

Brain-Based Classification of Youth with Anxiety Disorders: an ENIGMA-ANXIETY Transdiagnostic Examination using Machine Learning

Authors

Willem B. Bruin, MSc^{1,2}, Paul Zhutovsky, PhD^{1,2}, Guido A. van Wingen, PhD^{1,2}, Janna Marie Bas-Hoogendam, PhD^{3,4,5}, Nynke A. Groenewold, PhD^{6,7}, Kevin Hilbert, PhD⁸, Anderson M. Winkler, MD, PhD^{9,10}, Andre Zugman, PhD¹⁰, Federica Agosta, MD, PhD^{11,12,13}, Fredrik Åhs, PhD¹⁴, Carmen Andreescu, MD¹⁵, Chase Antonacci, MPhil¹⁶, Takeshi Asami, MD, PhD¹⁷, Michal Assaf, MD^{18,19}, Jacques P. Barber, PhD²⁰, Jochen Bauer, PhD²¹, Shreya Y. Bavdekar, ²², Katja Beesdo-Baum, PhD²³, Francesco Benedetti, MD²⁴, Rachel Bernstein, BA¹⁶, Johannes Björkstrand, PhD²⁵, Robert J Blair, PhD²⁶, Karina S. Blair, PhD²⁷, Laura Blanco-Hinojo, PhD^{28,29,30}, Joscha Böhnlein, PhD³¹, Paolo Brambilla, MD, PhD^{32,33}, Rodrigo A. Bressan, MD, PhD^{34,35}, Fabian Breuer, MSc³¹, Marta Cano, PhD^{36,37}, Elisa Canu, PhD¹¹, Elise M. Cardinale, PhD³⁸, Narcís Cardoner, MD, PhD^{36,39,37}, Camilla Cividini, PhD¹¹, Henk Cremers, PhD⁴⁰, Udo Dannlowski, MD, PhD³¹, Gretchen J. Diefenbach, PhD^{41,42}, Katharina Domschke, MD, PhD⁴³, Alexander G.G. Doruyter, PhD^{44,45}, Thomas Dresler, PhD^{46,47}, Angelika Erhardt, MD⁴⁸, Massimo Filippi, MD^{11,12,49,13}, Gregory A. Fonzo, PhD⁵⁰, Gabrielle F. Freitag, MSc⁵¹, Tomas Furmark, PhD⁵², Tian Ge, PhD^{53,54}, Andrew J. Gerber, MD, PhD^{55,56}, Savannah N. Gosnell, PhD⁵⁷, Hans J. Grabe, MD⁵⁸, Dominik Grotegerd, PhD³¹, Ruben C. Gur, PhD^{59,60,61}, Raquel E. Gur, MD, PhD^{59,60}, Alfons O. Hamm, PhD⁶², Laura K.M. Han, PhD^{63,64}, Jennifer C. Harper, MSc⁶⁵, Anita Harrewijn, PhD^{66,16}, Alexandre Heeren, PhD⁶⁷, David Hofmann, PhD⁶⁸, Andrea P. Jackowski, PhD^{34,35}, Neda Jahanshad, PhD⁶⁹, Laura Jett, BSc¹⁶, Antonia N. Kaczurkin, PhD⁷⁰, Parmis Khosravi, PhD¹⁰, Ellen N. Kingsley, MSc⁷¹, Tilo Kircher, MD, PhD⁷², Milutin Kostic, MD, PhD^{73,74}, Bart Larsen, PhD⁵⁹, Sang-Hyuk Lee, MD, PhD⁷⁵, Elisabeth J. Leehr, PhD³¹, Ellen Leibenluft, MD⁷⁶, Christine Lochner, PhD⁷⁷, Su Lui, PhD⁷⁸, Eleonora Maggioni, PhD^{79,80}, Gisele G. Manfro, MD, PhD^{81,82}, Kristoffer N.T. Månsson, PhD⁸³, Claire E. Marino, BA²², Frances Meeten, DPhil⁸⁴, Barbara Milrod, MD⁸⁵, Ana Munjiza Jovanovic, MD, PhD^{73,74}, Benson Mwangi, PhD⁸⁶, Michael J. Myers, BA⁶⁵, Susanne Neufang, PhD⁸⁷, Jared A. Nielsen, PhD^{88,89,90,91}, Patricia A Ohrmann, MD, PhD⁹², Cristina Ottaviani, PhD^{93,94}, Martin P. Paulus, MD⁹⁵, Michael T Perino, PhD⁹⁶, K. Luan Phan, MD⁹⁷, Sara Poletti, PhD²⁴, Daniel Porta-Casteràs, MSc^{36,98,39}, Jesus Pujol, MD, PhD^{28,29}, Andrea Reinecke, PhD^{99,100}, Grace V. Ringlein, MS¹⁶, Pavel Rjabtsenkov, BSc²², Karin Roelofs, PhD¹⁰¹, Ramiro Salas, PhD^{57,102,103}, Giovanni A. Salum, PhD^{104,35}, Theodore D. Satterthwaite, MD^{59,105}, Elisabeth Schrammen, MSc³¹, Lisa Sindermann, Dr. rer. nat.¹⁰⁶, Jordan W. Smoller, MD, ScD^{53,54}, Jair C. Soares, MD, PhD⁸⁶, Rudolf Stark, PhD^{107,108,109}, Frederike Stein, PhD^{72,110}, Thomas Straube, PhD⁶⁸, Benjamin Straube, PhD⁷², Jeffrey R. Strawn, MD^{111,112}, Benjamin Suarez-Jimenez, PhD²², Chad M. Sylvester, MD, PhD⁶⁵, Ardesheer Talati, PhD^{113,114}, Sophia I. Thomopoulos, BA⁶⁹, Raşit Tükel, MD¹¹⁵, Helena van Nieuwenhuizen, BSc¹¹⁶, Kathryn Werwath, BSc¹⁶, Katharina Wittfeld, PhD^{117,58}, Barry Wright, MD^{118,119}, Mon-Ju Wu, PhD⁸⁶, Yunbo Yang, PhD^{72,110}, Anna Zilverstand, PhD¹²⁰, Peter Zwanzger, MD^{121,122}, Jennifer U Blackford, PhD^{123,124}, Suzanne N Avery, PhD¹²⁵, Jacqueline A. Clauss, MD, PhD^{126,127}, Ulrike Lueken, PhD⁸, Paul M. Thompson, PhD⁶⁹, Daniel S. Pine, MD¹⁰, Dan J Stein, MD, PhD¹²⁸, Nic J.A. van der Wee, MD, PhD^{4,5,129}, Dick J. Veltman, MD, PhD¹³⁰, Moji Aghajani, PhD^{131,130}

