Overview of Genetic Research in Anorexia Nervosa: The Past, the Present and the Future

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ABSTRACT

Background: Even though the evidence supporting the presence of a heritable component in the aetiology of anorexia nervosa (AN) is strong, the underlying genetic mechanisms remain poorly understood. The recent publication of a genome-wide association study (GWAS) of AN (Boraska, Mol Psychiatry, 2014) was an important step in genetic research in AN.

Objective: To briefly sum up strengths and weaknesses of candidate-gene and genome-wide approaches, to discuss the genome-wide association studies of AN and to make predictions about the genetic architecture of AN by comparing it to that of schizophrenia (since the diseases share some similarities and genetic research in schizophrenia is more advanced).

Method: Descriptive literature review.

Results: Despite remarkable efforts, the gene-association studies in AN did not advance our knowledge as much as had been hoped, although some results still await replication.

Discussion: Continuous effort of participants, clinicians and researchers remains necessary to ensure that genetic research in AN follows a similarly successful path as in schizophrenia. Identification of genetic susceptibility loci provides a basis for follow-up studies. © 2015 Wiley Periodicals, Inc.

Keywords: genome-wide association; anorexia nervosa; genetic overlap between disorders; genetic architecture; heritability; candidate gene

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Introduction

Unravelling of the background of polygenic psychiatric disorders, such as AN, turned out to be more challenging than it had been envisioned in the early days of the genetic research in psychiatry. Nevertheless, the recent advancements in schizophrenia's (SCZ) research set an encouraging example. The present article overviews the past approaches to unravel the genetics underlying AN and discusses the current knowledge about the genetic architecture of AN by relating it to a field of psychiatry with more advanced research in genetics, i.e., SCZ. This discussion is particularly timely, as the largest genome-wide association study (GWAS) of AN to date has been published recently,[1] and the need for evaluation of past, present and future approaches is evident.

Heritability and Rationale for Gene-Association Studies

Several lines of evidence suggest that there is a substantial genetic component in the aetiology of AN. AN has been observed across many cultures.[2] Strong familiar aggregation of AN has been documented (relative risk of 11.3 in first-degree relatives of cases with AN, as compared to the general population[3,4]), and the heritability (h^2) has been estimated in several twin studies and one adoption study of disordered eating symptoms.[5] These estimates range from 0.56 (95% CI, 0.00–0.87)[6] to 0.74 (95% CI: 0.35–0.95),[7] depending on the studied population, definition of AN and applied methodology. Thus, a genetic component in the liability to

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AN has been demonstrated, although interpretation of h^2 can be problematic.

h^2 is an estimate of a fraction of phenotypic variance that can be attributed to the genetic variance. These estimates are often cited in papers as a rationale for embarking on a gene-association study (in a way that a high h^2 is supposed to suggest larger contribution of genes to the aetiology of the disease[8]). However, it needs to be remembered that h^2 is a relative value. It remains valid for the population under study, at a given time; when environmental factors are altered, h^2 changes as well. For instance, in a population at risk of developing AN, such as ballet dancers,[9] where the variation in exposure to environmental risk factors is low (so the environment is more uniform), the estimate of AN's h^2 will be higher than in the general population (high h^2 does not mean that the environmental factors are less important in the aetiology). h^2 informs about the proportion of the variation in a population that is due to genetic factors, and not about the fraction of cases attributable to genetic factors.[10] The exact value of h^2 is not as important as its qualitative interpretation. The h^2 estimate provides a hint about whether there is detectable genetic variance in a given trait,[8] but it does not say anything about how many genes might be involved, what their impact might be or whether it will be easy or not to identify the underlying variants.

With the advent of genome-wide genotype data it has become possible to estimate how much of the genetic variance is explained solely by SNPs captured on genotyping microarrays.[11] This so-called SNP-heritability is more informative in terms of the genetic architecture of a disease/trait than the h^2 calculated from the family studies. Unfortunately, to date it has not been possible to generate such an estimate for AN.

Nevertheless, aggregated evidence coming from several lines of research hints that genetic factors are pivotal in the aetiology of AN. No monogenic forms of AN have been found and the data suggest that the genetic underpinning of AN is multifactorial (i.e., multiple genetic variants with small effects, rather than one or a few potent variants, working in concert with environmental factors).[12] Two types of studies have been employed in search for those genetic factors.

The linkage approach, which investigates co-segregation of genetic regions with the disease status in large families, has been successful in detecting rare and very potent genetic variants involved in aetiology of single-gene disorders (Mendelian), e.g., cystic fibrosis or Huntington's disease.[13,14] However, its usefulness in unravelling common variants of small effects in complex, polygenic diseases or traits remains very limited.

