	105/282854
NR243	*RBM6*	c.515C>T	P172L	Deleterious	Benign	829/282838
NR243	*RBM6*	c.997G>A	E333K	Deleterious	Probably damaging	829/282772
NR243	*ALMS1*	c.4984A>G	T1662A	Tolerated	Benign	13/249100
NR243	*ALMS1*	c.5186A>G	E1729G	Tolerated	Benign	235/280392
NR86	*SCRIB*	c.4063C>T	R1355W	Deleterious	Probably damaging	11/227118
NR86	*UBA7*	c.923C>T	P308L	Deleterious	Possibly damaging	Unknown

Table 2: Rare variants (<1% in NM_; <0.01% in gnomAD (in grey)) identified in 185 genes involved in puberty-associated genes in 10 patients affected by anorexia nervosa (2 bulimia type NR86 and NR180; 8 restrictive type).

In contrast, after filtering data, 9 healthy women (9/10) had at least one variant (no variant was observed in one control) (Table 3). In these subjects, the number of variants varied from 2 to 7 for each control individual. In total, 35 variants were identified including 6 variants not previously described in all genomic databases (Table 3). One variant was a frameshift variant (*BDNF*), and all other variants were missense variants (Table 3). In total, variants were identified in 31 candidate genes.

Family	*Gene*	Substitution	Variant	SIFT	Polyphen	gnomAD
DIP131	*NFAT5*	c.1687T>G	S563A	Benign	Benign	Unknown
DIP131	*TAC3*	c.248A>G	H83R	Deleterious	Possibly damaging	42/282842
DIP131	*ARNTL*	c.1600C>T	P534S	Tolerated	Possibly damaging	14/282434
DIP131	*GHRHR*	c.53C>T	P18L	Tolerated	Benign	31/185148
DIP231	*ALOX15B*	c.22G>A	V8M	Deleterious	Probably damaging	2/250166
DIP231	*MAGEL2*	c.1357G>A	D453N	Tolerated	Benign	1/249240
DIP40	*EIF4G1*	c.4085T>C	M1362T	Deleterious	Probably damaging	133/282808
DIP40	*GALNT10*	c.1535G>A	R512Q	Deleterious	Probably damaging	3/251458
DIP40	*LRP1B*	c.11966C>T	S3989F	Deleterious	Possibly damaging	75/282328
DIP40	*UBA7*	c.2516G>A	R839H	Tolerated	Benign	86/282146
DIP40	*SEC16B*	c.2180C>T	S727L	Tolerated	Benign	131/254428
DIP40	*CADPS2*	c.31T>C	S11P	Tolerated	Benign	12/129752
DIP48	*DST*	c.5948A>G	K1983R	Benign	Benign	Unknown
DIP48	*LRP1B*	c.13114A>T	N4372Y	Deleterious	Benign	1532/282098
DIP48	*TCF7*	c.353A>C	H118P	Deleterious	Probably damaging	Unknown
DIP48	*CSMD1*	c.6671C>T	T2224M	Tolerated	Probably damaging	45/279852
DIP60	*BDNF*	c.99_100delAT				1/234032
DIP60	*SEC16B*	c.3145C>T	R1049C	Benign	Probably damaging	7/248758
DIP60	*TRPC6*	c.172C>T	R58W	Deleterious	Benign	269/277716
DIP60	*WSCD1*	c.313C>T	R105C	Deleterious	Probably damaging	4/149526
DIP60	*RAB29*	c.407G>A	R136Q	Tolerated	Benign	4/251448
DIP60	*WDR25*	c.397C>T	R133C	Tolerated	Benign	1224/282792
DIP60	*PTPRN2*	c.590C>G	A197G	Tolerated	Benign	987/277654
DIP286	*CSMD1*	c.8935G>A	G2979S	Benign	Probably damaging	1522/279098
DIP286	*ZNF131*	c.535G>A	V179M	Tolerated	Benign	Unknown
DIP50	*CADPS2*	c.2440A>C	N814H	Deleterious	Probably damaging	4/248764
DIP50	*PTPRD*	c.2920C>T	P974S	Benign	Benign	Unknown
DIP50	*UBA7*	c.1525G>A	V509M	Deleterious	Possibly damaging	542/282784
DIP50	*BRWD1*	c.3802A>G	N1268D	Tolerated	Benign	3/248862
DIP50	*HCRTR2*	c.1178G>A	R393Q	Tolerated	Benign	7/250738
DIP509	*NR0B1*	c.686A>G	E229G	Tolerated	Benign	Unknown
DIP509	*RMI1*	c.1000A>G	K334E	Tolerated	Benign	910/282232
DRTT4541	*DST*	c.2011A>C	I671L	Tolerated	Benign	79/282636
DRTT4541	*ALMS1*	c.11638C>T	H3880Y	Tolerated	Benign	272/280380
DRTT4541	*PRDM13*	c.710C>T	A237V	Tolerated	Benign	768/174856

Table 3: Rare variants (<1% NM_; <0.01% in gnomAD (in grey)) identified in genes involved in puberty-associated genes in 10 unaffected women (in 9/10 women).