Affiliations

¹Amsterdam UMC location University of Amsterdam, Department of Psychiatry, Meibergdreef 9, Amsterdam, The Netherlands. ²Amsterdam Neuroscience, Amsterdam, The Netherlands. ³Leiden University, Department of Developmental and Educational Psychology, Leiden, The Netherlands. ⁴Department of Psychiatry, Leiden University Medical Center, Leiden, the Netherlands. ⁵Leiden Institute for Brain and Cognition, Leiden, The Netherlands. ⁶SA-MRC Unit on Child & Adolescent Health & Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa. ⁷Department of Psychiatry & Mental Health, Neuroscience Institute, University of Cape Town, Cape Town, South Africa. ⁸Department of Psychology, Humboldt-Universität zu Berlin, Berlin, Germany. ⁹Department of Human Genetics, University of Texas Rio Grande Valley, Brownsville, TX, USA. ¹⁰Section on Development and Anxiety Disorders, National Institute of Mental Health Intramural Program, Bethesda, MD, USA. ¹¹Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy. ¹²Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy. ¹³Vita-Salute San Raffaele University, Milan, Italy. ¹⁴Department of Psychology and Social Work, Mid Sweden University, Östersund, Sweden. ¹⁵University of Pittsburgh, Department of Psychiatry, Pittsburgh, USA. ¹⁶Section on Development and Affective Neuroscience, National Institute of Mental Health, Bethesda, MD, USA. ¹⁷Department of Psychiatry, Yokohama City University School of Medicine, Yokohama, Japan. ¹⁸Olin Neuropsychiatry Research Center, Institute of Living, Hartford, CT, USA. ¹⁹Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA. ²⁰Derner School of Psychology, Adelphi University, Garden City, NY, USA. ²¹Department of Radiology, University of Münster, Münster, Germany. ²²The Del Monte Institute for Neuroscience, Department of Neuroscience, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA. ²³Behavioral Epidemiology, Institute of Clinical Psychology and Psychotherapy, Technische Universität Dresden, Germany. ²⁴Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy. ²⁵Department of Psychology, Lund University, Lund, Sweden. ²⁶Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region of Denmark, Copenhagen, Denmark. ²⁷Center for Neurobehavioral Research, Boys Town National Research Hospital, Boys Town, USA. ²⁸MRI Research Unit, Hospital del Mar, Barcelona, Spain. ²⁹CIBER de Salud Mental, Instituto de Salud Carlos III, Barcelona, Spain. ³⁰Fundació Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain. ³¹Institute for Translational Psychiatry, University of Münster, Münster, Germany. ³²Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy. ³³Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. ³⁴Department of Psychiatry, Universidade Federal de Sao Paulo, Sao Paulo, Brazil. ³⁵Instituto Nacional de Psiquiatria do Desenvolvimento, São Paulo, Brazil. ³⁶Sant Pau Mental Health Research Group, Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. ³⁷CIBERSAM, Carlos III Health Institute, Madrid, Spain. ³⁸Department of Psychology, The Catholic University of America. ³⁹Department of Psychiatry and Forensic Medicine, School of Medicine Bellaterra, Universitat Autònoma de Barcelona, Barcelona, Spain. ⁴⁰Department of Clinical Psychology, University of Amsterdam, Amsterdam, the Netherlands. ⁴¹Anxiety Disorders Center, The Institute of Living/Hartford Hospital, Hartford, CT, USA.

