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Altered DNA methylation associated with nervosa anorexia in males

Artem Kim[1], Brigitte Izac[2], Nicolas Lebrun[3], Nicolas Ramoz[3], Corinne Blanchet[4], Franck Letourneur[2], Marie Rose Moro[4], Philip Gorwood[3,5], Marie De Tayrac[1] and Thierry Bienvenu[3,6*]

[1]Institute of Genetics and Development of Rennes (Igdr), Rennes, France

[2]Cochin Institute, Genomics Platform, Inserm U1016, Paris, France

[3]Institute of Psychiatry and Neuroscience of Paris (IPNP), Inserm U1266, Paris, France

[4]Teenagers' House, Cochin Hospital, Paris, France

[5]CMME, St. Anne's Hospital, University Paris-Descartes, France.

[6]Laboratory of Molecular Genetics and Biology, Hôpital Cochin, HUPC, AP-HP, Paris, France

Abstract

Purpose: Anorexia nervosa (AN) is a serious psychiatric disorder characterized by abnormal eating behaviors, resulting in weight loss and increased mortality. Although more common in females, an estimated 5 to 10% of affected patients are males. Up to now, the exact cause of male AN is unknown. As with many psychiatric diseases, it's probably a combination of genetic, biological, psychological and environmental factors. Here, we used whole-genome bisulfite sequencing to determine the methylome of male individuals with AN.

Methods: We analyzed by bisulfite sequencing 3,340,894 biologically relevant CpG sites (Illumina TruSeqMethyl Capture EPIC kit) of 6 male patients affected with AN restrictive type. To reduce the environment effect, 4 related unaffected individuals were selected as controls.

Results: Comparisons between male patients affected with AN restrictive type and unaffected controls showed 153 differentially methylated regions and 1812 differentially methylated CpGs that corresponded to genes relevant to metabolic and nutritional status, psychiatric status and immune function. Moreover, the String network analysis software identified a subnetwork, related to MAPK signaling pathway, PI3K-Akt signaling pathway and neurotrophin signaling pathway.

Conclusions: Our findings replicate several results concerning several target genes such as *PRKAG2*, *RPTOR*, and *ICAM5* previously identified in female AN, and identified novel signaling pathways involving PI3K-Akt and neurotrophin signaling pathway disturbed in AN.

Introduction

Anorexia nervosa (AN) is a complex neuropsychiatric disorder characterized by weight loss, and an intense fear of gaining weight. Family and twin studies of AN have shown that genetic and environmental factors play important roles in the pathogenesis of AN. Twin studies have estimated the heritability to be~56% [1]. However, while several genes were identified by candidate gene studies and genome-wide association studies (GWAS), they often failed to replicate in other studies [2-5]. The same observation was found for major depression sharing common genetic and environmental risk factors with AN [6]. Indeed, young people who suffered from AN are at high risk of depression [7]. Epigenetics and environmental factors might play a crucial role in the development of AN as well as in major depressive disorder [8]. Recently, a genome-wide DNA methylation study was conducted in women with active AN, AN in remission and in non-AN controls. Global analysis of the 3 groups revealed 295 differentially methylated sites (DMS) representing 277 genes. Some of the identified genes were related to nutrition, to bone and tissue heath, immune function/inflammation, as well as general or transcriptional processes, and glia-neuron interaction [9].

As AN is nine times more often in females than in males, focusing on gender specificities might help to shed light on its overall nature. We therefore propose an analysis of several male AN patients, supporting in part the recent findings and identifying a novel disease-relevant pathway.

