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ORIGINAL ARTICLE

A genome-wide association study of anorexia nervosa

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Anorexia nervosa (AN) is a complex and heritable eating disorder characterized by dangerously low body weight. Neither candidate gene studies nor an initial genome-wide association study (GWAS) have yielded significant and replicated results. We performed a GWAS in 2907 cases with AN from 14 countries (15 sites) and 14 860 ancestrally matched controls as part of the Genetic Consortium for AN (GCAN) and the Wellcome Trust Case Control Consortium 3 (WTCCC3). Individual association analyses were conducted in each stratum and meta-analyzed across all 15 discovery data sets. Seventy-six (72 independent) single nucleotide polymorphisms were taken forward for *in silico* (two data sets) or *de novo* (13 data sets) replication genotyping in 2677 independent AN cases and 8629 European ancestry controls along with 458 AN cases and 421 controls from Japan. The final global meta-analysis across discovery and replication data sets comprised 5551 AN cases and 21 080 controls. AN subtype analyses (1606 AN restricting; 1445 AN binge–purge) were performed. No findings reached genome-wide significance. Two intronic variants were suggestively associated: rs9839776 ($P=3.01\times10^{-7}$) in *SOX2OT* and rs17030795 ($P=5.84\times10^{-6}$) in *PPP3CA*. Two additional signals were specific to Europeans: rs1523921 ($P=5.76\times10^{-6}$) between *CUL3* and *FAM124B* and rs1886797 ($P=8.05\times10^{-6}$) near *SPATA13*. Comparing discovery with replication results, 76% of the effects were in the same direction, an observation highly unlikely to be due to chance ($P=4\times10^{-6}$), strongly suggesting that true findings exist but our sample, the largest yet reported, was underpowered for their detection. The accrual of large genotyped AN case-control samples should be an immediate priority for the field.

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INTRODUCTION

Anorexia nervosa (AN) is a perplexing biologically influenced psychiatric disorder characterized by the maintenance of dangerously low body weight, fear of weight gain and seeming indifference to the seriousness of the illness.[1] AN affects ~1% of the population.[2,3] Females are disproportionately afflicted, although males also develop the condition.[4] The most common age of onset is 15–19 years;[5] however, the incidence appears to be increasing in the pre-pubertal period[6] and in older adults.[7] AN is often comorbid with major depressive disorder, anxiety disorders and multiple somatic complications.[8–12] Although most individuals recover, ~25% develop a chronic and relapsing course.[13] AN ranks among the ten leading causes of disability among young women[14] and has one of the highest mortality rates of any psychiatric disorder.[15–19] The evidence base for treatment of AN has been described as weak,[20,21] and treatment and extended inpatient hospitalizations for weight restoration are costly.[22,23] In sum, the public health impact of AN is considerable, and AN carries substantial morbidity, mortality, and personal, familial and societal costs.

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Table 1. List of ethnicities and numbers of samples for main case control and anorexia nervosa (AN) subtype analyses across discovery and replication data sets