⁴²Yale University School of Medicine, New Haven, CT, USA. ⁴³Department of Psychiatry and Psychotherapy, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany. ⁴⁴NuMeRI Node for Infection Imaging, Central Analytical Facilities, Stellenbosch University, Stellenbosch, South Africa. ⁴⁵Division of Nuclear Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa. ⁴⁶Department of Psychiatry and Psychotherapy, Tuebingen Center for Mental Health, University Hospital Tuebingen, Germany. ⁴⁷LEAD Graduate School & Research Network, University of Tuebingen, Germany. ⁴⁸Max Planck Institute for Psychiatry, Translational Department, Munich, Germany. ⁴⁹Neurorehabilitation Unit and Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy. ⁵⁰Department of Psychiatry and Behavioral Sciences, The University of Texas at Austin Dell Medical School, Austin, TX, USA. ⁵¹Center for Children and Families, Department of Psychology, Florida International University, Miami, FL, USA. ⁵²Department of Psychology, Uppsala University, Uppsala, Sweden. ⁵³Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA. ⁵⁴Center for Precision Psychiatry, Massachusetts General Hospital, Boston, MA, USA. ⁵⁵Department of Psychiatry, Columbia University Medical Center, New York, NY, USA. ⁵⁶Silver Hill Hospital, New Canaan, CT, USA. ⁵⁷Department of Psychiatry, Baylor College of Medicine, Houston TX, 77030, USA. ⁵⁸Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany. ⁵⁹Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. ⁶⁰Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. ⁶¹Philadelphia Veterans Administration Medical Center, Philadelphia, PA 19104. ⁶²Department of Psychology, University of Greifswald, Germany. ⁶³Centre for Youth Mental Health, The University of Melbourne, Parkville, VIC, Australia. ⁶⁴Orygen, Parkville, VIC, Australia. ⁶⁵Department of Psychiatry, Washington University, St. Louis, MO, USA. ⁶⁶Department of Psychology, Education & Child Studies, Erasmus University Rotterdam, Rotterdam, The Netherlands. ⁶⁷Psychological Sciences Research Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium. ⁶⁸Institute of Medical Psychology and Systems Neuroscience, University of Münster, Germany. ⁶⁹Imaging Genetics Center, Stevens Neuroimaging & Informatics Institute, Keck School of Medicine, University of Southern California, Los Angeles, California, USA. ⁷⁰Department of Psychology, Vanderbilt University, Nashville, TN 37240, USA. ⁷¹Child Oriented Mental Health Innovation Collaborative, Leeds and York Partnership Foundation Trust, NHS, York UK. ⁷²Department of Psychiatry and Psychotherapy, University of Marburg, Marburg, Germany. ⁷³Institute of Mental Health, Belgrade, Serbia. ⁷⁴Faculty of Medicine, University of Belgrade, Serbia. ⁷⁵Department of Psychiatry, CHA Bundang Medical Center, CHA University, 59 Yatap-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13496, Republic of Korea. ⁷⁶Section on Mood Dysregulation and Neuroscience, National Institute of Mental Health Intramural Program, Bethesda, MD, USA. ⁷⁷SAMRC Unit on Risk & Resilience in Mental Disorders, Dept of Psychiatry, Stellenbosch University, South Africa. ⁷⁸Huaxi MR Research Center (HMRR), Department of Radiology, West China Hospital of Sichuan University, Chengdu, China. ⁷⁹Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy. ⁸⁰Department of Neurosciences and Mental Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. ⁸¹Department of Psychiatry, School of Medicine, Universidade Federal do Rio Grande do Sul, Brazil. ⁸²Anxiety Outpatient Unit, Hospital de Clinicas de Porto Alegre, Brazil. ⁸³Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. ⁸⁴School of Psychology, University of Sussex, Falmer, UK. ⁸⁵Albert Einstein College of Medicine, Bronx, NY, USA. ⁸⁶Louis A. Failace, MD, Department of Psychiatry and Behavioral Sciences, The University of Texas Health Science Center at Houston, Houston, TX, USA. ⁸⁷Department of Psychiatry and Psychotherapy, Medical Faculty Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany. ⁸⁸Department of Psychology, Harvard University, Cambridge, Massachusetts, USA. ⁸⁹Center for Brain Science, Harvard University, Cambridge, Massachusetts, USA. ⁹⁰Department of Psychology, Brigham Young University, Provo, UT, USA. ⁹¹Neuroscience Center, Brigham Young University, Provo, UT, USA. ⁹²Department of Psychiatry, University of Münster, Münster, Germany. ⁹³Department of Psychology, Sapienza University of Rome, Italy. ⁹⁴Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy. ⁹⁵Laureate Institute for Brain Research, Tulsa OK, USA. ⁹⁶Washington University School of Medicine, St. Louis, MO. ⁹⁷Department of Psychiatry and Behavioral Health, The Ohio State University, Columbus, OH, USA. ⁹⁸Mental Health Department, Unitat de Neurociència Traslacional, Parc Taulí University Hospital, Institut d'Investigació i Innovació Sanitària Parc Taulí (I3PT), Barcelona, Spain. ⁹⁹Department of Psychiatry, University of Oxford. ¹⁰⁰Oxford Health NHS Foundation Trust. ¹⁰¹Donders Institute for Brain Cognition and Behaviour and Behavioural Science Institute, Radboud University, Nijmegen, The Netherlands. ¹⁰²Center for Translational Research on Inflammatory Diseases, Michael E DeBakey VA Medical Center, Houston Texas 77030, USA. ¹⁰³The Menninger Clinic, Houston, TX 77035, USA. ¹⁰⁴Department of Psychiatry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. ¹⁰⁵Center for Biomedical Image Computing and Analytics, University of Pennsylvania, Philadelphia, PA 19104, USA. ¹⁰⁶Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany. ¹⁰⁷Department of Psychotherapy and Systems Neuroscience, Justus Liebig University Giessen, Germany. ¹⁰⁸Bender Institute of Neuroimaging, Justus Liebig University Giessen, Germany. ¹⁰⁹Center for Mind, Brain and Behavior (CMBB), University of Marburg and Justus Liebig. ¹¹⁰Center for Mind, Brain and Behavior, University of Marburg, Germany. ¹¹¹University of Cincinnati, Cincinnati, Ohio, USA. ¹¹²Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA. ¹¹³Department of Psychiatry, Columbia University Irving Medical Center, New York, NY, USA. ¹¹⁴Division of Translational Epidemiology, New York State Psychiatric Institute. ¹¹⁵Department of Psychiatry, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkey. ¹¹⁶Department of Physics, Stony Brook University, Stony Brook, NY, USA. ¹¹⁷German Center for Neurodegenerative Diseases (DZNE), Site Rostock/ Greifswald, Greifswald, Germany. ¹¹⁸Hull York Medical School, University of York, York, UK. ¹¹⁹Child Oriented Mental Health Innovation Collaborative, Leeds and York Partnership Foundation Trust, NHS, Leeds UK. ¹²⁰Department of Psychiatry and Behavioral Sciences, University of Minnesota, USA. ¹²¹Department of Psychiatry, LMU Munich, Germany. ¹²²kbo-Inn-Salzach-Klinikum Wasserburg Germany. ¹²³University of Nebraska Medical Center, Omaha, Nebraska. ¹²⁴Vanderbilt University Medical Center, Nashville, TN. ¹²⁵Vanderbilt University Medical Center, Nashville, Tennessee. ¹²⁶Department of Psychiatry, Massachusetts General Hospital, Boston, MA. ¹²⁷Harvard Medical School. ¹²⁸SAMRC Unit on Risk & Resilience in Mental Disorders, Dept of Psychiatry & Neuroscience Institute, University of Cape Town. ¹²⁹Theme Neuroscience, Leiden University Medical Center, Leiden, the Netherlands. ¹³⁰Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Psychiatry, Amsterdam, the Netherlands. ¹³¹Leiden University, Institute of Education & Child Studies, Section Forensic Family & Youth Care, The Netherlands.