The second category is a population-based genetic-association study, which investigates whether frequencies of certain genotypes or alleles are different between cases and controls (significant difference implies association) or if they are correlated with a quantitative trait. This approach focuses on variants with small or medium effects, in a multifactorial model. Within this category, candidate-gene studies (CGSs) look into single-nucleotide polymorphisms (SNPs) in biologically plausible genes, whereas GWAS test common SNPs distributed throughout the whole genome. Although this category of studies uses data of unrelated individuals most often, inclusion of family trios or siblings is possible, as long as the level of relatedness is known to the researcher.

Candidate Gene Approach

The candidate-gene approach in AN, much like in other psychiatric disorders, turned out to be a primarily futile effort. The scarcity of successful replications can be explained by several reasons, such as genetic differences between the discovery population and the populations in the replication attempts, or by errors and biases leading to false positive results. These potential errors include:

- Imperfect matching of cases to controls in terms of the ethnic background. Different genetic backgrounds of cases and controls (population stratification) might lead to spurious associations. The CGSs offer no ways of attenuating this risk, except for relying on declarations of participants or inclusion of ancestry-informative markers[15] (the latter, however, is a relatively recent method, and it was not commonly applied when the most of the CGSs were performed).
- Some degree of relatedness between participants may lead to spurious associations, if it is not taken into account in the analysis.
- Technical biases might occur if DNA samples of cases and controls come from different sources (blood vs. buccal swabs).
- Various techniques of genotyping are prone to error, and ideally the samples of cases and controls should be randomly distributed across plates and genotyped under the same conditions.

The winner's curse might also be partially responsible for the lack of replications. It is a

statistical phenomenon of regression to the mean—the study which is first to identify a genetic association might find a larger effect size than it is in reality. Thus, the following studies might not confirm this association, because even if it is true, its effect is likely to be smaller than in the first published study (this is also often observed in multistage GWASs), and therefore the samples that are used in the replication studies are too small to detect the effect.

Overly optimistic expectations toward the potential effects of associated alleles might have led to overestimation of statistical power (a probability of not missing a true association), and the publication pressure might have resulted in the lack of appreciation for proper adjustment of results for multiple comparisons.[16] Finally, although, the CGSs focused on genes, often the common variation within the gene or adjacent to it was not well captured, due to insufficient SNP coverage (i.e., a density of probes throughout a genetic loci was not sufficient to cover all of the common SNPs).

Retrospectively, given the complexity and redundancy of biological pathways, and in light of what is now known about the genetic architecture of psychiatric diseases, the hypotheses about which genes could potentially harbor causative mutations had small chances to be proven right. Out of hundreds of associations indicated by CGSs in biomedical research only a few were replicated in GWASs.[17] This ratio is even less favorable in the field of psychiatry. A study by Ref. 18 found lack of enrichment of association signal in a large genome-wide dataset of cases with schizophrenia and controls after analysis of 732 autosomal genes indicated in 1,374 CGSs (investigation of signal enrichment involves collective testing of a selected group of variants in an independent dataset; it has much greater power, compared to testing of individual variants).

Candidate Gene Studies in Anorexia Nervosa

Comprehensive reviews of CGSs in AN are available elsewhere.[19,20] Although the selection of candidate genes for studies of AN was based on interesting hypotheses,[21] and >200 gene-association studies were performed in the context of EDs, up to date none of the initially promising findings have been convincingly replicated in the following candidate or genome-wide studies. Meta-analyses, which summarized and weighted the evidence from multiple studies, were also disillusioning.[22–25] Also the relatively recent CGS which used the modern standards of design, quality control, and statistical significance was negative.[26] Still, there are a few findings which await replication attempts, such as rs1473473 of TPH2,[27] the 5-HTTLPR polymorphism on SLC6A4,[28] rs7180942 in NTRK3[29] and *Ala67Thr* variant in AGRP[30] (these polymorphisms were no*t* tested in two recent GWASs of AN, because they were not present on the genotyping arrays used in those studies).

In parallel to the growing disillusionment about the candidate-gene method, a new approach towards investigation of genetic associations emerged. GWAS technology is relatively recent (first GWAS dates back to 2005[31]), but it already has had significant impact on the landscape of biomedical research and resulted in progression of aetiological knowledge about diseases and traits.[32]

