Characterization of genetic variation in the *VGLL4* gene in anorexia nervosa

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Anorexia nervosa (AN) is a chronic psychiatric disease characterized by a refusal to maintain body weight at or above 85% of that which is normal for height, body dysmorphia, and fear of gaining weight. Genetic studies have had limited success identifying risk loci and a recent genome-wide association study of 1033 AN cases and 3733 controls found no significantly associated loci; however, it identified several genes with single nucleotide polymorphisms (SNPs) of nominal significance: *AKAP6*, *FAM155A*, *LRP2*, *NTNG1*, *VGLL4*, and *ZNF804B* (Wang *et al.*, 2011). We attempted to replicate these associations in an independent cohort of 396 female AN cases of European descent (mean age = 32.4 ± 14.25) obtained from the NIMH Center for Collaborative Studies on Mental Disorders (Kaye *et al.*, 2008) and 690 age-matched and education-matched European controls (mean age = 26.34 ± 8.33). Controls were collected as part of the AN trios study, and never met the criteria for an eating disorder (Reba *et al.*, 2005) (more detailed methodology provided in online supplement, Supplemental digital content 1, *http://links.lww.com/PG/A106*). Overall, 12 SNPs were selected for genotyping, rs2383378 and rs12894779 in *AKAP6*, rs11842161 and rs4511387 in *FAM155A*, rs830998, rs830997, rs2075252, and rs4667591 in *LRP2*, rs10494067 in *NTNG1*, rs6782029 and rs2616551 in *VGLL4*, and rs6959888 in *ZNF804A*. Genotyping was performed using TaqMan genotyping assays (Applied Biosystems Inc., Foster City, California, USA) as per manufacturer protocol. χ^2-Tests of allelic association were performed using PLINK v1.04 (Purcell *et al.*, 2007) to test for allelic association with AN. rs2616551 in *VGLL4* was found to be nominally associated with AN [Minor allele frequency (MAF) in cases = 17%, controls = 21%, $\chi^2 = 4.3$, $P = 0.04$, odds ratio = 0.79]. These analyses did not correct for population stratification, however, rs2616551 was associated with AN in the genome-wide association study performed by Wang *et al.* (2011) ($P = 0.0005$, odds ratio = 0.78). Recent studies of complex genetic traits have found both common and rare genetic variation to influence liability to disease. Therefore, we performed next generation sequencing (NGS) of a 9.4 kb amplicon of *VGLL4* (capturing 80% of the coding region of *VGLL4*) with the aim of finding rare coding variation in *VGLL4* increasing risk for AN. An additional 554 AN samples (mean age = 26.2 ± 8.14) collected as part of the Price Foundation Anorexia Nervosa Affected Relative Pair dataset (Kaye *et al.*, 2000) and the AN Trios study (Reba *et al.*, 2005) were used, to provide 950 AN individuals in total for NGS. NGS was performed using SOLiD

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4 sequencing (Life Technologies, Grand Island, New York, USA) at the Penn Genome Frontiers Institute. Sequence reads were aligned to the reference sequence for *VGLL4* (build hg19) using Bowtie (version 0.12.7) (Langmead *et al.*, 2009), SAMtools (version 0.1.18), and VarScan (version 2.2.7). A total of 59 variants were identified and 40 of these were novel. Only one SNP was coding (synonymous), and therefore none of the 59 variants were genotyped in additional AN populations. This should be considered a limitation of this study as noncoding variation is known to influence disease risk. However, because of the nominal association of *VGLL4* in two independent AN cohorts, this remains a candidate gene worthy of future study in AN populations.

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Conflicts of interest

There are no conflicts of interest.

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