Country	*Cases (% of females)*	*AN restricting subtype cases*	*AN binge–purge subtype cases*	*Controls (% of females)*
Discovery data set[a]				
Canada	54	24	25	417 (46.52)
Czech Republic	72	40	29	331 (35.04)
Finland	131	39	29	404 (100)
France	293	137	135	619 (60.09)
Germany	475	147	55	1205 (49.13)
Greece	70	10	5	79 (100)
Italy-North	203	103	99	841 (52.19)
Italy-South	75	31	26	52 (100)
Netherlands	348	115	90	593 (51.26)
Norway	82	24	15	602 (67.44)
Poland	175	68	107	564 (29.43)
Spain	186	45	44	185 (75.14)
Sweden	39	28	11	975 (72.10)
UK	213	97	97	5163 (49.43)
USA	491	311	165	2830 (41.31)
Total discovery	2907	1219	932	14 860 (51.73)
In silico replication				
USA-Hakonarson	1033 (97.67)	0	0	3775 (45.85)
Estonia	31 (100)	0	0	106 (100)
De novo replication				
Austria	48 (100)	0	0	183 (65.03)
Czech Republic	32 (71.88)	0	0	22 (100)
Finland	15 (100)	0	0	94 (8.51)
France	55 (100)	0	0	123 (100)
Germany	174 (99.43)	31	64	380 (66.84)
Greece	16 (100)	0	0	53 (100)
Italy-South	156 (96.79)	32	24	63 (100)
Netherlands	229 (100)	45	23	380 (27.11)
Poland	52 (98.08)	0	0	93 (100)
Spain	10 (100)	0	0	328 (41.46)
UK	155 (100)	28	55	199 (65.83)
USA[b]	671 (100)	349	272	2830 (41.31)
Japan	458 (100)	213	240	421 (100)
Total replication	3135 (98.72)	698	678	9050 (50.08)
Total global meta-analysis	5551	1606	1445	21 080

[a]All AN cases from discovery data set were females. [b]USA samples from discovery data set were merged together with USA replication samples for replication analysis. The same USA control data set was used.

As with most idiopathic psychiatric disorders, the inheritance of AN is complex. The core features of AN (i.e., the ability and determination to maintain low body mass index (BMI)) are remarkably homogeneous across time and cultures.[24,25] Genetic epidemiological studies have documented the familiality of AN (relative risk 11.3 in first-degree relatives of AN probands)[26,27] and the estimated twin-based heritability of AN ranges from 33 to 84%.[28–32] Genome-wide linkage studies did not narrow the genomic search space in a compelling manner.[33–35] Findings from candidate gene studies of AN resemble those for most complex biomedical diseases—initial intriguing findings diminished by the absence of rigorous replication.[36–38]

Given the centrality of weight dysregulation to AN, genes implicated in the regulation of body weight might also be involved in the etiology of AN.[39,40] Therefore genetic variants with a profound effect on BMI are worthy of consideration.[38]

Two genome-wide association studies (GWAS) of AN have been conducted. One study that used DNA pooling and genotyping with a modest number of microsatellite markers with follow-up genotyping detected evidence for association with rs2048332 on chromosome 1, but this finding did not reach genome-wide significance.[41] A GWAS of 1033 AN cases from USA, Canada and Europe compared with 3733 pediatric controls yielded no genome-wide significant findings.[42] Recently, a sequencing and genotyping study of 152 candidate genes in 1205 AN cases and 1948 controls suggested a novel association of cholesterol metabolism influencing *EPHX2* gene with susceptibility to AN.[43]

In recognition of the need for large-scale sample collections to empower GWAS, we established the Genetic Consortium for Anorexia Nervosa (GCAN) in 2007—a worldwide collaboration combining existing DNA samples of AN patients into a single resource. As part of the Wellcome Trust Case Control Consortium 3 (WTCCC3), we have conducted the largest GWAS for AN to date.

MATERIALS AND METHODS

Discovery data set

We conducted a GWAS across 15 discovery data sets, comprising a total of 2907 AN cases and 14 860 ancestrally matched controls of European origin (Table 1). All AN cases were female. Diagnostic determination was via semi-structured or structured interview or population assessment strategy based on DSM diagnostic criteria. Cases met DSM-IV criteria for lifetime AN (restricting or binge–purge subtype) or lifetime DSM-IV eating disorders 'not otherwise specified' AN-subtype (i.e., exhibiting the core features of AN). We did not require the presence of amenorrhea as this criterion does not increase diagnostic specificity.[44,45] Given the frequency of diagnostic

crossover, a lifetime history of bulimia nervosa was allowed.[46] Exclusion criteria included the diagnosis of medical or psychiatric conditions that might have confounded the diagnosis of AN (e.g., psychotic disorders, mental retardation, or a medical or neurological condition causing weight loss). Controls were carefully selected to match for ancestry within each site and chosen primarily from existing GWAS genotypes through collaboration and genotyping repository (dbGAP) access. Each site obtained ethical approval from the local ethics committee, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Genotyping, imputation and quality control