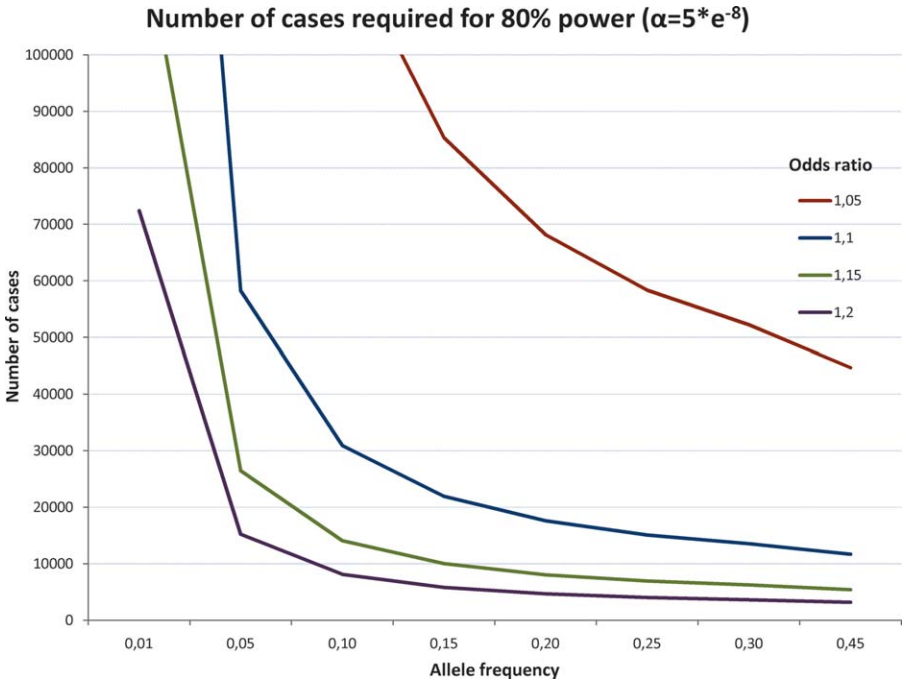
Genome-Wide Association Approach

GWAS is a hypothesis-free approach. It uses microarray platforms to examine the genotypic data from a large number of SNPs (from hundreds of thousands up to millions), which cover most of the human common SNP variation (a SNP is considered common if the frequency of its minor allele is larger than 1%). This coverage is increased via imputation—a procedure which uses statistical algorithms to infer the genotypes of the ungenotyped SNPs by employing the reference data coming from e.g., HapMap or 1000 Genomes Project populations. Genome-wide data also allows for investigation of copy number variants (CNVs; deleted or duplicated stretches of the genome).

Below is a list of the main goals of GWASs:

- Furthering the understanding of the biological mechanisms of the disease, by finding the genes and pathways involved in the aetiology. This is the foremost goal of GWASs.
- Learning about the genetic architecture. This includes the expected range of effect sizes, allelic frequencies of the associated variants, underlying genetic models (additive, dominant, recessive, overdominant, multiplicative) and the possibility of gene x environment and gene x gene interactions.
- Understanding of the genetic overlap between diseases and traits. This has a potential of enhancing the nosological system and treatment.
- Genetic screening to identify populations at risk (risk prediction) or individual genotyping of a patient to inform diagnosis and treatment (personalized medicine). As exciting as these prospects are, they are distant goals, and in view of a

FIGURE 1. Number of cases required for 80% power in relation to allele frequency, for different sizes of genetic effect, expressed in odds ratios, assuming an additive model of genetic effect. The calculation assumes 3:1 control:case ratio, 1% frequency of the disease in the general population and a genome-wide significance level ($\alpha = 5$*e-8). Calculated with Quanto 1.2.4.[37] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



highly polygenic nature of psychiatric diseases, they are unlikely to be achievable in the near future.[33]

There are many potential sources of systematic errors in these studies, much like in CGSs, but the nature of genome-wide data provides opportunity to detect such errors, and, in some cases, adjust for them. In particular, the danger of obtaining false results due to a mismatch between cases and controls (e.g., due to population stratification or a batch bias) can be identified and partially controlled for. Additionally, various quality control procedures may aid in the elimination of technical artifacts or covertly related participants. Reliability of results can be verified by post-hoc analyses, such as the LD-score regression approach.[34] Furthermore, it has become a standard for modern GWASs to include replication of top signals in independent samples.

Large numbers of statistical tests being carried out in GWASs mean that many of them will reject the null hypothesis due to chance. For example, for 500 k independent tests for association, 25 k tests will achieve significance of $p < 0.05$, even if they are not associated (leading to a type-I, false positive error). Alpha needs to be adjusted for multiple comparisons—a level of significance accepted as reliable in GWASs with ~1 million independent tests is $p < 5 \times 10^{-8}$. As a consequence, a true signal of association might be obscured in the midst of statistical noise (a type-II error—false-negative), especially since the effects of true associations in the studies of complex phenotypes tend to be small. Odds ratios (OR) are commonly used as a measure of effect size in GWASs of binary phenotypes. For example, ORs of SNPs associated with schizophrenia (SCZ) are typically in a range between 1.05 and 1.2 (reciprocally −0.83 to 0.95), with single SNPs explaining from 0.05% (SNP rs1344706, nearest gene *Znf804A*) to 0.67% (SNP rs7341475, nearest gene *RELN*) variance in the disease (the amount of explained variance depends on SNPs effect size and allelic frequency in population).[35,36] The way to avoid the risk of false-negative errors in GWASs is increasing the sample size.

The statistical power of a test informs about the likelihood of the type-II error (i.e., missing a true association). It is a function of the sample size, expected effect size, and frequency of the tested allele in the sample. It also depends on

assumptions about the genetic model underlying the association, and on the fact whether the tested variant is assumed to be causal or rather correlated with the causal variant.