Corresponding authors: Willem Bruin (willembenjamin@gmail.com) & Moji Aghajani

(m.aghajani@fsw.leidenuniv.nl)

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Structured Abstract

Background: Neuroimaging studies point to neurostructural abnormalities in youth with anxiety disorders. Yet, findings are based on small-scale studies, often with small effect sizes, and have limited generalizability and clinical relevance. These issues have prompted a paradigm shift in the field towards highly powered (i.e., big data) individual-level inferences, which are data-driven, transdiagnostic, and neurobiologically informed.

Methods: Here, we built and validated neurostructural machine learning (ML) models for individual-level inferences based on the largest-ever multi-site neuroimaging sample of youth with anxiety disorders (age: 10-25 years, N=3,343 individuals from 32 global sites), as compiled by three ENIGMA Anxiety Working Groups: Panic Disorder (PD), Generalized Anxiety Disorder (GAD), and Social Anxiety Disorder (SAD). ML classifiers were trained on MRI-derived regional measures of cortical thickness, surface area, and subcortical volumes to classify patients and healthy controls (HC) for each anxiety disorder separately and across disorders (transdiagnostic classification).

Results: Modest, yet robust, classification performance was achieved for PD vs. HC (AUC=0.62), but other disorder-specific and transdiagnostic classifications were not significantly different from chance. However, above chance-level transdiagnostic classifications were obtained in exploratory subgroup analyses of male patients vs. male HC, unmedicated patients vs. HC, and patients with low anxiety severity vs. HC (AUC 0.59-0.63). The above chance-level classifications were based on plausible and specific neuroanatomical features in fronto-striato-limbic and temporo-parietal regions.

Conclusions: This study provides a realistic estimate of classification performance in a large, ecologically valid, multi-site sample of youth with anxiety disorders, and may as such serve as a benchmark.

Introduction

Anxiety disorders are the most prevalent mental disorders among youth, with a life-time prevalence estimate of up to 30% (1,2). Most anxiety disorders develop during the critical transition from adolescence to young adulthood (10-25 years), affecting millions of youth worldwide and causing enormous emotional, societal, and economic burden (3,4). Critically, the COVID-19 pandemic has further exacerbated this alarming trend, with some experts now even talking about a “lost generation” of youth (5). Despite these concerns, the underlying neurobiology of anxiety disorders in youth remains elusive, making it difficult to pinpoint robust biomarkers, and formulate or test neurobiologically informed treatment/prevention strategies (4,6). Psychopathology is less differentiated in youth than in adults, and thus even less compatible with traditional diagnostic classification systems, with ongoing neuro-bio-psychosocial maturation further complicating the situation (7-9).

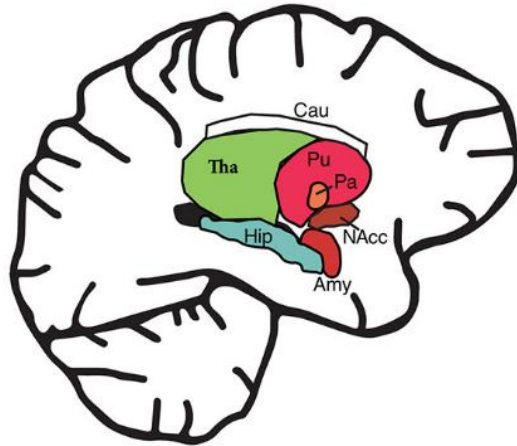
Neuroimaging studies point to anomalies in fronto-striato-limbic brain circuits and additional sensory territories in youth with anxiety disorders (6,7,10), which collectively affect the perception, processing and modulation of emotionally salient information (**Figure_1**). While promising, neurostructural findings are often difficult to replicate and small in effect size, with marginal clinical relevance (4,6,11-14). These issues may reflect limitations of our currently dominant analytic approach, which favors disorder-specific analyses rather than transdiagnostic ones, often within underpowered samples and with the use of traditional mass univariate analyses that preclude massively multivariate and individual-level analytics (11-15). These issues have prompted a paradigm shift in the field that aims to transcend classic case-control comparisons, and instead focuses on highly powered (i.e., big data) individual-level inferences that are data-driven, transdiagnostic, and neurobiologically informed (11,15).