Patients and methods

We analyzed more than 3.3 M biologically relevant CpGs (Illumina TruSeqMethyl Capture EPIC kit) of 6 male patients affected with AN restrictive type. This approach captures 3,340,894 CpG sites including 26,981 CpG islands (107 Mb), including American College of Medical Genetics (ACMG) genes, coding genes known to be involved in cancer, coding exons from Ensemble 70, and 100 promoters defined as being of high interest and difficult to sequence. To reduce the environment effect, 4 related unaffected individuals- parents of two AN individuals - were selected as controls. Briefly, libraries were prepared according to manufacturer protocol (using the TrueSeq DNA Methylation Kit (cat. EGMK81312, Illumina Inc., San Diego, CA), the NEXTflex

Correspondence to: Thierry Bienvenu, Institute of Psychiatry and Neuroscience of Paris, 102 rue de la Santé, 75014 Paris, France, E-mail: thierry.bienvenu@inserm.fr

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Bisulfite Sequencing Kit (cat. 5119–02) and the 24 NEXTflex Bisulfite Sequencing Barcodes (cat. 511913)). After PCR-amplification, the bisulfite-treated Libraries were clustered on a V3 paired-end read flow cell and sequenced for 100 cycles on an Illumina NextSeq500 System (Illumina). Fastq files were generated using Illumina's software CASAVA v1.8.2. Raw fastq reads were processed by a custom pipeline that consists of: (i) filtering raw fastq reads for pass filter reads, (ii) trimming adapter sequence by Trim Galore, (iii) genomic alignments performed using Bismark to reference human genome hg19, and (iv) methylation calling by a Bismark script (https://github.com/nf-core/methylseq). The R package methyKit was used for analysis and annotation of DNA [10]. After adjustment for potential confounders and exclusion of age-related CpG, differences were shown between AN individuals and controls. We initially investigated the differentially methylated regions (DMRs) in AN using a computational algorithm MethylKit with a stringent statistical cutoff of q-value (FDR adjusted p-value<0.01) and a minimum 25% change in methylation between AN patients and controls.

Results

This approach allowed us to identify a total of 153 DMRs in AN (Table 1). Of these, 53 DMRs were hypomethylated and 100 were hypermethylated, indicating that more genic regions tended to be methylated in AN individuals, similar to previous observations [9]. We further examined the genomic distribution of differentially methylated CpGs (DMCs). A total of 1812 DMC sites were identified in patients with AN compared to controls (Supplementary Table 1). Among the 1812 DMCs, 748 were hypomethylated, while 1065 were hypermethylated in the AN samples. Among these 1812 DMCs, 216functional genic regions included at least 2 DMCs. Interestingly, when we compared our DMCs list with the short list of Steiger and colleagues, 32 genes were found in both lists (Supplementary Table 2) [9]. Moreover, among the 100 top-ranked differentially methylated positions identified in patients with major depressive disorder, six were also found in our study (*GNG4, SLC39A12, CRTAC1, MYO7A, NTM* and *RMST*), four of them playing a role in neurite extension and neurogenesis (*GNG4, SLC39A12, NTM* and *RMST)* [8].

To identify the molecular pathways and functions potentially influenced by methylation changes in AN, we performed GO term and KEGG pathway enrichment analyses of the genes closest to the identified DMRs (within the gene body or within +/- 10kb of gene start/end sites) using the DAVID bioinformatics resources 6.8 (https://david.ncifcrf.gov/). When "Disease" was used for categorization, there were 5 charts categories (with a significant p value with Benjamini correction) of DMRs, the more significant charts being waist-hip ratio (n=9 counts, Benjamini 5.2e-3), tobacco use disorder (37 counts, Benjamini 2.8e-3),

Table 1. Identification of differentially methylated regions (DMR) was performed using the predefined regions of Illumina TruSeq Methylcapture EPIC kit presenting at least 25 % methylation difference between ANcases and controls (column *Change*) with a q-value (FDR corrected p-value) threshold of 0.01

