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Published in:
Scientific Reports

DOI:
10.1038/s41598-017-01674-8

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
A genome-wide association study of anorexia nervosa suggests a risk locus implicated in dysregulated leptin signaling

Dong Li1, Xiao Chang2, John J. Connolly1, Lifeng Tian3, Yichuan Liu4, Elizabeth J. Bhoj5, Nora Robinson1, Debra Abrams1, Yun R. Li1, Jonathan P. Bradfield1, Cecilia E. Kim1, Jin Li5, Fengxiang Wang1, James Snyder1, Maria Lemma3, Cuiping Hou1, Zhi Wei1, Yiran Guo1, Haijun Qiu1, Frank D. Mentch1, Kelly A. Thomas1, Rosetta M. Chiavacci1, Roger Cone2,5, Bingshan Li2, Patrick A. Sleiman1, Eating Disorders Working Group of the Psychiatric Genomics Consortium, Price Foundation Collaborative Group & Hakon Hakonarson1,3,4

We conducted a genome-wide association study (GWAS) of anorexia nervosa (AN) using a stringently defined phenotype. Analysis of phenotypic variability led to the identification of a specific genetic risk factor that approached genome-wide significance (rs929626 in EBF1 (Early B-Cell Factor 1); \( P = 2.04 \times 10^{-7} \); OR = 0.7; 95% confidence interval (CI) = 0.61–0.8) with independent replication (\( P = 0.04 \)), suggesting a variant-mediated dysregulation of leptin signaling may play a role in AN. Multiple SNPs in LD with the variant support the nominal association. This demonstrates that although the clinical and etiologic heterogeneity of AN is universally recognized, further careful sub-typing of cases may provide more precise genomic signals. In this study, through a refinement of the phenotype spectrum of AN, we present a replicable GWAS signal that is nominally associated with AN, highlighting a potentially important candidate locus for further investigation.

Anorexia nervosa (AN) is a complex and often chronic eating disorder characterized by inability to maintain a normal healthy body weight and a persistent fear of weight gain, resulting in extreme emaciation and even death in some cases. Previous genetic and epidemiological studies have indicated a multifactorial etiology, where both genetic and environmental factors contribute to disease risk. As sample sizes have increased, genome-wide association studies (GWASs) of AN have begun to identify risk variants. To further elucidate the genetic architecture of AN, we performed a GWAS using data from our previously published study consisting of 1,033 AN cases by excluding 212 patients with AN who experienced diagnostic crossover during the course of their illness. Specifically, we excluded patients who migrated from or to binge-eating disorder (BED) or bulimia nervosa (BN) as assessed with the Structured Interview for Anorexic and Bulimic Disorders. Although a previous study indicated women with BN were rarely to cross over to AN, we observed ~43% of AN/BN crossover cases falls into this category in our cohort, suggestive of a confounding factor. We hypothesized that this reduction in phenotypic heterogeneity, despite the fact that AN and BN may share some genetic risk factors, would enhance gene discovery.
Results

Our discovery cohort included a total of 692 female AN cases of non-Hispanic European (NHE) descent. Cases were included if they were diagnosed with restricting type and binge eating/purging type of AN as defined by DSM-IV. Both types are characterized by below-normal weight and restricted food intake. Individuals diagnosed as restricting type do not experience binge-eating episodes and do not engage in purging, such as vomiting or use of laxatives. Standard quality controls measures were applied, specifically, excluding potential cryptic relatedness and checking for population stratification (details described elsewhere). The average age of onset of the case subjects was 16.3 years with a standard deviation (SD) of 3 years (Interquartile Range; IQR = 16 (14–18)). The control group included 3,570 female matched healthy adolescents of NHE ancestry that had an average age of 18.3 years at the time of data analysis (SD = 5.7; IQR = 19 (13–23)) (Supplementary Table 1). Associations were assessed with 507,999 SNPs genotyped on either Illumina HumanHap550 or Human610-Quad BeadChips in an additive model using logistic regression analyses with principal components adjustment, based on the principal component analysis of cases and controls (Supplementary Figure 1), resulting in significantly low level of genomic control inflation factor of 1.03 (Supplementary Figure 2). The analysis yielded one SNP (rs929626) with a P value of 2.04 × 10−7 and 4 other SNPs with marginally larger P values that are in strong linkage disequilibrium (r2 > 0.8); these SNPs were selected for further analysis (Supplementary Figure 3; Supplementary Table 2).