4.2 Prediction of pathogenicity of variants

Among the 52 puberty-related (PR) missense variants, 12 were considered as deleterious and probably damaging by all bioinformatic softwares. Among these potential pathogenic variants, two (*NPTXR*, *PTPRN2*) were not described in all genomic databases, and 4 (*FAAH2*, *LAMB2*, *GHRHR*, *SCRIB*) were considered as extremely rare (<0.1%). Moreover, 18 variants were considered as deleterious or probably damaging by one bioinformatic software (*GHR*, *DST (x3)*, *TNRC6A (x2)*, *PROKR2*, *LRP1B*, *CSMD1*, *NPHP3*, *WDR11*, *VDR*, *TENM2*, *GALNT10*, *WWP2*, *IGF2R*, *RBM6*, *UBA7*).

In contrast, in controls, among the 35 PR variants, only 6 were considered as deleterious and probably damaging by all bioinformatic softwares (*ALOX15B;EIF4G1; GALNT10; TCF7; WSCD1; CADPS2*). Among these potential pathogenic variants, only one (*TCF7*) was not described in all genomic databases, and all were considered as extremely rare (<0.1%). Moreover, 8 variants were considered as deleterious or probably damaging by one bioinformatic software.

4.3 Variants in candidate genes with significant sex-biases expression

The fact that AN patients were predominantly female (with nine females affected for each male case observed) and that onset of its condition is classically around puberty highly suggested that genes with sex biases expression were potential candidates to explain AN vulnerability. Recently, O'Brien and colleagues (http://dx.doi.org/10.1101/483636) identified 2726 autosomal transcripts with significant sex biases expression. Among these transcripts, we found 8 of our 38 candidate genes (*CSMD1*, *TNRC6A*, *LRP1B*, *NPHP3*, *WDR11*, *PEX2*, *SCRIB*, *RBM6*). Moreover, using the SAGD database (http://bioinfo.life.hust.edu.cn/SAGD), we found that 18 of our candidate genes were found to be sex-associated genes which varied expression between females and males in Human (*PTPRD*, *CSMD1*, *VDR*, *GHRHR*, *MKRN3*, *HNF4A*, *ALOX15B*, *TENM2*, *PTPRK*, *SIX6*, *NPTXR*, *WSCD1*, *GPRC5B*, *PROKR2*, *LAMB2*, *UBA7*, *BEGAIN*, *GALNT10*). Interestingly, *BEGAIN*, *GALNT10*, *GHRHR*, *GPRC5B*, *HNF4A*, *LAMB2*, *MAGEL2*, *PTPRK*, *SIX6*, *UBA7*, and *VDR* showed a pituitary-specific sex-biased expression [19].

5. Discussion

Puberty is a critical risk period for psychiatric disorders and more specifically for eating disorders (i.e., anorexia nervosa, bulimia nervosa) and their symptoms [7]. ED incidence increases across the pubertal period and becomes female predominant, and genetic influences on eating disorders significantly increase [20]. Ten years ago, a twin study showed that genetic factors might be important for pubertal risk for eating disorders in girls [21]. This study showed that there was essentially no genetic influence on eating disorder symptoms in pre-adolescent female twins, but significant genetic effects in twins during late adolescence. This conclusion was confirmed by two other twin studies [13, 22]. Moreover, investigations of shared genetic risk factors between eating disorder symptoms (e.g.,

overall eating disorder symptoms, dieting) and age at menarche or early pubertal timing showed that genetic risk factors (rather than environmental influences) entirely account for the co-occurrence of early menarche and eating disorder behaviors in girls [13].

To identify genetic factors that appear to become "activated" during the pubertal period increasing the phenotypic risk for AN in girls, we searched for rare variants in genes associated with age at menarche in 10 women affected by anorexia nervosa.