DMR	Chr	Nb Genes	Genes	*p* value	*q* value	Change
chrX_114502896_114503051	chrX	0	-	2.22451E-15	3.71584E-14	59.78484933
chr14_54375093_54375328	chr14	1	**AL138479.**	2.65207E-24	1.18277E-22	48.00682879
chr8_80036078_80036321	chr8	0	-	2.44128E-36	2.55548E-34	46.08190008
chr11_79289117_79289197	chr11	0	-	2.09678E-48	3.89352E-46	44.01889299
chr2_165342265_165342368	chr2	1	**GRB14**	5.17941E-11	4.44995E-10	43.62139918
chr5_118811413_118811668	chr5	2	**HSD17B4|snoU1**	4.63731E-11	4.01928E-10	43.59922516
chr19_45113900_45114110	chr19	2	**CEACAM22P|IGSF23**	9.32156E-32	7.39337E-30	40.63822929
chr12_39885082_39885307	chr12	0	-	1.48466E-54	3.57503E-52	40.49521423
chr11_99564881_99564954	chr11	1	**CNTN5**	9.37129E-15	1.42916E-13	39.62334329
chr20_46228572_46228783	chr20	1	**NCOA3**	1.11712E-13	1.47266E-12	39.56217898
chr12_9507242_9507307	chr12	0	-	1.05726E-24	4.90641E-23	38.46786372
chr2_238213149_238213414	chr2	0	-	1.58339E-06	5.42033E-06	37.58090232
chr9_88891303_88891317	chr9	1	**ISCA1**	1.08415E-06	3.8617E-06	37.31409719
chr7_134279040_134279370	chr7	0	-	7.42127E-19	1.90865E-17	36.8404498
chr11_125477842_125477944	chr11	1	**STT3A**	1.37342E-52	3.02928E-50	36.14363165
chr4_85873367_85873551	chr4	1	**WDFY3**	8.771E-29	5.54172E-27	35.86458464
chr1_228243499_228243750	chr1	1	**WNT3A**	7.15126E-21	2.28614E-19	35.584194
chr2_131046231_131046636	chr2	5	**MTND4P2|MTND5P2|MTND6P|AC068137.1|AC068137.1**	1.32673E-40	1.75348E-38	35.46103678
chr7_39723763_39723940	chr7	1	**RALA**	2.01448E-11	1.85339E-10	34.0085943
chr6_132297421_132297621	chr6	1	**RP11-69I8.**	0.001384883	0.002169711	33.37310303
chr3_108880309_108880502	chr3	0	-	7.64309E-11	6.36725E-10	33.06601128
chr1_66753890_66754101	chr1	1	**PDE4B**	0.00182074	0.00275054	32.96104032
chr2_66654487_66654810	chr2	2	**MEIS1-AS3|MEIS1**	2.10022E-69	7.72486E-67	32.72071839
chr5_97764439_97764614	chr5	0	-	0.000291866	0.000557559	32.43193392
chr9_115957266_115957508	chr9	1	**FKBP15**	1.69011E-15	2.87555E-14	32.29050602
chr15_50209373_50209588	chr15	1	**ATP8B4**	3.01371E-25	1.45926E-23	31.98261372
chr17_11608628_11608839	chr17	1	**DNAH9**	6.97091E-30	4.8207E-28	31.79815222
chr4_3040168_3040507	chr4	2	**GRK4|RNU6-204**	7.3598E-14	9.96918E-13	31.65690685
chr6_88956453_88956584	chr6	0	-	9.99041E-12	9.68676E-11	31.54201058
chr21_15436054_15437354	chr21	3	**AP001347.|ANKRD20A18|RNA5SP48**	0	0	31.16688815
chr3_49712243_49712522	chr3	4	**BSN|APEH|MST1|AC099668.**	8.07314E-25	3.79115E-23	31.03180163
chr12_25264152_25264371	chr12	2	**LRMP|CASC1**	8.78331E-27	4.8313E-25	30.29058703
chrX_9121569_9121778	chrX	1	**FAM9B**	0.000426217	0.000777831	30.08088109