The power estimations in the earlier studies were often overly optimistic, as they were based on the hope of finding moderate-to-large effects (i.e., OR > 1.25). If SNPs of common frequency and with moderate-to-large effects were to exist in AN, they would have likely been detected by now. Nowadays, it is known that the majority or all of the associated variants with common allelic frequencies will have small effect sizes. With the benefit of hindsight, it is clear that carrying-out an underpowered study or not including an independent replication is likely to lead to unreliable results, and, in fact, power as high as 90% is recommended.[32] This means that in the field of psychiatry tens of thousands of cases are necessary for a GWAS to succeed (Fig. 1).

What needs to be remembered when interpreting a GWAS is that its results inform about association but do not determine causality, and that a statistical strength of association at a given locus should not be confused with its biological relevance (the most significant finding in GWASs might not be the most informative).

Genome-Wide Association Studies in Anorexia Nervosa

The first genome-wide study of AN was published in 2009.[38] It was based on a DNA-pooling approach, in which allele frequencies were estimated from pools of DNA of cases vs. controls (in contrast to classical individual genotyping in cases and controls). The authors used a set of microsatellite markers to search for genetic loci associated with AN in groups of Japanese cases and controls. This method has several disadvantages.[39] Most importantly, it does not provide a way to guard against population stratification and does not allow inclusion of covariates. The analysis of microsatellite markers led to identification of 10 potentially associated loci. The second stage of the study was a single SNP fine-mapping analysis of indicated regions (96.6% of 331 cases with AN overlapped with the first stage of the study), which found a SNP rs2048332 (downstream of SPATA17 gene) to be most strongly associated with AN (p values = 0.0001). However, these interesting results need to be viewed in the context of several limitations, such as using a technique which is prone to errors (DNA-pooling), small sample sizes in both stages of the study and lack of control for population stratification and cryptic relatedness (i.e., relatedness between participants unknown to the researcher and not taken into account in the analysis). The implication of SPATA17 was not replicated by two later GWA studies in AN, but the most significant SNP from the study of Nakabayashi et al. was not present on their genotyping microarrays (also, those studies were performed on participants with European descent and they used different methodology).

In 2011 Wang et al. published a GWAS of a sample of 1,033 cases with AN (98% female) and 3,733 pediatric controls (46% females).[40] In retrospect, the fact that this study found no genome-wide significant associations comes as no surprise. Underpowered as for the current standards, it was unable to detect associations with small effect sizes. With this sample size, the study had 80% power to detect an association of a SNP with minor allele frequency of 10% and OR > 1.58, at genome-wide significance, whereas the ORs typically observed in the GWASs of psychiatric disorders (with a few exceptions) are below 1.25.[32] The control group was composed of pediatric participants, which could have decreased the power even further, since some of the children might develop AN or other psychiatric disorder later in life.

Additionally, the genome-wide genotype data allowed the authors to test a hypothesis whether rare and large CNVs, which were previously associated with several psychiatric disorders,[41] associate with AN. None of those CNVs were found to associate with AN, but again, insufficient sample size was a major limitation. Even though some of those CNVs were found to have ORs ranging from 5 to 20 in SCZ,[32] their extremely low frequencies drastically decrease statistical power.

Recently, the Genetic Consortium for Anorexia Nervosa (GCAN) and the Wellcome Trust Case Control Consortium 3 performed a GWAS of AN which included 2,907 female cases with AN and 14860 mixed-sex *in silico* controls in the first stage of the study, and 2,677 cases and 8,629 controls in the replication phase.[1] *In silico*, in this context, means that the controls were not genotyped along with the cases but retrieved from the already existing databases, which reduced the overall costs of genotyping, but came at the price of difficulties in the analysis and interpretation. Cases and controls came from 14 countries and they were ancestrally matched to each other within multiple strata, to protect against population stratification. After the discovery stage of the study, 76 most promising SNPs were carried forward (prioritized) to a

replication stage in an independent sample. None of those SNPs reached genome-wide significance. The two most strongly associated variants were rs9839776 in *SOX2OT* ($p = 3.01 \times 10^{-7}$) and rs17030795 in *PPP3CA* ($p = 5.84 \times 10^{-6}$).

Subtype analyses were also carried out, since AN is categorized into AN-restricting type and AN-binging/purging type (both types are underweight and restrict calorie-intake but the former does not have binging episodes and do not engage in purging, such as vomiting or use of laxatives). The hope behind these analyses was that the benefit of increased phenotypic homogeneity of the subsets would outweigh the loss of power due to decreased sample size. Subtyping worked well in, for instance, a GWAS of ischaemic stroke.[42] In this case; however, the analyses did not reveal any variants with association signals stronger than in the main analysis. Subtype analyses might offer a promising approach, but not before the size of the sample becomes sufficient to detect small effect sizes.