AN cases from the 15 sites were genotyped using Illumina 660 W-Quad arrays (Illumina Inc., San Diego, CA, USA) at the Wellcome Trust Sanger Institute. Funding was available only for genotyping AN cases. Thus, control genotypes were selected from existing data sets matched as closely as possible to the ancestry of cases and Illumina arrays as similar as possible to the 660 W array (Supplementary Table S1). Quality control (QC) of directly typed variants was performed within each of the 15 case-control data sets (Supplementary Table S2, Supplementary Information).

Phasing and imputation were performed separately for each of the 15 data sets using a common set of single nucleotide polymorphisms (SNPs) passing QC (Supplementary Table S2) using the program Impute2 v2.1.2 (Supplementary Information).[47] The imputation reference panel was HapMap 3 release 2. We used all available HapMap3 populations for imputation as it was shown that the increase in the reference panel decreases error.[48,49] Post-imputation filters were applied to remove SNPs with INFO scores < 0.4 or with MAF < 0.05. We observed high imputation accuracy (as captured by the INFO score) across a range of minor allele frequencies (Supplementary Figure S1). There was high concordance between directly genotyped variants with imputed dosages of the same variants after masking (Supplementary Figure S2).

Statistical analysis

Single-SNP association analyses were performed under an additive genetic model separately within each of the 15 data sets (Supplementary Information). We tested for association across the autosomes and the non-pseudoautosomal region of the X chromosome. Imputation and association analysis of the non-pseudoautosomal region of the chromosome X data were based on females (2907 AN cases and 10 594 controls). Association analyses were performed using SNPTEST v2.2.0[49] under an additive model and using a score test. To guard against false positives due to population stratification, we carried out association analysis within each data set and then combined the results using meta-analysis (for the French data set, the first principal component was added as a covariate). Fixed-effects meta-analyses were performed using GWAMA.[50] All 15 discovery data sets were corrected for the genomic control inflation factor prior to performing meta-analysis (Supplementary Table S2; Supplementary Information).

Replication

We prioritized directly genotyped and imputed SNPs for replication based on statistical significance ($P < 10^{-4}$), robust QC metrics and vicinity to plausible candidate genes. In total, 96 SNPs (95 autosomal and one on chromosome X) in 66 genomic regions showed nominal evidence for association. We selected 72 independent, uncorrelated variants representing each of the 66 associated genomic regions and added 4 proxies for the most-associated SNPs resulting in 76 SNPs for replication. Cluster plots of all prioritized SNPs were examined using Evoker[51] in cases and controls separately to minimize the possibility of spurious association due to genotyping error. We included 27 ancestry-informative markers (AIMs) for genotyping in the replication data sets, to guard against population stratification (Supplementary Information).[52]

Our replication data included 15 data sets—two existing *in silico* data sets and 13 data sets for *de novo* genotyping (Table 1). The *in silico* data set from the USA came from an existing GWAS of AN genotyped using the Illumina HumanHap610 platform (Illumina, San Diego, CA, USA)[53] and the other *in silico* data set came from Estonian Genome Center (www.biobank.ee) and was genotyped using the Illumina OmniExpress array. *De novo*-genotyped samples included newly collected AN cases and controls from members of the GCAN and samples from the same sites as the discovery samples that had failed GWAS QC (including saliva and whole-genome amplified samples). *De novo* SNP genotyping was carried out using the iPLEX Gold Assay (Sequenom Inc., San Diego, CA, USA). SNPs with poor Sequenom design metrics were replaced with high-LD proxies. Sample and SNP QC were performed within each replication data set. QC included checking for sex inconsistencies and exclusions based on sample call rate $< 80\%$, SNP call rate $< 90\%$ and exact Hardy–Weinberg Equilibrium, $P < 0.0001$. In total, replication genotypes (*in silico* and *de novo*) of 76 prioritized SNPs and 27 AIMs were available from 2677 AN cases and 8629 controls of European ancestry and 458 AN cases and 421 controls from Japan.