chr3_84932517_84932772	chr3	2	**LINC00971|LINC02025**	2.07283E-18	5.07715E-17	29.94169954
chr7_123928236_123928461	chr7	2	**RP5-921G16.|RP11-264K23.**	3.518E-31	2.67311E-29	29.91216787
chr9_106087270_106087465	chr9	1	**LINC01492**	8.36825E-19	2.13729E-17	29.78985686
chr5_120388958_120389191	chr5	2	**AC008565.|CTD-2613O8.**	7.61105E-44	1.17667E-41	29.58350371
chr11_8927411_8927650	chr11	2	**ST5|AKIP1**	4.49366E-10	3.25802E-09	29.52623011
chr6_159572123_159572413	chr6	0	**-**	7.42108E-39	8.88692E-37	29.31934038
chr22_49625975_49626187	chr22	0	**-**	2.92003E-11	2.61571E-10	29.27879223
chr12_9776968_9777167	chr12	2	**LOC374443|RNU6-700**	1.12651E-09	7.58502E-09	29.08923252
chr3_170332310_170332590	chr3	1	**SLC7A14-AS1**	1.8717E-08	9.81278E-08	29.01098901
chr4_77986307_77986536	chr4	1	**CCNI**	3.91084E-14	5.48555E-13	28.97085991
chr22_42176013_42176129	chr22	1	**MEI1**	0.000206038	0.000410706	28.81962113
chr10_25240859_25240994	chr10	2	**PRTFDC1|RP11-165A20.**	6.26124E-28	3.71906E-26	28.60587752
chr3_79966821_79966915	chr3	0	**-**	1.15638E-10	9.34284E-10	28.60217066
chr3_45649141_45649463	chr3	1	**LIMD1**	6.28165E-62	1.8899E-59	28.50314686
chr6_25166613_25166879	chr6	1	**CMAHP**	2.17351E-96	1.46968E-93	28.37986243
chr3_152213629_152213846	chr3	1	**RP11-362A9.**	6.8342E-05	0.000155335	28.26746276
chr11_59836664_59836958	chr11	2	**MS4A3|RP11-736I10.**	3.63343E-09	2.21257E-08	28.0516934
chr20_14124320_14124383	chr20	1	**MACROD2**	1.40341E-20	4.35847E-19	28.02413196
chr22_32728242_32728474	chr22	1	**RP1-149A16.1**	8.85421E-11	7.29047E-10	27.91646442
chr2_183106034_183106226	chr2	1	**PDE1A**	9.65506E-09	5.3791E-08	27.85254866
chr6_118401762_118401938	chr6	2	**SLC35F1|LOC105377967**	4.64459E-19	1.22408E-17	27.79487179
chr10_91369814_91369992	chr10	1	**PANK1**	1.57036E-21	5.3785E-20	27.77056277
chr12_49121182_49121257	chr12	1	**TEX49**	8.24722E-13	9.54004E-12	27.60425962
chr5_95362159_95362388	chr5	1	**LOC101929710**	4.61645E-19	1.21738E-17	27.51574796
chr17_16935456_16935522	chr17	0	**-**	4.58885E-09	2.74027E-08	27.46293683
chr3_42093299_42093629	chr3	2	**TRAK1|RP11-193I22.**	6.20321E-37	6.69223E-35	27.41943731
chr3_149867507_149867632	chr3	1	**LOC105374313**	3.07268E-11	2.74327E-10	27.29693742
chrX_43503885_43504133	chrX	0	**-**	4.40393E-09	2.64009E-08	27.18926273
chr14_77035752_77035960	chr14	1	**RP11-187O7.**	6.2288E-12	6.23127E-11	27.15821169
chr10_52771069_52771259	chr10	1	**PRKG1**	3.87096E-16	7.14936E-15	27.04932754
chr2_38055293_38055466	chr2	1	**LINC00211**	7.48568E-28	4.4228E-26	27.03032591