We identified an excess of rare variants in genes associated with age at menarche in AN patients as compared to controls. When its number is corrected by the total number of variant identified in the exome, the difference was not significant (Table 4). However, interestingly, we identified a significant enrichment of extremely rare variants and variants in genes previously identified associated with age of menarche (i.e. *TNC6CA, LAMB2* and *FAAH2*) (Table 4) [15]. Indeed, in 2015, Lunetta and colleagues found that five missense variants (in *ALMS1, LAMB2, TNRC6A, TACR3, PRKAG1*) and two X-chromosome loci (*IGSF1, FAAH2*) were associated with age of menarche [15]. In the present study, one AN patient carried three rare variants in these genes (p.G1225D in *LAMB2*, p.G1602S in *TNRC6A*, and p.R274C in *FAAH2*) and another AN patient two rare variants in *TNRC6A* (p.Y1681C, p.S1927C). TNRC6A encodes a trinucleotide repeat-containing gene 6A protein, a member of the trinucleotide repeat containing 6 protein family. It functions in post-transcriptional gene silencing through the RNA interference (RNAi) and microRNA pathways [23]. It associates with messenger RNAs and Argonaute proteins in cytoplasmic bodies known as GW-bodies or P-bodies [24]. Variants identified in our two AN patients (NR15, NR224) were located in the C-terminal part of the protein (amino acids 1603-1622, PABPC1-interacting motif-2), known to be important for translational repression, and may impair RNAi and microRNA-induced gene silencing affecting metabolic pathways [25]. LAMB2 encodes the Laminin subunit beta-2 protein, a member of the family of extracellular matrix glycoproteins, major non collagenous constituent of basement membranes implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, and neurite outgrowth. Recently, compound heterozygous missense variants were identified in patients with congenital nephrotic syndrome, ocular abnormalities and neurodevelopmental delay [26]. Interestingly, Laminin b2 is highly expressed during pituitary development and may explain the abnormal parenchyma of the anterior pituitary gland in this patient. Taking into account the role of the hypothalamus-pituitary-adrenal axis in eating disorders, variant in *LAMB2* may play a role in the biological vulnerability to ED. Finally, FAAH2 encodes the Fatty acid amide hydrolase 2 (EC 3.5.1.99, oleamide hydrolase 2, anandamide amidohydrolase 2), a member of the serine hydrolase family of enzymes. Fatty acid amide hydrolase 2 degrades endocannabinoids and defects in this enzyme have been associated with neurologic and psychiatric disorders [27]. Patient carrying the missense variant p.A458S in this gene presented hypotonia, autistic features, seizures, but also anxiety disorder, suicidal ideas and feeding difficulties. Interestingly, we previously identified a rare variant (p.H312N) at the hemizygous state in a male patient with AN [28].

Parameters	Anorexia nervosa (n=10)	Healthy Controls (n=10)	*p Value*
Number of mutated individuals	9	9	-
Number of variants per individual	2 to 11	2 to 7	-
Number of total variants (<5%) in the exome	14692	13536	-
Number of total variants (<1%) in the exome	10307	9489	0.1487
Number of variants	52	35	0.1494
Number of unknown variant	11	6	0.6991
Number of mutated genes	38	31	0.0802
Number of deleterious variants	13	6	0.3844
Number of extremely rare variants	19	20	0.0581
Number of variants in genes associated with age of menarche*	5	0	0.0588
Number of variants in puberty diseases genes**	4	2	0.7211
Number of variants in GWAS candidate genes***	29	32	0.0004
Number of variants in literature-based genes $	3	1	0.5248

Table 4: Comparison between the frequency of variants (taking into account their frequency in gnomAD database) in the cohort of AN patients and of control women.* genes associated with age of menarche (n=7) (15), ** genes involved in rare Mendelian disorders of puberty (n=23) [29], *** genes associated with age of menarche (n=145) [29], $ Literature-based genes [16-17, 30-32] (n=10).

In the present study we identified rare variants in genes initially associated with age of menarche by GWAS analysis and confirmed by other approaches. This is the case for *MKRN3* (NR227) and *VGLL3* (NR224).Up to now, one of the most relevant gene underlying central precocious puberty (CPP) is the makorin ring finger protein 3 gene (*MKRN3*) [33]. MKRN3 is a single-exon, maternally imprinted gene, which is expressed only from the paternal allele. Consequently, only paternally-inherited loss-of-function mutations in MKRN3 cause CPP. Interestingly, MKRN3 is expressed in the mouse and human hypothalamus, a structure containing integrative systems that support physiological processes such as food intake and energy expenditure [34]. VGLL3 is a transcriptional co-factor that binds TEAD family transcription factors to regulate events ranging from wing development in fly, to muscle fibre composition and immune function in mice. Interestingly, Vgll3 play a role in the sex-biased disorders such as autoimmune diseases and may participate to the sex-biased rate of anorexia nervosa. We hypothesize that these genes account for, at least part of, the underlying shared genetic factors between age at menarche and eating disorders [13].