Association analyses of prioritized SNPs were performed under an additive genetic model within each replication data set with and without adjustment for AIMs. AIMs that showed nominally significant *P*-values for allele frequency differences between *de novo*-typed cases and controls were used for conditional analysis (Supplementary Table S3). As there were no qualitative differences between these results, the main text reports the unadjusted results. The USA replication data set contained individuals who were related to individuals from the USA discovery data set. As such, those samples were excluded from the discovery data set and combined with replication USA samples to correctly account for relatedness between samples for the final global meta-analysis and sign test. Software packages GenABEL[54] and GEMMA[55] were used for replication analysis of the USA data set. Fixed-effects meta-analysis across the replication data sets was performed using GWAMA[50] (with and without adjustment for AIMs and in samples of European ancestry only, i.e., excluding Japan, also with and without adjustment for AIMs). We also performed meta-analyses across the discovery and replication data sets, comprising a total of 5551 AN cases and 21 080 controls (USA discovery samples were included only once as part of the replication phase). We calculated the power of the final global meta-analysis using QUANTO.[56]

Seventy-two independent SNPs were used to compare the direction of effects between the discovery and replication meta-analyses using R.[57] For this analysis, the USA samples were used only once as part of the replication meta-analysis.

Additional analyses

We performed three additional analyses: a genome-wide complex trait analysis designed to estimate the proportion of phenotypic variance explained by genome-wide SNPs for complex traits,[58] a network analysis and a gene-based association test (Supplementary Information).

AN subtype analyses

Two subtype (Supplementary Information) association analyses were performed for the 76 prioritized SNPs across the discovery and replication data sets (Table 1). In total, the AN restricting subtype global meta-analysis included 1606 cases and the AN binge–purge subtype analysis included 1445 cases. Both analyses used the same set of 16 303 controls (Supplementary Information).

Related traits

Using the discovery meta-analysis, we investigated evidence for association using SNP results from published studies: 9 SNPs with nominal evidence of association with AN,[42] 14 SNPs suggestively associated with eating disorder-related symptoms, behaviors or personality traits,[59,60] 89 SNPs with genome-wide significance in studies of BMI or obesity,[61,62] and 15 SNPs related to morbid obesity.[61] We also investigated evidence for association across the 72 replication SNPs using published GWAS results from the Psychiatric Genomics Consortium (https://pgc.unc.edu) for attention-deficit hyperactivity disorder (ADHD), schizophrenia, bipolar disorder and major depressive disorder.[63–66]

Expression studies

We prioritized the top 20 SNPs in terms of statistical significance and quantified the expression of the two nearest genes per SNP (Supplementary Table S4) in 12 inbred strains of mice. We obtained publicly available RNAseq data from whole-brain tissue samples and used standard software to map and count the sequence reads (Supplementary Information).

Table 2. Global meta-analysis results of SNPs with the greatest evidence of association for the main anorexia nervosa (AN) case-control analysis