chr1_108245511_108245769	chr1	1	**VAV3**	3.17027E-12	3.3165E-11	26.94594044
chr11_132891631_132891819	chr11	1	**OPCML**	4.6077E-05	0.000109674	26.80024478
chr8_8538073_8538307	chr8	0	**-**	6.60262E-14	8.99085E-13	26.64249158
chr8_107757803_107757980	chr8	1	**OXR1**	0.000716855	0.001224528	26.62186488
chr21_36250641_36250940	chr21	2	**LOC100506403|RUNX1**	3.11176E-25	1.50565E-23	26.55673748
chr9_14910433_14910528	chr9	1	**FREM1**	8.33159E-21	2.64892E-19	26.53151371
chr3_10851357_10851586	chr3	1	**SLC6A11**	0.004410223	0.005890646	26.40737551
chr9_73216788_73217020	chr9	1	**TRPM3**	3.0921E-15	5.06212E-14	26.40133464
chr17_77906666_77906803	chr17	3	**LINC01979|LINC01978|TBC1D16**	3.90756E-08	1.9197E-07	26.38634157
chr15_101507155_101507328	chr15	1	**LRRK1**	5.16927E-09	3.05155E-08	26.37814064
chr10_87988149_87988359	chr10	1	**GRID1**	0.003497861	0.004828383	26.35618749
chr13_46978725_46978934	chr13	2	**RUBCNL|RNU6-68**	2.29746E-12	2.46329E-11	26.04182226
chrX_149197068_149197285	chrX	1	**LINC00894**	2.44211E-07	1.01031E-06	26.00779648
chr16_66453623_66453908	chr16	2	**LINC00920|BEAN1**	3.68957E-06	1.15579E-05	25.85630262
chr1_165186330_165186523	chr1	3	**LMX1A|RP11-38C18.|RP11-38C18.**	0.000275984	0.000530947	25.72822521
chr12_6662473_6662654	chr12	3	**IFFO1|RP5-940J5.|NOP2**	9.5204E-29	6.00387E-27	25.71991419
chr4_129308317_129308548	chr4	1	**LINC02615**	2.02201E-09	1.29361E-08	25.68972744
chr4_102756899_102757268	chr4	1	**BANK1**	8.27941E-46	1.39081E-43	25.66642434
chr6_101852800_101852969	chr6	1	**GRIK2**	1.14283E-19	3.23959E-18	25.50742035
chr7_147500652_147500831	chr7	1	**CNTNAP2**	4.69478E-06	1.43535E-05	25.47112462
chr2_130683180_130683508	chr2	2	**PLAC9P1|LINC01856**	2.6716E-16	5.03565E-15	25.46823547
chr3_69157750_69157970	chr3	2	**ARL6IP5|LMOD3**	6.39788E-06	1.89017E-05	25.46542143
chr12_104676677_104676883	chr12	2	**TXNRD1|RP11-818F20.**	4.32249E-15	6.92567E-14	25.4273867
chr9_4375797_4376098	chr9	1	**AL162419.**	3.07166E-06	9.81114E-06	25.41113806
chr6_55377549_55377764	chr6	1	**HMGCLL1**	4.38355E-45	7.21874E-43	25.36445879
chr8_80816076_80816210	chr8	0	**-**	2.50289E-06	8.16528E-06	25.34385329
chr5_171810643_171810847	chr5	1	**SH3PXD2B**	2.88223E-10	2.16508E-09	25.2488391
chr2_66648614_66648933	chr2	1	**MEIS1-AS3**	1.28929E-22	4.90242E-21	25.21383356
chr14_96583490_96583604	chr14	0	**-**	0.003078364	0.004325989	25.19935377
chr22_16864741_16864893	chr22	1	**ABCD1P**	0.002259786	0.003313591	25.19298246
chr10_90483503_90483709	chr10	2	**LIPK|KRT8P3**	3.47526E-44	5.45461E-42	25.15294597

