Title

Characterization of genetic variation in the VGLL4 gene in anorexia nervosa

Journal

Psychiatric Genetics, 24(4)

ISSN

0955-8829

Authors

Clarke, Toni-Kim
Crist, Richard C
Doyle, Glenn A
et al.

Publication Date

2014-08-01

DOI

10.1097/ypg.0000000000000040

Peer reviewed

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

Toni-Kim Clarke[a], Richard C. Crist[a], Glenn A. Doyle[a], Amy R.D. Weiss[a], Harry Brandt[c], Steve Crawford[c], Scott Crow[d], Manfred M. Fichter[q,r], Katherine A. Halmi[e], Craig Johnson[f], Allan S. Kaplan[s,t,u], Maria La Via[g], James E. Mitchell[h,i], Michael Strober[j], Alessandro Rotondo[v], Janet Treasure[w], D. Blake Woodside[s,t,u], Pamela Keel[n], Kelly L. Klump[o], Lisa Lilenfeld[p], Katherine Plotnicov[b], Pierre J. Magistretti[x], Andrew W. Bergen[k], Walter H. Kaye[l], Nicholas J. Schork[m] and Wade H. Berrettini[a]

Psychiatric Genetics 2014, **24**:183–184

[a]Department of Psychiatry, Center for Neurobiology and Behavior, University of Pennsylvania, Philadelphia, [b]Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania, [c]Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland, [d]Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota, [e]New York Presbyterian Hospital-Westchester Division, Weill Medical College of Cornell, University, White Plains, New York, [f]Eating Recovery Center, Denver, Colorado, [g]Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, [h]Neuropsychiatric Research Institute, Fargo, [i]Department of Clinical Neuroscience, University of North Dakota School of Medicine and Health Sciences, Fargo, North Dakota, [j]Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, [k]Center for Health Sciences, SRI International, Menlo Park, [l]Department of Psychiatry, University of California at San Diego, San Diego, [m]Scripps Genomic Medicine, The Scripps Research Institute, La Jolla, California, [n]Department of Psychology, Florida State University, Tallahassee, Florida, [o]Department of Psychology, Michigan State University, East Lansing, Michigan, [p]Clinical Psychology Program, American School of Professional Psychology at Argosy University, Washington, District of Columbia, USA, [q]Roseneck Hospital for Behavioral Medicine, Prien, [r]Department of Psychiatry, University of Munich (LMU), Munich, Germany, [s]Center for Addiction and Mental Health, [t]Department of Psychiatry, Toronto General Hospital, University Health Network, [u]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada, [v]Department of Psychiatry, Neurobiology, Pharmacology, and Biotechnology, University of Pisa, Pisa, Italy, [w]Department of Academic Psychiatry, Bermondsey Wing Guys Hospital, University of London, London, UK and [x]Brain Mind Institute EPFL – Lausanne and Center for Psychiatric Neuroscience, Department of Psychiatry, University of Lausanne Medical School, Lausanne, Switzerland

Correspondence to Toni-Kim Clarke, PhD, Translational Research Laboratory, University of Pennsylvania, 125 S. 31st Street, Philadelphia, PA 19104, USA
Tel: +44 131 537 6531; e-mail: toni.clarke@ed.ac.uk

Received 25 April 2013 **Revised** 19 February 2014 **Accepted** 20 April 2014

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

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (*www.psychgenetics.com*).

0955-8829 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/YPG.0000000000000040

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.

Acknowledgements

This work was supported by grants from the Klarman Family Foundation, the Price Foundation and the Hilda & Preston Davis Foundation.

The data and collection of biomaterials for the Genetics of Anorexia Nervosa study have been supported by the National Institutes of Health Grants (MH066122, MH066117, MH066145, MH066296, MH066147, MH066289, MH066193, MH066287, MH066288, MH066146).

Conflicts of interest

There are no conflicts of interest.

References

Kaye WH, Lilenfeld LR, Berrettini WH, Strober M, Devlin B, Klump KL, *et al.* (2000). A search for susceptibility loci for anorexia nervosa: methods and sample description. *Biol Psychiatry* **47**:794–803.

Kaye WH, Bulik CM, Plotnicov K, Thornton L, Devlin B, Fichter MM, *et al.* (2008). The genetics of anorexia nervosa collaborative study: methods and sample description. *Int J Eat Disord* **41**:289–300.

Langmead B, Trapnell C, Pop M, Salzberg SL (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **10**:R25.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al.* (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**:559–575.

Reba L, Thornton L, Tozzi F, Klump KL, Brandt H, Crawford S, *et al.* (2005). Relationships between features associated with vomiting in purging-type eating disorders. *Int J Eat Disord* **38**:287–294.

Wang K, Zhang H, Bloss CS, Duvvuri V, Kaye W, Schork NJ, *et al.* (2011). A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa. *Mol Psychiatry* **16**:949–959.