SNP information						*Global meta-analysis across discovery and replication data sets*							
CHR	*POS*	*MARKER*	*NEAREST GENE*	*EA*	*NEA*	*EAF*	*OR*	*OR_95L*	*OR_95U*	P	I^2	*N_st*	*N_sa*
3	182794261	rs9839776	*SOX2OT*	T	C	0.270	1.158	1.095	1.225	3.01E−07	0	27	21857
4	102267099	rs17030795	*PPP3CA*	G	A	0.192	1.149	1.082	1.220	5.84E−06	0	24	23111
8	19584542	rs11204064	*CSGALNACT1*	G	A	0.477	1.118	1.063	1.176	1.57E−05	0.008	28	21477
3	12013264	rs2618405	7.5 kb from *SYN2*	C	A	0.218	1.152	1.079	1.229	2.03E−05	0.244	22	18566
13	23433988	rs1886797	18 kb from *SPATA13*	T	C	0.301	1.133	1.070	1.200	2.18E−05	0.317	25	15827
21	21257379	rs10482915	35 kb from *NCAM2*	A	G	0.074	1.193	1.097	1.297	3.96E−05	0	28	26164
7	106473684	rs2395833	*PRKAR2B*	T	G	0.334	1.101	1.051	1.154	5.62E−05	0.132	29	26511
2	80768625	rs1370339	39 kb from *CTNNA2*	C	T	0.472	1.098	1.049	1.149	5.68E−05	0	29	26508
13	63470128	rs9539891	255 kb from *OR7E156P*	C	T	0.332	0.891	0.842	0.942	5.88E−05	0	23	20389
2	225017222	rs1523921	26 kb from *CUL3* / 42 kb from *FAM124B*	T	C	0.210	1.131	1.065	1.201	5.95E−05	0.162	26	21858
19	11650015	rs206863	*ZNF833P*	A	G	0.899	0.864	0.804	0.928	6.47E−05	0.076	28	26402
23	107578961	rs5929098	*COL4A5*	T	C	0.771	1.135	1.066	1.210	8.37E−05	0.002	29	19249
7	146565029	rs6943628	*CNTNAP2*	A	G	0.097	1.161	1.077	1.251	9.38E−05	0	29	26377

Abbreviations: CHR, chromosome; POS, position in hg18; EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; OR, odds ratio; OR_95L, lower 95% confidence interval; OR_95U, upper 95% confidence interval; P, *P*-value; I^2, measure of heterogeneity; N_st, number of contributing studies; N_sa, number of contributing samples.

RESULTS

Main association results

Of 1 185 559 imputed SNPs that passed QC, 287 showed evidence for association in the discovery stage with $P < 10^{-4}$. These variants represented 66 independent signals and had frequencies and effect sizes commensurate with observations in other common complex diseases. One variant, not surrounded by other SNPs achieving low p-values and for which genotypes were only available in two of the 15 initial study groups, surpassed genome-wide significance (rs4957798, $P = 1.67 \times 10^{-12}$) but was not subsequently replicated in the global meta-analysis across discovery and replication samples. The overall genomic control inflation factor was 1.03 (Supplementary Figures S3 and S4). Seventy-six SNPs (of which 72 were independent) were prioritized for follow-up through *in silico* and *de novo* replication (Supplementary Table S5). Nine SNPs showed association with $P < 0.05$ (minimum *p*-value was 0.003) in the replication data set meta-analysis (binomial $P = 0.0135$) (Supplementary Table S5). On the basis of 72 independent SNPs taken forward, we would expect $0.05 \times 72 = 3.6$ SNPs to reach $P = 0.05$ by chance. The 0.0135 *P*-value reflects this enrichment in signal. No signals surpassed genome-wide significance ($P = 5 \times 10^{-8}$) in the final global meta-analysis across all discovery and replication samples (Supplementary Table S5) or in the AN subtype analyses (Supplementary Tables S6-S7).

Of critical importance, we observed significant evidence of SNP effect sizes in the replication data in the same direction as the discovery set (55/72 signals, sign test binomial $P = 4 \times 10^{-6}$). This enrichment was also observed for the AN restricting (58/72, $P = 8 \times 10^{-8}$) and binge–purge (56/72, $P = 1 \times 10^{-6}$) subtype analyses. These results strongly indicate that the prioritized set of variants is likely to contain true positive signals for AN but that the current sample size is insufficient to detect these effects.