chr6_7699982_7700169	chr6	0	-	1.47903E-12	1.64125E-11	25.12462285
chr3_195501977_195502276	chr3	1	**MUC4**	1.31243E-47	2.3649E-45	25.08813062
chr18_29303346_29303538	chr18	3	**RN7SKP4|LRRC37A7|RP11-549B18.**	1.62011E-27	9.3655E-26	25.03570419
chr3_95424692_95425326	chr3	0	-	3.3968E-14	4.80683E-13	25.03516155
chr3_180102299_180102384	chr3	0	-	9.13491E-07	3.3066E-06	25.01885857
chr22_35587422_35587713	chr22	2	**LINC01399|COX7BP**	1.08406E-10	8.79565E-10	-25.01400968
chr20_36132841_36133069	chr20	1	**BLCAP**	1.55091E-18	3.84306E-17	-25.14666861
chr19_47082930_47083060	chr19	2	**PPP5D1|AC011551.**	6.29188E-21	2.02349E-19	-25.24712874
chr19_49572906_49573157	chr19	4	**NTF4|CTB-60B18.1|CTB-60B18.1|KCNA7**	5.89203E-07	2.23171E-06	-25.34019384
chr3_11489044_11489192	chr3	1	**ATG7**	7.90562E-10	5.47842E-09	-25.40198715
chr17_75631717_75631979	chr17	0	-	1.84137E-05	4.85349E-05	-25.53803605
chr1_19176711_19177105	chr1	2	**TAS1R2|RP13-279N23.**	0.000375134	0.000695164	-25.58525242
chr11_5840963_5841191	chr11	2	**TRIM5|OR52N2**	2.88714E-66	9.68772E-64	-25.69185678
chr2_55378165_55378300	chr2	0	-	9.89458E-19	2.51065E-17	-25.78431373
chr13_19315890_19316198	chr13	3	**ZNF965|LINC00417|CYP4F34**	8.90044E-14	1.19079E-12	-25.87364383
chr18_61669993_61670289	chr18	1	**SERPINB8**	8.41432E-63	2.5838E-60	-26.04262437
chr20_32885922_32886673	chr20	1	**AHCY**	2.8332E-44	4.47838E-42	-26.45197851
chr6_106252261_106252558	chr6	0	-	6.2211E-107	4.9875E-104	-26.54101932
chr2_25383849_25384399	chr2	3	**EFR3B|RP11-509E16|POMC**	4.25503E-85	2.26062E-82	-26.70759651
chr4_131633369_131633569	chr4	0	-	1.21778E-09	8.14225E-09	-26.8218303
chr4_26789871_26790007	chr4	0	-	9.76527E-06	2.75795E-05	-27.15229384
chr1_55370080_55370265	chr1	0	-	1.33489E-60	3.89366E-58	-27.2302102
chr19_45619267_45619497	chr19	2	**MARK4|PPP1R37**	3.61011E-48	6.62101E-46	-27.29034787
chr16_33588844_33589010	chr16	1	**ENPP7P13**	8.03476E-26	4.0933E-24	-27.38040136
chr4_15907926_15908145	chr4	0	-	2.8442E-50	5.76083E-48	-27.44818039
chr10_67155111_67155236	chr10	0	-	4.83108E-11	4.17264E-10	-27.80333564
chr17_4278292_4278786	chr17	1	**UBE2G1**	9.00939E-24	3.82074E-22	-27.82640422
chr11_116344108_116344147	chr11	0	-	0.000326633	0.000615342	-27.8388828
chr19_34245878_34246069	chr19	1	**CHST8**	6.69284E-06	1.96741E-05	-27.92649973
chr17_16864290_16864524	chr17	1	**TNFRSF13B**	1.26001E-12	1.41214E-11	-28.2842299