The application of machine learning (ML) may be particularly useful for this endeavor (11,15,16). ML is well-suited to dealing with high-dimensional data in a data-driven manner, extracting complex multivariate patterns that best predict individual-level clinical outcomes (11,15,16). Prior brain-based classification studies of anxiety disorders show that neuroimaging data can distinguish patients from controls with varying success (17-24). While encouraging, most of these studies are limited by their use of small single-site samples, so it is unclear whether results generalize to data from other sites with different demographic (i.e., age/sex distribution), clinical (i.e., medication, symptom severity, comorbidity) or technical (i.e., scanner, acquisition protocols, and diagnostic assessment) characteristics. To overcome this, large multi-site collaborations have begun to pool neuroimaging and clinical datasets for coordinated analyses, wherein all data are preprocessed and analyzed according to

harmonized and standardized protocols. The Enhancing Neuro-Imaging Genetics through Meta-Analysis (ENIGMA) consortium is such an initiative with a massive global reach (25,26), and hence used for the current study. The large-scale multi-site ENIGMA datasets offer ecologically valid and clinically representative information, which ML algorithms can exploit to identify multivariate patterns generalizable to the majority of patients, in a realistic fashion (27-29). While increasing sample size typically benefits ML performance and generalizability, the use of retrospectively pooled multi-site data may also complicate performance, due to the substantial heterogeneity (i.e., sample characteristics and methodology) that is inherently introduced (27-30). On the other hand, a multi-site design may give more realistic estimates of performance, and can be used to explicitly test the robustness of predictive models which is a necessary prerequisite for implementation into routine care.

Here, we built and validated structural magnetic-resonance imaging (MRI)-based ML models based on the largest-ever multi-site sample of neuroimaging data from young anxiety patients and healthy controls (HC) worldwide (N=3,343 from 32 sites). The sample was aggregated from three subgroups of the ENIGMA-Anxiety Working Group: Panic Disorder (PD), Generalized Anxiety Disorder (GAD) and Social Anxiety Disorder (SAD) (10). We focused on individuals between 10-25 years old, thereby capturing the adolescence period and young adulthood (31). ML classifiers were trained on brain MRI-derived cortical and subcortical gray matter features (regional cortical thickness/surface area, and subcortical volumes) to classify patients vs. HC for each anxiety disorder separately (disorder-specific), and across anxiety disorders (further referred to as transdiagnostic classification). Our hypothesis was that the classifiers would correctly classify patients vs. HC above chance-level. Exploratory analyses additionally examined the influence of sex, medication usage and symptom severity on classification performance.

Subcortical



Cortical

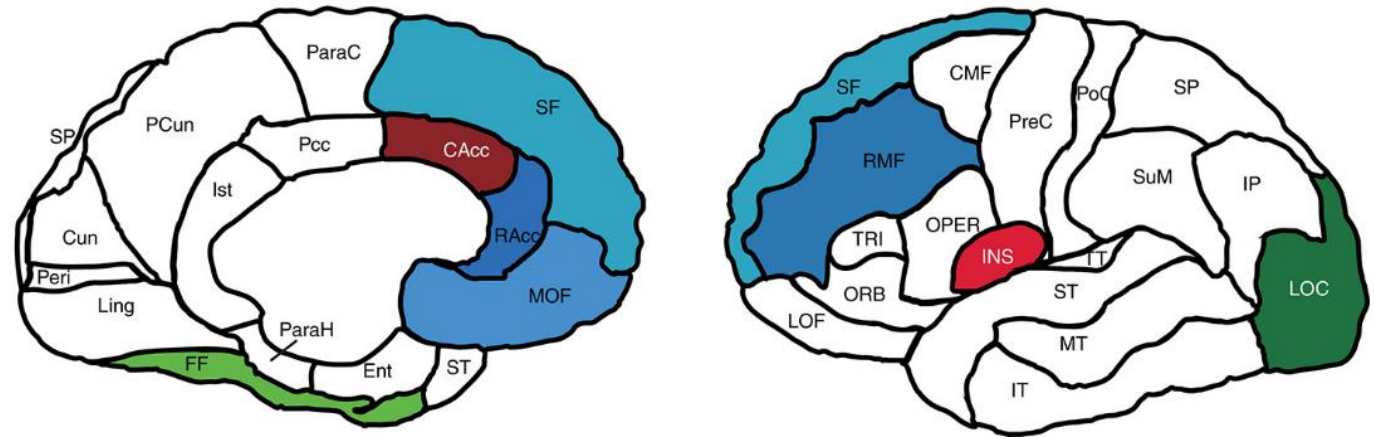


Figure 1. Putative neurocircuitry of anxiety disorders. Anomalies in these regions tend to collectively impede how emotionally salient information is perceived, processed, and modulated. This includes brain areas involved in sensory processing (occipital cortex, fusiform gyrus, thalamus; *green*), emotion generation and processing (striatum, amygdala, insula, dorsal anterior cingulate cortex; *red*) and emotion modulation (medial prefrontal cortex, hippocampus, dorsolateral prefrontal cortex, subgenual/rostral anterior cingulate cortex; *blue*). Adapted and reprinted with permission from Wiley Periodicals, Inc.: Human Brain Mapping (Bas-Hoogendam et al., 2022). Amy, Amygdala; Cau, Nucleus Caudatus; Hip, Hippocampus; NAcc, Nucleus Accumbens; Pa, Pallidum; Pu, Putamen; Tha, Thalamus; CAcc, Caudal Anterior Cingulate Cortex; FF, Fusiform; INS, Insula; LOC, Lateral Occipital; MOF, Medial Orbitofrontal; RAcc, Rostral Anterior Cingulate Cortex; RMF, Rostral Middle Frontal; SF, Superior Frontal.