Using imputation analysis based on data from the 1000 Genomes Project (Phase I integrated variant set, v2, March 2012), we subsequently tested associations with SNPs (imputed info > 0.5, minor allele frequency (MAF) > 0.05) located in a 200-kb window centered on the SNP rs929626. We observed association with a series of markers around this region, of which 34 SNPs supported suggestive associations ($P < 1.0 \times 10^{-4}$) with both imputed and genotyped SNPs, which were in high LD with AN (Fig. 1; Supplementary Table 3). This suggests that the single markers demonstrating association in the GWAS are part of a larger haplotype in the genome.

We further explored this finding using the meta-analysis results from 15 previously reported AN cohorts. Interestingly, two SNPs were also nominally significant (rs929626 with $P = 0.037$ and rs17543752 with $P = 0.05$) in the same direction as in the GWAS (Table 1). Meta-analysis results in a P value of 1.52 × 10−7.

We next used the ENCODE project data to predict possible functional effect of the SNPs identified in this study. The top SNP, rs929626, and other significant markers located in the 6th intron of the EBF1 gene (Early B-Cell Factor 1), as well as two SNPs (rs113252656 and rs1081071) flanking the top SNP rs929626 at r2 > 0.5 function as binding sites for EBF1 itself (HaploReg v4.1; ref. 15). This suggests that these genetic variants may modulate the expression of EBF1. Indeed, we observed a positive correlation with the rs929626 C allele carriers compared with TT homozygotes on the EBF1 expression level in nine independent subjects (the FPKM [counts per million] expression level in nine independent subjects (the FPKM expression level in nine independent subjects (the FPKM) in mice) (Supplementary Table 4; Supplementary Fig. 4). Using imputation analysis based on data from the 1000 Genomes Project (Phase I integrated variant set, v2, March 2012), we subsequently tested associations with SNPs (imputed info > 0.5, minor allele frequency (MAF) > 0.05) located in a 200-kb window centered on the SNP rs929626. We observed association with a series of markers around this region, of which 34 SNPs supported suggestive associations ($P < 1.0 \times 10^{-4}$) with both imputed and genotyped SNPs, which were in high LD with AN (Fig. 1; Supplementary Table 3). This suggests that the single markers demonstrating association in the GWAS are part of a larger haplotype in the genome.

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Discussion

$EBF1$ encodes a transcription factor that originally thought to function as necessary for the development of the immune system, but it has since been shown to regulate the development of both osteoblast and adipocyte lineages. Two $EBF1$ variants, rs11953630-T and rs9317727-T, showed significant association at genome-wide level ($P < 5 \times 10^{-8}$) in a study testing blood pressure in European whites. In addition, rs17056278-C was also identified as a metabolic risk allele, interacting with psychosocial stress to contribute to increased hip circumference ($P = 3 \times 10^{-8}$). However none of these is in LD with any markers in our identified locus. In animal studies, $Ebf1$ mice showed increased adipose tissue within marrow, whereas peripheral white adipose tissue was severely reduced. Circulating levels of leptin, a hormone released by adipocytes and one of the major players in food intake regulation, were also decreased in $Ebf1$ mice compared with controls. This concurs with the reported generalized loss of accumulation of subcutaneous and visceral adipose accompanied by significant increases in yellow marrow in AN patients. Also notable is the finding that circulating levels of leptin are very low in AN patients and a decline in levels of circulating leptin can lead to changes in brain activity in areas involved in regulatory, emotional, and cognitive control of appetite.