chr11_82354426_82354705	chr11	2	**MIR4300HG|RP11-179A16.**	6.09835E-07	2.30172E-06	-29.18358119
chr5_153676389_153676633	chr5	1	**GALNT10**	1.43406E-68	5.14737E-66	-29.70725527
chr10_134778418_134779164	chr10	3	**LINC01166|LINC01167|LINC01168**	5.1864E-178	1.2399E-174	-29.73911375
chr8_36995835_36995986	chr8	1	**MIR1268A**	3.40507E-09	2.0846E-08	-29.75107115
chr2_213316451_213316721	chr2	1	**ERBB4**	3.25312E-05	8.05895E-05	-30.06050559
chr3_105601223_105601548	chr3	0	-	3.99011E-26	2.08106E-24	-30.45696192
chr2_130693963_130694229	chr2	3	**PLAC9P1|LINC01856|AC079776.**	1.8989E-114	1.6726E-111	-30.54653298
chr11_110986763_110986975	chr11	1	**RP11-89C3.**	0	0	-30.61868687
chr4_184296182_184296485	chr4	2	**RP11-451F20.|AC093844.**	3.2812E-82	1.64531E-79	-30.6235367
chr6_21452696_21452935	chr6	0	-	4.66098E-09	2.77827E-08	-30.7652865
chr14_56669107_56669307	chr14	1	**PELI2**	0.005344129	0.006946025	-31.10639369
chr13_25141770_25142091	chr13	3	**TPTE2P6|PSPC1P|RP11-556N21.**	2.11697E-25	1.04092E-23	-31.10709988
chr2_238796929_238797268	chr2	1	**RAMP1**	9.04001E-24	3.83251E-22	-31.35072812
chr17_6901611_6901751	chr17	4	**ALOX12-AS1|RP11-589P10.|RP11-589P10.|ALOX12**	0.00014541	0.000302279	-31.60823695
chr15_79299672_79299707	chr15	1	**RASGRF1**	0.004476538	0.005967375	-32.72230999
chr1_223353214_223353363	chr1	1	**RP11-239E10**	7.13359E-18	1.64468E-16	-33.00889209
chr1_113221602_113221746	chr1	2	**CAPZA1|MOV10**	8.44636E-34	7.62526E-32	-33.03827068
chr19_21860552_21861267	chr19	1	**RP11-420K14.**	1.3469E-284	1.127E-280	-33.08420719
chr2_145505959_145506291	chr2	2	**TEX41|AC023128.**	2.36911E-23	9.63501E-22	-33.13077409
chr21_29565178_29565285	chr21	1	**LINC01695**	0.000196292	0.000393653	-33.4915241
chr22_47442504_47442671	chr22	1	**TBC1D22A**	2.44869E-31	1.87658E-29	-34.87001855
chr8_119449323_119449547	chr8	1	**SAMD12**	2.1869E-06	7.23312E-06	-36.3138769
chr3_6706652_6706860	chr3	2	**AC069277.|GRM7-AS3**	0.00028562	0.000547251	-39.63810451
chr8_129286309_129286584	chr8	0	-	3.30084E-09	2.02608E-08	-39.75752508
chr12_64067778_64067878	chr12	3	**MIR548Z|DPY19L2|RP11-415I12.**	0.000389652	0.000718873	-40.8170893
chr15_71187717_71187868	chr15	2	**LRRC49|THAP10**	0.004522921	0.006020138	-42.45762712
chr3_115099771_115100023	chr3	0	-	0.002555418	0.003683188	-42.60471914
chr17_57187586_57187924	chr17	3	**TRIM37|AC099850.|SKA2**	2.39899E-60	6.93704E-58	-46.21784352