Table 1. Demographic and clinical characteristics for included patients and controls of the three ENIGMA Anxiety Workings groups.

Characteristic	ENIGMA-Anxiety Working Group						Transdiagnostic (32 sites)	
	PD (12 sites)		GAD (16 sites)		SAD (13 sites)			
	Patients (N=112)	Controls (N=813)	Patients (N=465)	Controls (N=1084)	Patients (N=259)	Controls (N=610)	Patients (N=823) ^e	Controls (N=1969) ^e
Age, Years	21.68 ± 2.78	21.73 ± 3.01	18.39 ± 4.28	16.06 ± 4.35	21.36 ± 2.45	22.17 ± 2.30	19.79 ± 3.90	18.79 ± 4.75
Sex (N, % female)	87 (77.68)	538 (66.17)	317 (68.17)	589 (54.34)	181 (69.88)	422 (69.18)	575 (69.87)	1196 (60.74)
STAI-T ^a	50.07 ± 12.25	-	45.25 ± 11.14	-	52.12 ± 11.08	-	48.10 ± 11.72	-
Medication, (N, %) ^b	37 (35.58)	-	91 (20.13)	-	35 (13.57)	-	160 (19.98)	-
- Antidepressants	31 (30.39)	-	59 (13.05)	-	30 (12.35)	-	118 (14.96)	-
- Benzodiazepines	6 (8.22)	-	30 (6.80)	-	0 (0)	-	36 (4.59)	-
- Antipsychotics	9 (10.98)	-	25 (5.67)	-	4 (1.80)	-	37 (5.02)	-
Comorbid Anxiety (N, %) ^c								
- PD	-	-	38 (9.43)	-	8 (3.09)	-	36 (5.55)	-
- GAD	38 (34.86)	-	-	-	12 (4.63)	-	46 (12.78)	-
- SAD	32 (28.57)	-	223 (49.34)	-	-	-	223 (43.98)	-
- Mixed ^d	20 (18.35)	-	23 (5.71)	-	1 (0.39)	-	41 (5.41)	-

PD = Panic Disorder, GAD = Generalized Anxiety Disorder, SAD = Social Anxiety Disorder; STAI_T = The State-Trait Anxiety Inventory – Trait Index scores

^a Available data on STAI-T: PD = 88/GAD = 306/SAD = 182

^b Available data on psychotropic medication use at the time of scan: PD = 104/GAD = 452/SAD = 258

^c Available data on comorbid anxiety disorders: PD = 109/GAD = 403/SAD = 259. Assigned primary anxiety disorder diagnoses correspond to respective working groups.

^d Patients diagnosed with all three anxiety disorders.

^e Participants present in more than one working group were excluded from transdiagnostic classifications to avoid the use of duplicated data entries.

Methods and Materials

Study Sample

Participants were included from three ENIGMA-Anxiety Working Groups: Panic Disorder (PD/12 sites): 112 patients/813 controls; Generalized Anxiety Disorder (GAD/16 sites): 465 patients/1,084 controls; and Social Anxiety Disorder (SAD/13 sites): 259 patients/610 controls. All participants included in the analysis were between 10-25 years of age. HC were free of past and present psychopathology and psychotropic medication use at the time of scan. Comorbid anxiety disorders (PD/GAD/SAD) were present in some patients, and their assigned primary (DSM-IV/5 anxiety disorder) diagnosis corresponds to their respective working group. Participants' demographic and clinical characteristics are summarized in **Table_1**. For an overview per site, see **Table_S1**. This study was conducted in accordance with the Declaration of Helsinki; participating sites obtained approval from their local institutional review boards or ethics committees. All participants or caretakers provided written informed consent.

Image Acquisition and Processing

Structural T1-weighted 3D brain magnetic resonance imaging (MRI) scans were acquired, and processed either locally at each site (PD and SAD data) or centrally (GAD data) using standardized protocols for harmonized analysis and quality control. Images were acquired at different field strengths (1.5T/3.0T); sample-specific acquisition parameters are listed elsewhere (10). Regional mean cortical thickness (CT), cortical surface area (SA) and subcortical volumes (SCV), were extracted from the brain images using FreeSurfer (version 5.3/6.0) (32). Parcellations/segmentations were visually inspected and statistically evaluated for outliers. For each subject, SA and CT were calculated for 68 cortical Desikan-Killiany atlas-based regions (34 per hemisphere) (33). In addition, gray matter SCVs were extracted for seven structures per hemisphere (nucleus accumbens, putamen, pallidum, caudate, thalamus, amygdala, hippocampus), along with lateral ventricle volume. This yielded a total of 152 FreeSurfer features per subject for classification.

Multivariate Classification

1. Primary Analyses

As shown in **Figure_2**, classification tasks were performed with linear support vector machines (SVM), one of the most commonly and successfully used algorithms in the field (15,34,35). We performed disorder-specific case-

control classification for each working group separately (PD vs. HC, GAD vs. HC, SAD vs. HC). We also performed a transdiagnostic classification for patients with any anxiety disorder (PD/GAD/SAD) vs. HC using pooled samples across the three working groups. 561 participants (545 HC, 5 GAD and 11 SAD patients) whose data were present in more than one working group were included only once in transdiagnostic classifications to avoid the use of duplicated data. Participants with >75% missing FreeSurfer features were excluded (14 HC and 3 PD).