Several important conclusions came from this study. A sign test was performed to determine whether alleles of SNPs present in both the discovery and the replication stage showed the same direction of effect in both stages (i.e., if they increase or decrease the risk for a given phenotype). In case of no enrichment of association signal among the prioritized SNPs, 50% of considered SNPs should have the same direction of effect in both stages. It was demonstrated via a sign test that there was a true signal of association among the 76 prioritized SNPs, but the statistical power of the study was insufficient to detect it on a level of individual SNPs. This means that the effect sizes of truly associated SNPs are expected to be small. Nevertheless, the term "suggestive association of a SNP" in an underpowered GWAS should be taken with great caution. Not only does low power reduce the chances of discovering a true effect but it also decreases the likelihood that a nominally significant finding reflects a true association.[43] Nine variants with the lowest *p* values found in Wang et al. and 12 variants from the studies of ED-related traits[44] were tested in this GWAS, but they showed no evidence of association (individually, or collectively via a sign test). The findings reported in those earlier studies were all below genome-wide significance.

Another observation concerned the fact that 60 SNPs associated with four psychiatric disorders (SCZ, attention deficit hyperactivity disorder (ADHD), bipolar disorder (BD) and major depressive disorder (MDD)) showed no enrichment of the association signal in the genome-wide data of cases with AN and controls.[1] Similarly, there was no enrichment of the signal for 89 SNPs known to associate with BMI in the general population.[45,46] Interestingly, evidence of slight enrichment was found for 13 SNPs previously associated with morbid obesity,[45] which suggests some overlap in the genetic aetiology of AN and the opposite, extreme end of the BMI spectrum (but not the non-extreme part of this spectrum).

These analyses provide no evidence for genetic overlap between determinants of AN and other psychiatric disorders or AN and BMI (in a normal range), but they were not sufficient to claim that such overlap does not exist at the level of common variation. More comprehensive ways of testing for genetic overlap will become possible when much larger sets of genome-wide data of individuals with AN become available.

To sum up, the outcomes of this largest up-to-date GWAS in AN were only modestly informative. However, the success of a genome-wide approach in a given disease cannot be judged fairly as long as the sample size is not sufficiently large.[47]

Recent Progress of Gene-Association Studies in Schizophrenia

GWAS technology is relatively recent (the first GWAS dates back to 2005[31]), but it already has had a tremendous impact on the landscape of biomedical research.[32] For instance, the identification of inflammatory pathway regulation in macular degeneration[31] or autophagy pathway in inflammatory bowel disease[48] are examples of how GWASs implicated unexpected biological mechanisms in the aetiology of diseases. In psychiatric disorders, the genetics of SCZ is arguably the most advanced field. By referring to this encouraging example, we intend to discuss what the future of the genetic studies in AN might be.

The two recent GWASs of SCZ have led to remarkable progress in the understanding of the genetics of SCZ.[49,50] A study from 2013, which combined the data of 21 k cases and 38 k controls, reported twenty-two robust association signals. These findings gave basis to follow-up analyses, where a gene or a pathway became a unit of analysis, rather than individual SNPs. Although a gene-association study does not determine causality, it can indicate it, especially when the number of independently associated SNPs becomes larger. Four main implications about the aetiology of SCZ came from this study and they pertain to calcium channel genes (also implied in autistic spectrum

disorder—ASD, and BD[49]), to the major histocompatibility complex region of the genome (MHC, fundamental for the immune system), to the mRNA-137 pathway and to the long intergenic non-coding RNAs. It is a good example of how a successful GWAS advances biological knowledge and generates targets for subsequent studies. This was possible even though the 22 significant association signals constitute only a tiny fraction of the population of independent SNPs expected to be associated with SCZ.

Using these genome-wide data, Ripke et al. performed analyses which delineated the genetic architecture of SCZ. By analyzing the genetic similarities between case–case, case–control, and control–control pairs (testing whether cases are genetically more similar to each other than to controls[51]) the authors were able to estimate the SNP-heritability of SCZ (i.e., a portion of heritability explained solely by SNPs) to be 32%. They also report that about 80% of all SNPs associated with SCZ are expected to have a frequency larger than 1%.[11] An alternative method[52] produced a slightly higher estimate of SNP-heritability and indicated that 8,300 SNPs (6,300–10,200 95% CI) contribute to the genetic underpinnings of SCZ. This method also projected that for 60 k cases and 60 k controls about 794 independent SNP associations would be expected. These analyses should be interpreted with caution, as they rely on various assumptions—their exact numerical output is not as important as the conclusion that a substantial proportion of the genetic heritability to SCZ is explained by thousands of SNPs with small effect sizes and predominantly in the range of common frequency.