chemdependency (n=40 counts, Benjamini 1.2e-3), metabolic (n=49, Benjamini 3.1e-3) and neurological (32 counts, Benjamini 3.8e-3).

We next focused on the 153 DMRs, the String network analysis software identified a subnetwork, related to MAPK signaling pathway (hs04010, FDR 0.0026), PI3K-Akt signaling pathway (hsa04151, FDR 0.0232) and neurotrophin signaling pathway (hsa04550, FDR 0.0251)(https://string-db.org/cgi/network.pl?taskId=07ri9Goe1SzI) (Figure 1).

Discussion

To the best of our knowledge, the present study presents the first genome-wide DNA methylation profiling of 6 male AN patients, using a high-throughput DNA methylation sequencing covering a large number of CpG sites on the human genome. Patients affected with AN restrictive type showed many differentially methylated sites, with significant between group differences corresponding to genes implicated in metabolic and nutritional status, psychiatric status and immune function. When we compared our DMCs list with the short list of Steiger and colleagues obtained in female AN patients, 32 genes were found in both lists [9]. Moreover, because the interaction between depression and anorexia nervosa was significant, we also identified six genes previously found in patients with major depressive disorder [8]. Taking into account that our approach was based on genome-wide methylation sequencing, we also identified additional candidate genes involved in psychiatric disorders (*GRID1, NAALADL2-AS3, PDZD, CEP85L, GRIK3, SLC7A14*), in metabolism regulation (lipids, *ZFP36L1, CERK, ACSF3, EEPD1*; glucose, *KCNA7*), and finally, in addiction (*GDE1, CCKAR, PDYN*). Moreover, we identified few genes previously associated with anorexia nervosa in genome-wide association studies (*VGLL4, GRID1, WWOX, CAMK1D, SORCS2*) (https://www.ebi.ac.uk/gwas/search?query=anorexia%20nervosa). Interestingly, the String network analysis software revealed a subnetwork related to neurotrophin signaling pathway (Figure 1). The reported data, in addition to the previous reported findings for *BDNF*, *NTRK2* and *NTRK3*, point again neurotrophin signaling genes as key regulators of eating behavior.

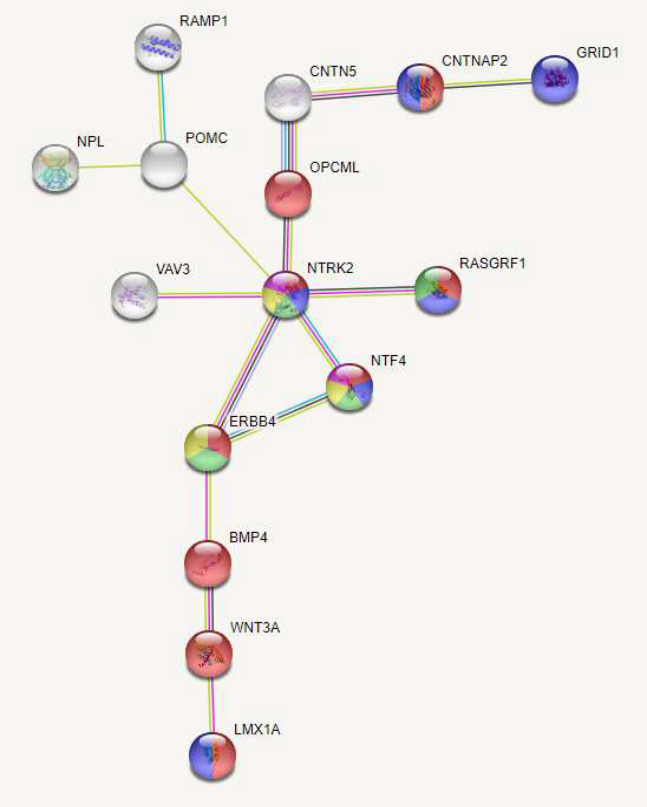

Figure 1. Protein subnetwork of the identified proteins constructed with the STRING software for enrichment analysis of proteins (PPI enrichment value 7.04e-08) showing a dysmethylation status (15 DMRs among the 119 identified DMRs including at least one coding or non-coding genes). Each node represents a protein entity. Gene Ontology identified functional enrichments for several biological process including neuron differentiation (GO:0030182, FDR 8.15e-06) and behavior (GO:0007610, FDR 0.00025)(in black), and KEGG pathways identified enrichments for several pathways including MAPK signaling pathway (hs04010, FDR 0.0026)(grey square), PI3K-Akt signaling pathway (hsa04151, FDR 0.0232)(white square) and Neurotrophin signaling pathway (hsa04550, FDR 0.0251) (grey triangle)(https://string-db.org/cgi/network.pl?taskId=07ri9Goe1SzI)

To conclude, our data replicates several results concerning several target genes such as *PRKAG2*, *RPTOR*, and *ICAM5* previously discussed [9], and identified novel signaling pathways involving PI3K-Akt and neurotrophin signaling pathway disturbed in anorexia nervosa. Future replication of findings in male AN patients will be a determinant.

Compliance with ethical standards

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procédures performed in this study were in accordance with the ethical standards of our national research committee and with the 1964 Helsinki declaration and its later amendments. Informed written consent was obtained from all patients and parents included in the study.

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