Classification performance was evaluated using stratified 5-times-repeated-5-fold cross-validation (CV), in which the proportion of patients and controls from each site was (approximately) maintained for each fold. Additionally, we evaluated to what extent the aforementioned classifiers were able to generalize to unseen sites using leave-one-site-out (LOSO) CV. In each LOSO-CV fold, one site is left out for model testing and remaining sites are used for training, and this is repeated so that each site is used as a test set once. LOSO-CV represents a realistic clinical scenario where a model has to generalize to entirely new samples (36). Classification performance was measured using the area-under-the-receiver-operating-characteristic-curve (AUC), balanced accuracy, sensitivity, and specificity, which were calculated for each CV iteration on the testing set and averaged across CV iterations.

Features were standardized using the training set in a site-wise manner by calculating the mean and standard deviation of each feature on the HC from each site separately, and applied to standardize test and training data for both patients and HC of the same site to obtain harmonized and comparably interpretable features. This was done to account for site-effects (i.e., differences in data acquisition protocols and inclusion criteria) that could affect classification performance (27,37). There are alternative methods to handle site-effects, such as ComBat and normative modeling (37), however, the assumptions for these techniques are violated here, rendering them unsuitable for this study (this is further addressed in the discussion). Only participants from sites that had data for both HC and patients, and at least ten HC were included for each classification task to ensure sufficient data for standardization. Missing features were mean-imputed using the full training set. Site-wise undersampling was applied on the training set to account for class imbalance for each site separately so that an equal number of samples was used from both classes. The undersampling procedure was repeated ten times for each training set within the CV procedure, resulting in ten SVM models trained using different (balanced) training sets and evaluated on the same test set (no undersampling). Classifications across the resulting SVM models were combined using an ensemble approach by taking the median across the decision values obtained for the predictions of the test set. We applied the SVM classification with the regularization parameter (C) set to 1,

following general recommendations from the field (38). More details on the classification procedure are provided in the **Supplementary_Methods**. Label permutation testing with 1000 iterations (39) was used to test whether classification performance (AUC) was significantly above chance-level ($\alpha=0.05$), with our hypothesis being above chance-level classification of patients vs. HC. To assess which brain regions contributed most to the classification model, we applied sign-based consistency mapping as per Gomez-Verdejo et al., 2019 (**Supplementary_Methods** for details) (40). We only report sign-based feature importance for classifications that passed label permutation testing.

2. Exploratory Subgroup Analyses

To explore the effects of demographic and clinical heterogeneity on classification performance, we performed subgroup analyses on sex, current psychotropic medication use, and anxiety severity (State-Trait Anxiety Inventory–Trait Index: STAI-T (41); median-split on patients' scores (median=48) produced high/low severity groups). Subgroup analyses were restricted to the transdiagnostic sample (any anxiety disorder vs. HC across working groups), as limited data would remain when investigating these subgroups in each disorder group separately. The classification procedure itself (i.e., site-wise scaling, imputation, undersampling and ensemble learning) was as described above. Classifications included: female patients vs. female HC, male patients vs. male HC, medicated patients vs. HC, unmedicated patients vs. HC, unmedicated patients vs. medicated patients, low severity patients (STAI-T \leq 48) vs. HC, high severity patients (STAI-T $>$ 48) vs. HC and low severity patients vs. high severity patients.