We also identified 29 GWAS puberty-associated genes via coding variation, without significant enrichment as compared to general population and our control sample including 10 unaffected women. The majority of these genes

are localized in candidate regions for age of menarche and puberty, but were not clearly involved in puberty. For example, this is the case for the rare variant in *PTPRN2* (NR224). PTPRN2 is also known la-2beta and was identify as a candidate associated with pediatric obesity [35, 36].

6. Conclusions

To conclude, although pubertal timing increases risk for eating disorders, the nature of the association between pubertal timing and eating disorders is unclear [37-39]. Puberty involves many physical, psychological, and emotional changes all of which likely play an important and complex role in the association between pubertal timing and eating disorders. The genes involved in puberty timing likely interact with aspects of the environment to increase vulnerability to develop ED at puberty, and previous reports have shown that the association between puberty and eating disorders is due to genetic factors [13]. Our results suggest that rare variants in genes involved in puberty timing such as *TNRC6A*, *LAMB2* and *FAAH2* may contribute to the vulnerability to AN during puberty and support causal links with anorexia nervosa susceptibility.

Disclosure and Conflict of Interest

The authors report no conflict of interest.

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Credit Author Statement

Thierry Bienvenu and Nicolas Ramoz had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and Design: Thierry Bienvenu, Nicolas Ramoz; Acquisition, analysis and interpretation of the data: Thierry Bienvenu, Nicolas Lebrun, Philip Gorwood, Nicolas Ramoz; Drafting of the manuscript: Thierry Bienvenu, Philip Gorwood, Nicolas Ramoz; Patient's inclusion: Julia Clarke, Philibert Duriez; Technical analysis: Nicolas Lebrun.

Compliance with Ethical Standards

Ethical approval All procedures performed in this study were in accordance with the ethical standards of our national research committee (Comité Protection Personnes Paris Pitié Salpétrière N°33-03) and with the 1964

Helsinki declaration and its later amendments. Informed written consent was obtained from all patients included in the study.

References

1. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. Metab 82 (1997): 1066-1070.
2. Cesario SK, Hughes LA. Precocious puberty: a comprehensive review of literature. J. Obst. Gynecol. Neonat. Nursing 36 (2007): 263-274.
3. Hughes IA. Releasing the brake on puberty. N. Engl. J. Med 368 (2013): 2513-2515.
4. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol 4 (2016) : 254-264.
5. Day FR, Thompson DJ, Helgason H, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat. Genet 49 (2017): 834-841.
6. Witchel SF. Genetics, Genome-Wide Association Studies, and Menarche. Sem.Reprod. Med 34 (2016): 205-214.
7. Hayward C, Killen JD, Wilson DM, et al. Psychiatric risk associated with early puberty in adolescent girls. J.Am. Acad. Child Adolesc. Psychiatry 36 (1997): 255-262.
8. Klump KL, Kaye WH, Strober M. The evolving genetic foundations of eating disorders. Psy. Clin. North Am 24 (2001): 215-225.
9. Klump KL, McGue M, Iacono WG. Differential heritability of eating attitudes and behaviors in prepubertal versus pubertal twins. Int. J. of Eat. Disord 33 (2003): 287-292.
10. Gorwood P, Kipman A, Foulon C. The human genetics of anorexia nervosa. Eur. J. Pharmacol 480 (2003): 163-170.
11. Culbert KM, Burt SA, McGue M, et al. Puberty and the genetic diathesis of disordered eating attitudes and behaviors. J. Abnormal Psy 118 (2009): 788-796.
12. Klump KL, Culbert KM, Slane JD, et al. The effects of puberty on genetic risk for disordered eating: evidence for a sex difference. Psy. Med 42 (2012): 627-637.
13. Baker JH, Thornton LM, Bulik CM, et al. Shared genetic effects between age at menarche and eating disorders. J. Adolesc Health 51 (2012): 491-496.
14. Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a metaanalysis of genome-wide association studies. Nat. Genet 42 (2010): 1077-1085.
15. Lunetta KL, Day FR, Sulem P, et al. Rare coding variants and X-linked loci associated with age at menarche. Nat. Commun 6 (2015): 7756.