Understanding the genetics of AN is currently a major within-field initiative, in parallel to other neuropsychiatric/neurodevelopmental disorders such as schizophrenia, bipolar disorder, and autism spectrum disorders. Although the clinical and etiologic heterogeneity is universally recognized, in practice, many studies still failed to account for sample heterogeneity. In this study, by focusing on individuals with AN who have not crossed over to other eating disorder presentations, we have identified a marginally replicating GWAS signal that approached genome-wide significance. This would represent a conservative bias and underscores the importance of further investigation of this locus in the future focusing on individuals with lifetime AN who have never crossed over to other eating disorder presentations.

Methods

Discovery data set and quality control. We conducted a GWAS using data from our previously published study consisting of 1,033 AN cases by excluding 212 patients with AN who experienced diagnostic...
crossover during the course of their illness (i.e. migrated from or to binge-eating disorder (BED) or bulimia nervosa (BN) as assessed with the Structured Interview for Anorexic and Bulimic Disorders\textsuperscript{11}) plus 100 patients without such information. A total of 692 female AN cases and 3,570 female matched controls that were carefully selected from Center for Applied Genomics (CAG) database were included in the analysis after Standard quality controls, namely, excluding potential cryptic relatedness and checking for population stratification by using the PLINK software\textsuperscript{31} version 1.90a. The Research Ethics Board of CHOP and other participating centers approved the study. Informed consent was obtained from all adult participants and from a parent or legal guardian in the case of children and all work followed was in accordance with an IRB-approved protocol.

Association tests. For the genome-wide association analysis for SNPs, we utilized the PLINK software\textsuperscript{31} version 1.90a, through Cochran–Armitage trend test.

Expression studies. The extended locus around associated SNP was then defined by identification of all SNPs showing $r^2 > 0.5$. Linkage disequilibrium (LD) was defined with the HaploReg v4.1 (ref. 15) based on Phase I of the 1000 Genomes project. Variants showing evidence of LD with associated AN variants were explored for impact on gene function via regulatory function (including eQTLs) by HaploReg v4.1, which both collate data from the Encyclopedia of DNA Elements (ENCODE)\textsuperscript{14}. We also referred to the Genotype-Tissue Expression Portal database (http://www.gtexportal.org) for eQTLs analysis.

References


Acknowledgements

We gratefully thank all the patients and their families who were enrolled in this study, as well as all the control subjects who donated blood samples to The Children's Hospital of Philadelphia (CHOP) for genetic studies. Dong Li was funded in part by 2012–2015 Davis Foundation Postdoctoral Fellowship Program in Eating Disorders Research Award. Bingshan Li was partially supported by Klairman Family Foundation for eating disorders. All genome-wide genotyping for controls was funded by an Institute Development Award to Center for Applied Genomics from CHOP. We thank the Eating Disorders Working Group of the Psychiatric Genomics Consortium (PGC-ED) for providing summary results data for the replication analysis.

Author Contributions

D.L. and H.H. were leading contributions in the design, analysis and writing; D.L., X.C., Y.L., J.P.B. and PS contributed to data analysis. J.J.C., L.T., N.R., D.A., Y.R.L. contributed samples and phenotypes. C.E.K., J.L., F.W., J.S., M.L., C.H., Z.W., Y.G., H.Q., F.M., K.T., R.C., B.L., and R.C. provided assistance with samples and data processing. Eating Disorders Working Group of the Psychiatric Genomics Consortium and Price Foundation Collaborative Group provided data for the replication and helped with the discussion; D.L. drafted the manuscript. D.L., J.J.C., E.J.B. and H.H. revised the manuscript. All authors approved final version of manuscript.