Our analysis revealed two notable variants: rs9839776 ($P = 3.01 \times 10^{-7}$) in *SOX2OT* (SOX2 overlapping transcript) and rs17030795 ($P = 5.84 \times 10^{-6}$) in *PPP3CA* (protein phosphatase 3, catalytic subunit, alpha isozyme) (Table 2). Two additional signals emerged from the analysis focused on European replication samples only: rs1523921 ($P = 5.76 \times 10^{-6}$) located between *CUL3* (cullin 3) and *FAM124B* (family with sequence similarity 124B) and rs1886797 ($P = 8.05 \times 10^{-6}$) located 18 kb from *SPATA13* (spermatogenesis associated 13) (Supplementary Table S5). Four signals were in neurodevelopmental genes regulating synapse and neuronal network formation (*SYN2, NCAM2, CNTNAP2* and *CTNNA2;* Table 2).

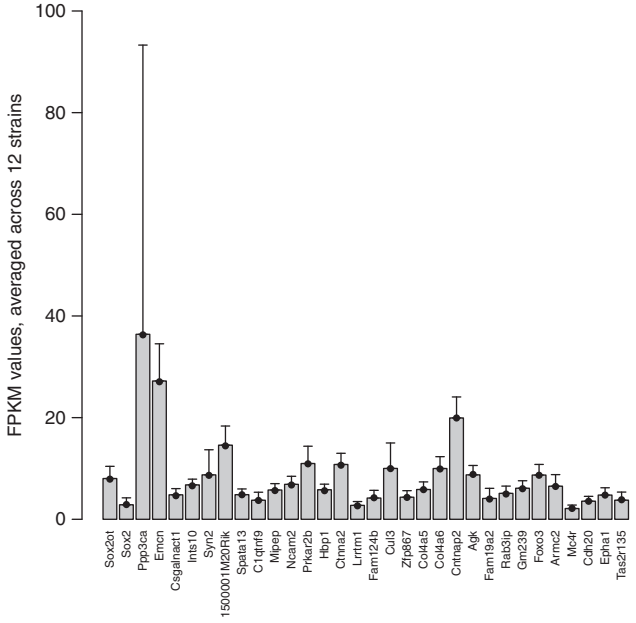

Figure 1. Analysis of RNAseq data for whole-brain tissue obtained from 12 different mouse strains for 32 mouse orthologs of the 34 human genes for which association to anorexia nervosa (AN) was identified. The average FPKM (Fragments Per Kilobase of exon per Million fragments mapped) values for 32 genes across 12 mouse strains are shown.

AN subtype analyses

In the AN restricting subtype analyses, the two most significant signals were rs1523921 (as in the main analysis, $P = 8.39 \times 10^{-5}$) and rs10777211 ($P = 8.95 \times 10^{-5}$) located 333 kb from *ATP2B1* (ATPase, calcium transporting, plasma membrane 1), both detected in the European-only analysis (Supplementary Table S6). The most significant result for AN binge–purge analysis was rs9839776 (as in the main analysis, $P = 3.97 \times 10^{-4}$) in *SOX2OT*, also in Europeans only (Supplementary Table S7). Overall, signals from the main AN case-control analysis display similar levels of association across both AN subtypes (Supplementary Table S8).

Additional analyses

Genome-wide complex trait analysis is technically challenging when synthesizing data across multiple strata with small individual sample sizes. When we applied it to our data, we saw great variability in the estimates of variance and did not judge the results reliable. Results of the gene-based association test and network analysis are presented in their entirety in Supplementary Information and Supplementary Figure S5, both of which were unremarkable.

Related traits

Nine out of the 11 previously reported variants suggestively associated with AN[42] were found in our discovery meta-analysis, and six of these nine SNPs had the same direction of effect as originally reported ($P = 0.508$) (Supplementary Table S9). Twelve out of 14 variants previously reported to be associated with eating disorder-related symptoms, behaviors and personality traits[59,60] were found in our discovery meta-analysis and seven had the same direction of effect ($P = 0.774$) (Supplementary Table S10), with one SNP (inside *RUFY1*) having $P < 0.05$ (binomial $P = 0.459$). We did not find evidence for signal enrichment in the 60 independent SNPs found in the Psychiatric Genomics Consortium data for ADHD, schizophrenia, bipolar disorder or major depressive disorder[63–66] (Supplementary Table S11).