16. Yermachenko A, Dvornyk V. UGT2B4 previously implicated in the risk of breast cancer is associated with menarche timing in Ukrainian females. Gene 590 (2016): 85-89.
17. Yang L, Li L, Peters SAE, et al. Age at Menarche and Incidence of Diabetes: A Prospective Study of 300,000 Women in China. Am. J. Epidemiol 187 (2018): 190-198.
18. Bienvenu T, Lebrun N, Clarke J, et al. De novo deleteriousvariants that may alter the dopaminergic reward pathway are associated with anorexia nervosa. Eat Weight Disord (2019).
19. Kassam I, Wu Y, Yang J, et al. Tissue-specific sex differences in human gene expression. Hum. Mol. Genet 28 (2019): 2976-2986.
20. Hudson JI, Pope HG Jr. Genetic epidemiology of eating disorders and co-occurring conditions: the role of endophenotypes. Int. J. Eat Disord 40 (2007): S76-S78.
21. Klump KL, Keel PK, Sisk C, et al. Preliminary evidence that estradiol moderates genetic influences on disordered eating attitudes and behaviors during puberty. Psy. Med 40 (2010): 1745-1753.
22. Harden KP, Mendle J, Kretsch N. Environmental and genetic pathways between early pubertal timing and dieting in adolescence: distinguishing between objective and subjective timing. Psy. Med 42 (2012): 183-193.
23. Nishi K, Nishi A, Nagasawa T, et al. Human TNRC6A is an Argonaute-navigator protein for microRNA-mediated gene silencing in the nucleus. RNA 19 (2013): 17-35.
24. Suzawa M, Noguchi K, Nishi K, et al. Comprehensive Identification of Nuclear and Cytoplasmic TNRC6A-Associating Proteins. J. Mol. Biol 429 (2017): 3319-3333.
25. Zipprich JT, Bhattacharyya S, Mathys H, et al. Importance of the C-terminal domain of the human GW182 protein TNRC6C for translational repression. RNA 15 (2009): 781,793.
26. Tahoun M, Chandler JC, Ashton E, et al. Mutations in LAMB2 associate with albuminuria and Optic Nerve Hypoplasia with Hypopituitarism. J. Clin. Endocrinol. Metab (2019): dgz216.
27. Sirrs S, van Karnebeek CD, Peng X, et al. Defects in fatty acid amide hydrolase 2 in a male with neurologic and psychiatric symptoms. Orphanet J Rare Dis 10 (2015): 38.
28. Lombardi L, Blanchet C, Poirier K, et al. Anorexia nervosa is associated with Neuronatin variants. Psy. Genet 29 (2019): 103-110.
29. Hou H, Uusküla-Reimand L, Makarem M, et al. Gene expression profiling of puberty-associated genes reveals abundant tissue and sex-specific changes across postnatal development. Hum. Mol. Genet 26 (2017): 3585-3599.
30. Demerath EW, Liu CT, Franceschini N, et al. Genome-wide association study of age at menarche in African-American women. Hum. Mol. Genet 15 (2013): 3329-3346.
31. Pyun JA, Kim S, Cho NH, et al. Genome-wide association studies and epistasis analyses of candidate genes related to age at menarche and age at natural menopause in a Korean population. Menopause 21 (2014): 522-529.

32. Cohen E, Belkacem S, Fedala S, et al. Contribution of functionally assessed GHRHR mutations to idiopathic isolated growth hormone deficiency in patients without GH1 mutations. Hum. Mutat 40 (2019): 2033-2043.
33. Valadares LP, Meireles CG, De Toledo IP, et al. MKRN3 Mutations in Central Precocious Puberty: A Systematic Review and Meta-Analysis. J. Endocr. Soc 3 (2019): 979-995.
34. Florent V, Baroncini M, Jissendi-Tchofo P, et al. Hypothalamic structural and functional imbalances in anorexia nervosa. Neuroendocrinology (2019).
35. Selvanayagam T, Walker S, Gazzellone MJ, et al. Genome-wide copy number variation analysis identifies novel candidate loci associated with pediatric obesity. Eur. J. Hum. Genet 26 (2018): 1588-1596.
36. Lee S. The association of genetically controlled CpG methylation (cg158269415) of protein tyrosine phosphatase, receptor type N2 (PTPRN2) with childhood obesity. Sci. Rep 9 (2019): 4855.
37. Stice E, Presnell K, Bearman S. Relation of early menarche to depression, eating disorders, substance abuse, and comorbid psychopathology among adolescent girls. Dev. Psy 37 (2001): 608-619.
38. Kaltiala-Heino R, Marttunen M, Rantanen P, et al. Early puberty is associated with mental health problems in middle adolescence. *Soc.* Sci. Med 57 (2003): 1055-1064.
39. Klump KL. Puberty as a critical risk period for eating disorders: a review of human and animal studies. Hormones Behav 64 (2013): 399-410.

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