Additional Information

Supplementary information accompanies this paper at doi:10.1038/s41598-017-01674-8

Competing Interests: The authors declare that they have no competing interests.
Consortia
Eating Disorders Working Group of the Psychiatric Genomics Consortium (PGC-ED)

Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

Department of Child and Adolescent Psychiatry, Institute of Psychiatry and Neurology, Warsaw, Poland

Department of Child and Adolescent Psychiatry, Department of Psychiatry, Poznan University of Medical Sciences, Poznan, Poland

Hjelt Institute, University of Helsinki, Helsinki, Finland

Institute of Molecular Medicine, University of Helsinki, Helsinki, Finland

Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland

Department of Adolescent Psychiatry, Helsinki University Central Hospital, Helsinki, Finland

Center for Eating Disorders Ursula, Leiden, The Netherlands

Leiden University Medical Centre, Department of Psychiatry, Leiden, The Netherlands

Leiden University Medical Centre, Molecular Epidemiology Section (Department of Medical Statistics), Leiden, The Netherlands

Department of Psychiatry, McLean Hospital/Harvard Medical School, Belmont, MA, USA

Department of Genetics, Environment and Mental Health, Norwegian Institute of Public Health, Oslo, Norway

Institute of Clinical Medicine, University of Oslo, Oslo, Norway

Department of Psychiatry, University of Naples SUN, Naples, Italy

Chair of Psychiatry, University of Salerno, Salerno, Italy

Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada

Department of Psychiatry, University of Toronto, Toronto, Canada

Eating Disorders Unit, Department of Child and Adolescent Psychiatry, Medical University of Vienna, Vienna, Austria

Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

Department of Molecular and Experimental Medicine and The Scripps Translational Science Institute, The Scripps Research Institute, La Jolla, CA, USA

Department of Psychosomatic Research, National Institute of Mental Health, NNCNP, Tokyo, Japan

Department of Molecular Life Sciences, Tokai University School of Medicine, Kanagawa, Japan

Estonian Genome Center, University of Tartu, Tartu, Estonia

Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia

Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Seattle University College of Nursing, Seattle, WA, USA

Kartini Clinic, Portland, OR, USA

Centre de Psychiatrie et Neurosciences – Inserm U894, Paris, France

Department of Genetics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King’s College London, London, UK

UCL Genetics Institute, Department of Genetics, Evolution and Environment, University College London, London, UK

Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Clinics RWTH Aachen, Aachen, Germany

Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Charité, Berlin, Germany

Department of Psychosomatic Medicine and Psychotherapy, Hannover Medical School, Hannover, Germany

Department of Psychosomatic Medicine and Psychotherapy, University of Erlangen-Nuremberg, Erlangen, Germany

Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Würzburg, Würzburg, Germany

Department of Child and Adolescent Psychiatry, University Hospital Carl Gustav Carus, Dresden University of Technology, Dresden, Germany

Massachusetts General Hospital/Harvard Medical School, Athinoula A. Martinos Center for Biomedical Imaging, Psychiatric Neuroimaging Research Program, Charlestown, MA, USA

Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Clinics RWTH Aachen, Aachen, Germany

Departments of Psychosocial and Internal Medicine, Heidelberg University, Heidelberg, Germany

Parklandklinik, Bad Wildungen, Germany

Institute for Medical Informatics, Biometry and Epidemiology, Universitätssklinikum Essen, University of Duisburg-Essen, Essen, Germany

Department of Internal Medicine VI, Psychosomatic Medicine and Psychotherapy, University Medical Hospital Tübingen, Tübingen, Germany

Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

Center for Neurobehavioral Genetics, University of California, Los Angeles, Los Angeles, CA, USA

Brain Center Rudolf Magnus, Department of Psychiatry, University Medical Center Utrecht, The Netherlands

Department of Social Sciences, Utrecht University, Utrecht, The Netherlands

Clinical Genetics Unit, Department of Woman and Child Health, University of Padova, Padova, Italy

M. Skłodowska-Curie Cancer Center and Institute of Oncology, Warsaw, Poland

Department of Epidemiology, Institute of Occupational Medicine, Department of Epidemiology, Lodz, Poland

Department of Clinical Nutrition, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

Netherlands Consortium for Healthy Ageing, Leiden University Medical Center, The Netherlands

Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
First Department of Psychiatry, Athens University Medical School, Athens, Greece
Eating Disorders Unit, 1st Department of Psychiatry, Athens University Medical School, Athens, Greece
Adolescent Health Unit (AHU), 2nd Department of Pediatrics – Medical School, University of Athens ‘P & A Kyriakou’ Children's Hospital, Athens, Greece
Department of Psychiatry, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
Department of Pediatrics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
Institute of Human Genetics, Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany
Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany
Division of Medical Genetics, Department of Biomedicine, University of Basel, Basel, Switzerland
Martin-Luther-Universität Halle-Wittenberg, Klinikum der Medizinischen Fakultät, Halle/Saale, Germany
Institute of Clinical Molecular Biology, University of Kiel, Kiel, Germany
Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University, Munich, Germany
CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France
McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada
Division of Nephrology, Department of Internal Medicine and Medical Specialties, Columbus-Genelly Hospitals, Catholic University, Rome, Italy
Unitat de Recerca de Reumatologia (URR), Institut de Recerca Hospital Universitari Vall d’Hebron, Barcelona, Spain
Genetic Epidemiology Group, International Agency for Research on Cancer (IARC), Lyon, France
Virginia Institute for Psychiatric and Behavioral Genetics, Department of Psychiatry, Virginia Commonwealth University, Virginia, VA, USA
The Finnish Institute of Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland
The Program for Human and Population Genetics, The Broad Institute of MIT and Harvard, Cambridge, MA, USA
Finnish Institute of Occupational Health, Province of Southern Finland, Helsinki, Finland
NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital & Institute of Clinical Medicine, University of Oslo, Oslo, Norway
Department of Psychology, University of Oslo, Oslo, Norway
Department of Biological and Medical Psychology, University of Bergen, Bergen, Norway
Kavli Research Centre for Aging and Dementia, Haraldsplass Deaconess Hospital, Bergen, Norway
K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, Bergen, Norway
KG Jebsen Centre for Psychosis Research, Norwegian Centre For Mental Disorders Research (NORMENT), Department of Clinical Science, University of Bergen, Bergen, Norway
Dr. Einar Martens Research Group for Biological Psychiatry, Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway
Institute of Hygiene and Epidemiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic
Palacký University, Olomouc, Czech Republic
University Health Network and Mount Sinai Hospital, Toronto General Hospital, and Samuel Lunenfeld Research Institute, Toronto, ON, Canada
Departments of Psychiatry, and Genetics and Genomics Sciences, Seaver Autism Center, and the Mindich Child Health and Development Institute, Mount Sinai School of Medicine, New York, NY, USA
The Centre for Applied Genomics and Program in Genetics and Genomics Biology, The Hospital for Sick Children, Toronto, ON, Canada
Department of Psychiatry and Psychotherapy, Medical University Vienna, Vienna, Austria
The Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
Rheumatology Unit, Department of Medicine at the Karolinska University Hospital, Solna, Sweden
Department of Nutrition, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

The Price Foundation Collaborative Group


Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA
Department of Psychiatry, University of Minnesota, Minneapolis, MN, USA
Rosenock Hospital for Behavioral Medicine, Prien, Germany
Department of Psychiatry, University of Munich (LMU), Munich, Germany
New York Presbyterian Hospital, Westchester Division, Weill Medical College of Cornell University, White Plains, NY, USA
Laureate Psychiatric Clinic and Hospital, Tulsa, OK, USA
Center for Addiction and Mental Health, Toronto, Canada
Department of Psychiatry, Toronto General Hospital, University Health Network, Toronto, Canada
Neuropsychiatric Research Institute, Fargo, ND, USA
127 Department of Clinical Neuroscience, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, USA
128 Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA
129 Neuropsychiatric Research Biotechnologies, University of Pisa, Pisa, Italy
130 Eating Disorders Section, Institute of Psychiatry, King's College, University of London, London, England
131 Department of Psychology, Florida State University, Tallahassee, FL, USA
132 Department of Psychology, Georgia State University, Atlanta, GA, USA
133 Center for Health Sciences, SRI International, Menlo Park, CA, USA
134 Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA
135 Department of Psychiatry, University of California at San Diego, San Diego, CA, USA
136 Department of Psychiatry, Brain Mind Institute EPFL—Lausanne, Center for Psychiatric Neuroscience, University of Lausanne Medical School, Lausanne, Switzerland