The most recent GWAS of SCZ corresponds with the findings of the study discussed above.[50] In an unprecedented effort to analyze the data of almost 37 k cases and over 113 k controls, the authors confirm that increasing the sample size leads to new findings (128 independent associations, ascribed to 108 genetic loci, 83 of which reported for the first time in this study). Some of the associations point toward genes and regions previously implicated in GWAS and rare-variant studies (voltage-gated calcium channel subunits, MHC, genes involved in glutamatergic neurotransmission and synaptic plasticity[49]). Other findings suggest aetiological involvement of G-protein coupled receptor genes (including DRD2 gene, a target of antipsychotic medications) and genes related to other ion channels and to neurodevelopment. To some extent, the implications of these results converge with the proposed aetiological hypotheses of SCZ (although, it should be stressed again that GWAS do not determine causality).

This is still the beginning of a long way to reveal a substantial portion of SNPs associated with SCZ. Nevertheless, these encouraging results have already led to meaningful biological inferences and important follow-up studies. For instance, it has been shown that expression of the *ZNF804A* gene in the dorsolateral prefrontal cortex is dependent on the genotype of SNP rs1344706 (previously associated with SCZ).[53] Also, the availability of genome-wide data for several psychiatric diseases, including SCZ, made it possible to study their genetic overlap, which led to discovery that the genetic diathesis of SCZ have many points of convergence with ASD, BD and other psychiatric disorders.[54] Interestingly enough, a recent study found positive genetic correlation between SCZ and AN.[55]

Genetic Architecture of Anorexia Nervosa, as Compared to Schizophrenia

The genetic landscape of AN has been explored less than that of SCZ. The authors of the recent GWAS of AN attempted to estimate the SNP-heritability (similar to what Ripke et al. did in the SCZ study[49]), but the results of this analysis were not judged reliable—the *in silico* controls were matched to the cases within multiple small-sized strata, which did not provide a solid basis for this estimation.[1] Therefore, we will discuss the genetic architecture of AN based on indirect premises, such as its epidemiological characteristics, and comparisons with SCZ.

Estimates of lifetime prevalence of AN in women range from 0.9 to 2.2%,[56,57] whereas the lifetime prevalence of SCZ (for both sexes) is estimated to be between 0.4[58] and 1.6%.[59,60] Both diseases have high mortality rates. The standardized mortality ratio (SMR) is very high in AN (SMR of 6, i.e. nearly 6 times greater mortality than in the general population[61]), whereas SMR in SCZ is 2.6[62]). AN and SCZ have generally an early age of onset (median ages of onset are 15 and 22, respectively[63]), but the pubertal period appears to be pivotal in AN.[64] The affected individuals have markedly reduced fertility ratio (FR; measured as a number of children in comparison to the general population); this reduction is greater in SCZ ($FR_{men} = 0.23$ and $FR_{women} = 0.47$) than in AN ($FR_{men} = 0.54$ and $FR_{women} = 0.81$).[60] Thus, both diseases are associated with survival and reproductive disadvantage. Also, similarly to SCZ, no Mendelian forms of AN have been identified thus far (unlike ASD, Alzheimer's disease and mental retardation). Altogether,

it suggests that common variants with moderate or strong effects are unlikely to be involved in the aetiology of those disorders, because such variants are pruned out of the genetic pool due to the evolutionary pressures (purifying selection). On the other hand, common and rare variants with small effects remain nearly invisible to purifying selection (they "behave" like neutral mutations), especially in the light of the recent population expansion.[65] Their individual effects are almost negligible, but collectively such variants are responsible for a substantial portion of the risk to the disease. This model fits well with what has been observed in the GWASs of SCZ and, with less certainty due to scarcity of data, in AN. An alternative model posits that the susceptibility variants remain in the population because they have ambivalent effects on fitness (e.g., they used to increase fitness under specific environmental circumstances in the evolutionary past but are deleterious in the modern ages or they are deleterious in the affected individuals but might have beneficial effects in their unaffected relatives). This model, which relies on balancing selection, has found less empirical support (siblings of individuals affected with SCZ or AN do not seem to have increased fecundity),[60,63] but it cannot be ruled out.

It was demonstrated that de novo variants also contribute to the susceptibility to SCZ. This is known from the recent genetic studies using sequencing[66] and was also suspected on basis of the increased age of fathers of individuals with SCZ (in comparison to the general population).[67] The association between advanced paternal age and SCZ (and some other psychiatric disorders) is likely due to a higher rate of de novo mutations coming from the male germline.[68] Whether this could also be true for AN remains an open question, due to the scarcity of data. One study found a positive correlation between paternal age and disordered eating in offspring, in particular in the case of fathers older than 40 years of age,[69] which suggests a possible role of de novo mutations in the aetiology of AN. However, large-scale sequencing studies are required to verify that.

SCZ has been robustly associated with several rare, large and recurrent CNVs, which are also known to associate with other psychiatric and non-psychiatric disorders. A possible role of those CNVs in AN remains unknown. One study attempted to test some of those CNVs in AN, and found no association, but in view of the insufficient power its conclusions were limited.[40]

Another observation concerns the fact that AN is far more frequently observed in females than in males (studies report the female:male ratio of lifetime prevalence to range between 3:1[56] to more than 10:1[70]). This sexual dimorphism is stronger than in other psychiatric disorders and it might have consequences for the genetic architecture (e.g., a greater involvement of chromosome X or sex steroid-responsive genes in the aetiology).