When we compared 76 (53 independent) SNPs from the AN results with 89 established BMI/obesity SNPs,[61,62] five SNPs (inside *NEGR1*, *PTBP2*, *TMEM18*, *FTO* and *MC4R*) had $P < 0.05$ (binomial $P = 0.1906$). Twenty-six of these 53 SNPs had the same direction of effect as originally reported (binomial *P*-value = 1) (Supplementary Table S12). Thirteen of 15 SNPs associated with extreme obesity were extracted from our data set and nine of these were independent. Four of these nine SNPs had the same direction of effect as originally reported (binomial *P*-value = 1) (Supplementary Table S13). Three SNPs (in *TMEM18*, *FTO* and *MC4R*) had $P < 0.05$ (binomial *P*-value = 0.0084), indicating modest enrichment of nominally associated SNPs from extreme obesity in our discovery data set.

Expression studies

We analyzed RNAseq data for whole-brain tissue obtained from 12 different mouse strains (Figure 1). We performed this analysis for 32 mouse orthologs of the 34 human genes identified (Supplementary Table S4). All 32 genes were expressed in the brain, above an average of two FPKM (Fragments Per Kilobase of exon per Million fragments mapped). Specifically, we found extremely high expression levels for *Ppp3ca* (FPKM value 36.40). Further, we found high expression for *Sox2ot,* with an FPKM value of 8.02, and similar expression values for *Cul3* (10.01) and *Ctnna2* (10.79).

DISCUSSION

Given that the evidence base for the treatment of AN remains weak and that no effective medications for its treatment exist,[20,67] advances in our understanding of the underlying biology of the disorder are essential in order to develop novel therapeutics and to reduce the loss of life and diminution of quality of life associated with the disorder. The GCAN/WTCCC3 investigation represents an unprecedented international genetic collaboration in the study of AN, which sets the foundation for further genetic studies.

Our final global meta-analysis had 80% power to detect SNPs with allele frequency of 0.35 and genotypic relative risk of 1.15 ($\alpha = 5 \times 10^{-8}$, additive model).[68] The AN subtype meta-analysis had 80% power to detect SNPs with allele frequency of 0.35 and genotypic relative risk 1.27 for the AN restricting subtype and 1.28 for the AN binge–purge subtype. Given these limitations in power, our strongest indicator that larger sample sizes could detect genetic variants associated with AN was revealed in the sign tests. The strong and significant evidence for SNP effect sizes in the same direction between discovery and replication sets ($P = 4 \times 10^{-6}$) clearly suggests that larger sample sizes could successfully identify variants associated with AN and with the AN subtypes potentially enabling differentiation on a genetic level between restricting and binge–purge subtypes.

Several genetic variants were suggestively associated with AN ($P < 10^{-5}$) (Table 2). Two variants, rs9839776 in *SOX2OT* and rs17030795 in *PPP3CA*, were identified through analysis of all discovery and replication data sets. Two additional variants with $P < 10^{-5}$, rs1523921 located between *CUL3* and *FAM124B* and rs1886797 located near *SPATA13*, were identified through analysis of individuals of European descent only (Supplementary Table S5), suggesting either heterogeneity in the effects of these SNPs by ancestry or low power. The genes displayed in Table 2 are discussed in greater detail in the Supplementary Information; however, we highlight that four of these variants are neurodevelopmental genes that regulate synapse and neuronal network formation (*SYN2, NCAM2, CNTNAP2* and *CTNNA2)* and two have been associated with Alzheimer's disease *(SOX2OT* and *PPP3CA)*. In addition, one of our prioritized SNPs (rs6558000) (Supplementary Table S5) is located in close vicinity (9 kb upstream) of the *EPHX2* gene that was recently identified as a susceptibility locus to AN through candidate gene sequencing study of early-onset severe AN cases and controls.[43]