Eating disturbance and severe emaciation are distinguishing features of AN. The hope that due to apparent involvement of food-regulatory circuitry and measurable phenotype (body weight) the complexities of genetic underpinnings of AN would be revealed easier (i.e., the effect sizes would be larger and phenotypic heterogeneity lower), than in the case of other psychiatric disorders, did not prove right. All in all, it is reasonable to suspect that on a general level, the genetic architecture of AN is not fundamentally different from that of SCZ. Thousands of independently associated SNPs with small effect sizes and from the common range of allelic frequencies, which point toward hundreds of loci, are likely to account for a sizeable part of the variance in liability to AN. It is clear by now that there are no common variants of moderate-to-large effects (should they exist, they would have been found by now—the study by Boraska et al. had 80% power to detect association with OR = 1.32 for a SNP with a minor allele frequency of 10%, at a genome-wide significant level). Associated variants are expected to be present across the whole range of allelic frequencies (de novo, rare and common). It remains unclear whether variants with very low allele frequencies might have larger effect sizes than the common variants.[35]

Additional Phenotypes and Genetic Overlap between Disorders

Various degrees of genetic overlap were demonstrated across psychiatric entities, at the levels of de novo, rare and common variation. For instance, Fromer et al.[66] showed in a sequencing study of cases with SCZ and their parents that loss-of-function de novo mutations were enriched in cases with SCZ within the group of genes implicated into aetiology of autism. Rare variants, such as CNVs, are also known to associate with several psychiatric disorders and epilepsy.[41] Purcell at al. showed that the aggregate polygenic contribution of many common alleles with small effects on the liability to SCZ (polygenic risk score) also increased the risk for BD.[71] In another study, the genome-wide data of common variants allowed for investigation and indication of shared genetic aetiology in SCZ, BD, ASD, MDD and ADHD[72] (interestingly, calcium-channel activity genes emerged again and

appeared to have pleiotropic effects on psychopathology) or in SCZ and multiple sclerosis.[73] Other study quantified the extent of the overlap in genetic variation between disorders, and showed that it was substantial between e.g., SCZ and BD or SCZ and MDD.[74] The analyses of genetic overlap between AN, other eating disorders and psychiatric disorders are likely to be performed in the future, but, for the moment, the number of genome-wide genotyped individuals with AN remains too small.

These observations support the notion that psychiatric diagnoses require revision, since they do not reflect the recent advancement of our understanding of relationships between disorders. This problem is apparent in the field of EDs.[75] In a longitudinal study, most of the patients with AN experienced diagnostic cross-over (more than half migrated between AN subtypes and 34% migrated to BN).[76] Also, eating disorder not-otherwise-specified is the most often established diagnosis in the clinical practice, meaning that the criteria for core diagnoses (AN, bulimia nervosa, binge-eating disorder) are failing at discriminating the majority of patients.[77] Psychiatric comorbidities in EDs are ubiquitous. Depression and anxiety disorders are similarly frequent across ED categories, whereas diagnoses of OCD are more frequent in AN.[78] ASD and ADHD are also observed among patients with AN.[77] The specific diagnoses within the category of EDs are likely to have highly overlapping aetiologies, and aetiological mechanisms shared with other psychiatric disorders are also plausible.[3]

There exist cross-diagnostic phenotypes that reflect the general psychopathological domains of psychiatric diseases.[66,79] Furthermore, it has already been suggested many years ago that psychiatric research and taxonomy could benefit from focusing on phenotypes which are intermediate between the genetic causes and the diagnoses (so-called endophenotypes or intermediate phenotypes (IP).[80] IP are thought to be quantitative traits, which are less complex, more accurately measured and closer to the genetic substrate than the diagnoses. In view of the substantial genetic overlap between several psychiatric disorders, it is plausible that the postulated domains of psychopathology represent such IP.

Additional phenotyping in genetic studies of psychiatric disorders can be very useful in the future research, but it has drawbacks. Positive aspects of application of valid IP in genetic research include:

- More reliable ascertainment of cases, due to higher accuracy of measurement (lesser phenotypic heterogeneity)
- Possibility of ranking of individuals within diagnoses according to the IP level
- Possibility of merging of groups of cases with different diagnoses, according to the IP level
- Possibility of inclusion of covariates in the model
- Possibility of inclusion of individuals from the general (non-clinical) population, according to the IP level
- In general, tests of quantitative traits have higher power than tests of binary traits
- Possibly, larger effect sizes of associated genetic variants
- Simpler interpretation of results in terms of mechanisms of action.

On the other hand, the negative aspects include:

- Practical difficulties with collecting large samples of individuals with IP measurement
- The increase in power from improved phenotyping (better phenotypic homogeneity) could be less than decrease in power due to a smaller sample
- Possibility of inconsistent recording of phenotypes across collaborating centers
- Some of the proposed IP in psychiatry may not be less complex than the diagnoses
- Danger of confusing biomarkers and epihenomena with IP (which are supposed to be involved in aetiology)
- It is not clear which phenotypes could be valid and useful IP of psychiatric diseases.

Type-II diabetes is an example of how an application of quantitative IP (in this case glycemic traits or BMI) complemented the typical case-control approach and led to new discoveries (e.g., association of GCK loci with fasting glucose levels).[81] To date, attempts to use suspected IP in the psychiatric genetics (either cross-diagnostically or within particular diagnoses) were less fruitful,[82] although there were some encouraging examples. For instance, a GWAS of ASD, which, besides the diagnoses, used quantitative measures of autistic symptoms, found multiple previously unknown associations.[83] In the field of SCZ, one study used a combined score of multiple variants associated or nominally associated with SCZ (polygenic risk score) and found a modest association with quantitative measures of psychosis,[84] whereas another studies used the same score and detected association with total brain and white matter volume[85] or with working memory-related prefrontal brain activation.[86] There are also examples of cross-diagnostic applications—Ruderfer et al. found an

association of a BD polygenic risk score with dimension of mania in patients with SCZ.[87] Investigation of additional phenotypes can be useful both at the genome-wide level (in the discovery phase) and in the follow-up studies of the associated variants.

Bulik et al. reviewed and evaluated subphenotypes and potential IP in AN and EDs[75] (e.g., perfectionism, cognitive set-shifting, obssessionality or impulsivity), but the current state of research does not give a clear indication about which of them is the most promising.

There are recent CGSs[88,89] and a GWAS[44,90] which investigated ED-related temperamental and psychometric phenotypes. None of them, however, found significant associations.

To date, the search for relevant IP in EDs focused on behavioral and psychometric traits. In the future, all levels of human organism functioning need to be taken into account, including the transcriptome, proteome, neuroanatomy and neurological functioning (imaging genetics). It will become easier to determine valid IP once the knowledge about the aetiological pathways and mechanisms of AN improves. Studies of physiological, neurobiological or neurocognitive IP are likely to follow successful GWAS focused on diagnostic categories.

Discussion

The early stage of GWASs in psychiatric disorders was considered largely unsuccessful. Lack of significant results came as a disappointment and criticism was raised that investing resources in GWASs does not pay off.[91] At that time, much less was known about the underlying genetic architecture of psychiatric diseases; nowadays it is clear that these studies were unlikely to yield significant findings, predominantly due to their insufficient sample sizes. As many as five GWASs in SCZ found no significant SNP associations.[32] A study which was first to report genome-wide significant findings used the data of 8,000 cases with SCZ and twice as many controls.[65] Looking back at the year 2011, the accrual of robust findings in SCZ in relation to the increasing sample was even greater than expected.[32] One of the reasons why the recent GWASs are increasingly successful (besides the massive samples sizes) is the rigorous, uniform and transparent methodology (methodological homogeneity).

The field of genetics in AN and other EDs is still in an early stage. It has the advantage of being able to learn from the more advanced fields and, thus, divert the resources for research in optimal directions. Cooperation of GCAN with the Psychiatric Genomics Consortium (PGC) creates opportunities for cross-disorder analyses and is likely to greatly increase the pace of genetic discoveries in AN. It is likely to shed light on AN's connections with other comorbid and genetically related disorders (especially that the genotyping platform which will be used in the future research—Illumina's PsychArray BeadChip—was designed for studies of psychiatric disorders). Before that comes to fruition, however, sample sizes of more than 25,000 (at minimum) individuals with AN are necessary, so that a barrier of at least several genome-wide significant SNP associations is breached. More distant goals, envisioned by PGC, include amassing of 100,000 cases per disorder of interest. Further follow-up studies of the variants identified in a GWAS should be based on statistically significant and replicated results (suggestive association in an underpowered study is not enough). At this early stage of genetic research in AN, investing effort in genome-wide genotyping of large number of individuals with AN will probably result in more insight into biology than more costly sequencing of smaller number of individuals.[35]

Substantial progress in the understanding of the genetic substrate of AN and its relations to other diseases is bound to come. Nevertheless, patience is advised and the hopes should not be inflated by unrealistic promises. Such progress will require a tremendous effort and it will not be quick. Valuable achievements rarely come easily. Continuous collection of DNA samples and unified phenotypic characterization of patients is a *conditio sine qua non*. The Psychiatric Genomics Consortium-Anorexia Nervosa Working Group (PGC_AN) is already in place, and it constitutes a framework which can expedite and organize this process. The most important and challenging aspect of this job, however, lies on the part of the patients and clinicians. We encourage them to contribute to this effort, as GWASs are an indispensable step of what has already started to change the face of the psychiatry.

Readers who would like to learn about how to contribute are advised to contact the PGC at http://www.med.unc.edu/pgc.

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