Our expression studies further extend the GWAS findings. It is reasonable, although perhaps not essential, to expect that genes implicated in AN be expressed in the brain. Supporting this assumption, 32 mouse orthologs of 34 human genes identified as being of interest were expressed at least at a low level in mouse brain. Moreover, genes corresponding to the more strongly associated genetic variants tended to be more highly expressed. For example, high FPKM values for *Ppp3ca, Cul3* and *Sox2ot* underscore that these genes may have a neuropsychiatric role.

AN subtype analyses were included to determine whether differences might exist between the classic restricting subtype of AN and the subtype marked by dysregulation characterized by binge eating and/or purging behavior. These analyses had lower power due to the smaller sample sizes. Only two SNPs, rs1523921 (also found to be suggestively associated in the main case-control analysis) and rs10777211 located 333 kb upstream of *ATP2B1*, showed association at the 10^{-5} significance level (Supplementary Table S6). Similarly, subsequent analyses pertaining to associated phenotypes (weight regulation: BMI/obesity loci,[40,61,69,70] and loci for extreme obesity;[61,71,72] psychiatric comorbidities: ADHD, schizophrenia, bipolar disorder and major depressive disorder) or previous equivocal association findings for AN or eating disorders (AN variants,[42] eating disorder-related symptoms, behaviors and personality trait variants[59,60]) did not reveal significant findings. More adequately powered analyses that could allow us to detect variants that can distinguish between these two subtypes could be clinically meaningful in predicting clinical course and outcome and eventually in designing targeted therapeutics.

Our understanding of the fundamental genetic architectures of complex medical diseases and psychiatric disorders has expanded rapidly.[73] It has also become manifestly clear that genomic searches for common variation via GWAS can successfully uncover biological pathways of etiological relevance. The major limitation to discovery is sample size.[74] A recent GWAS for schizophrenia reported the identification of 22 genome-wide significant loci for schizophrenia (21 000 cases and 38 000 controls), and the results yielded multiple themes of clear biological and translational significance (e.g., calcium biology and miR-137 regulation).[75] Moreover, given that cases and controls were derived from multiple sources and genotyped on multiple platforms, imputation was essential. Although effective, the preferred approach will always be to have samples genotyped on the same platform to maximize comparability and the capacity to identify genomic associations.

Although the underlying biology of AN remains incompletely understood, the relative homogeneity of the phenotype, replicated heritability estimates and encouraging results of the sign tests presented herein strongly encourage continuing this path of discovery. Phenotypic refinement and the identification of biomarkers of illness (independent of biomarkers of starvation) could assist with identification of risk loci. We believe that the surest and fastest path to fundamental etiological knowledge about the biological basis of AN is via GWAS in larger samples.[74] This path is notably safe given that it relies on off-the-shelf technology whose utility has been proven in empirical results for multiple biomedical and psychiatric disorders. This approach is cost-effective due to recent sharp decreases in genotyping pricing. Therefore, we believe that accrual of large genotyped AN case-control samples should be an immediate priority for the field.

CONFLICT OF INTEREST

Patrick F Sullivan was on the SAB of Expression Analysis (Durham, NC, USA). Cynthia Bulik was a consultant for Shire Pharmaceuticals at the time the manuscript was written. Federica Tozzi was a full-time employee of GSK at the time when the study was performed. David A Collier was employed by Eli Lilly, UK for a portion of the time that this study was performed. James L Kennedy has received honoraria from Eli Lilly and Roche. Robert D Levitan has received honorarium from Astra-Zeneca. The remaining authors declare no conflict of interest.

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APPENDIX

WELLCOME TRUST SANGER INSTITUTE: THE WTCCC3

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