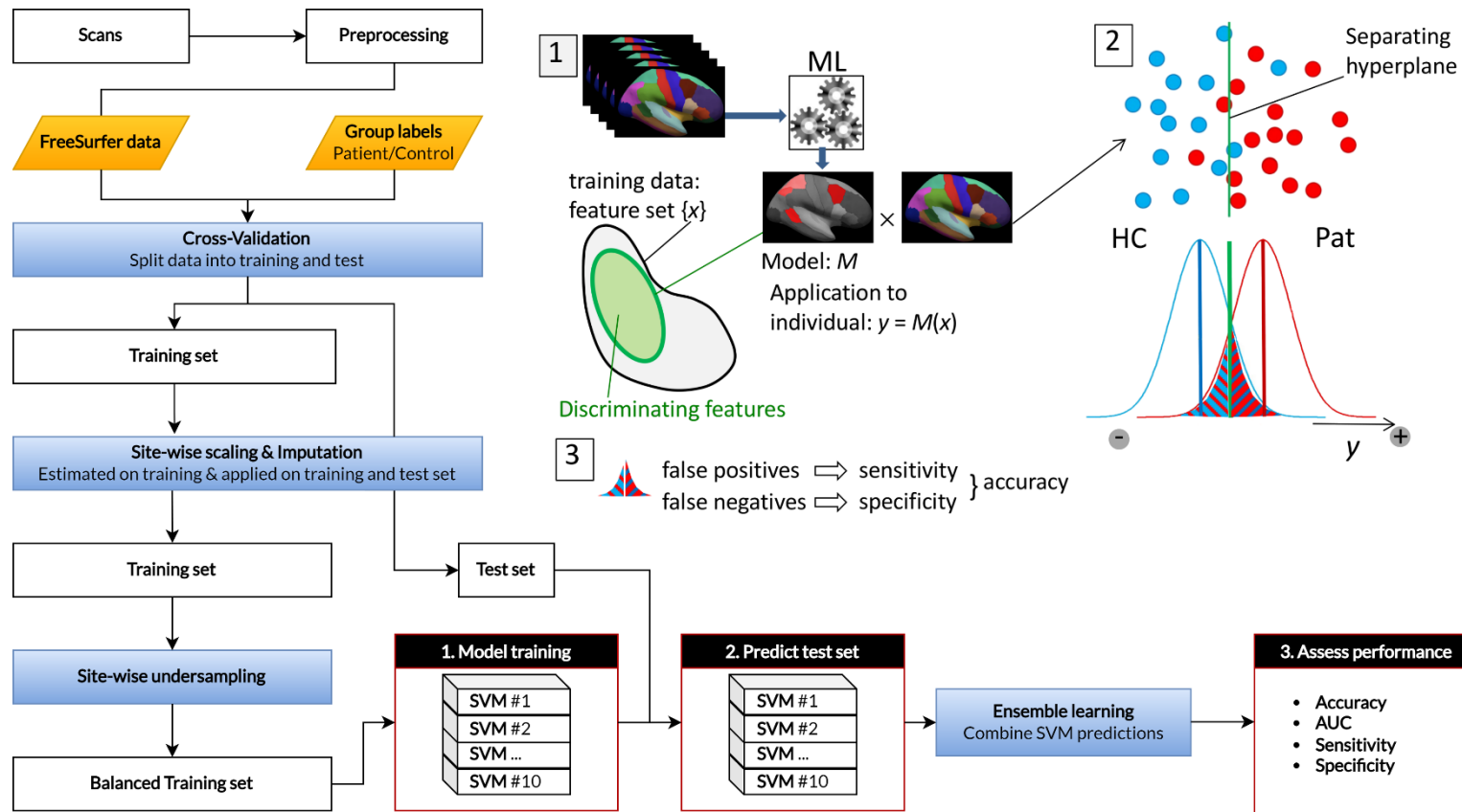


Figure 2. Simplified visual representation of the ML pipeline. 1) ML algorithm is trained on a set of labeled, preprocessed MRI structural measures, resulting in a model M that classifies patients and controls based on a discriminative subset of the features (feature vector). 2) The classification is achieved using an (optimal) separating hyperplane in the (high-dimensional) feature space. Application of the model to an individual scan yields an output value y that is proportional to the distance of the subject's feature vector to that plane: *blue* (HC) and *red* (Patients) dots. The y -values of all subjects form two distributions. 3) A threshold halfway between the distributions separates the two groups; the overlapping parts below and above the threshold represent the false negatives and false positives, respectively. Adapted and reprinted with permission from Frontiers Media SA: Frontiers Psychiatry (Schnack & Kahn, 2016).

Results

1. Primary Analyses

Case-control classification performances are summarized in **Figure_3A**, and a complete overview of results (AUC, balanced accuracy, sensitivity, specificity, permutation p -values) is provided in **Table_S2**. The best classification performance was obtained for PD vs. HC (112 PD/813 HC/12 sites) with an average AUC=0.62 ($p=.027$). None of the other disorder-specific classification performances were significantly different from chance: GAD classification (465 GAD/1,084 HC/16 sites/average AUC=0.55, $p=.605$), SAD classification (259 SAD/610 HC/13 sites/average AUC=0.57, $p=.32$), and transdiagnostic classification (823 any anxiety disorder/1,969 HC/32 sites/average AUC=0.56, $p=.093$). Classification performances obtained using LOSO-CV were similar to those obtained with repeated 5-Fold CV, in which only PD classification performed significantly above chance-level (average AUC=0.63, $p=.003$), while the SAD, GAD, and transdiagnostic classifications failed to surpass chance-level (**Table_S3**).

2. Exploratory Subgroup Analyses

2.1 Sex

We assessed transdiagnostic case-control classification (across ENIGMA-Anxiety subgroups) separately for males and females. Performances obtained are summarized in **Figure_3B/Table_S2**. Whereas above chance-level performance was achieved for male patients with any anxiety disorder vs. male HC classification (167 patients/678 HC/15 sites) (average AUC=0.63, $p=.007$), female patients with any anxiety disorder vs. female HC classification (524 patients/1,133 HC/24 sites) failed to surpass chance-level (average AUC=0.57, $p=.218$).

2.2 Psychotropic Medication

Results for transdiagnostic classifications based on current psychotropic medication use are summarized in **Figure_3C/Table_S2**. Unmedicated patients vs. HC classification (641 patients/1,969 HC/32 sites) led to above chance-level performance (average AUC=0.59, $p=.013$), whereas medicated patients vs. HC classification (160 patients/1,307 HC/17 sites) failed to surpass chance-level (average AUC=0.59, $p=.22$). Likewise, classification of medicated patients vs unmedicated patients (251 medicated patients/160 unmedicated patients/17 sites) failed to surpass chance-level (average AUC=0.51, $p=.38$).

2.3 Severity

Results for transdiagnostic classifications based on severity (STAI-T: low severity \leq 48, high severity $>$ 48) are summarized in **Figure_3D/Table_S2**. Low severity patient vs. HC classification (299 patients/1,422 HC/21 sites) performance was above chance-level (average AUC=0.59, $p=.016$), while high severity patients vs. HC classification (272 patients/1,449 HC/22 sites) failed to surpass chance-level (average AUC=0.57, $p=.305$). Likewise, classification of low vs. high severity patients (299 low severity patients/270 high severity patients/21 sites) also failed to surpass chance-level (average AUC=0.52, $p=.235$).

3. Feature Importance

3.1 PD Classifier

We investigated which brain regions contributed most to above chance-level classifications via sign-based consistency mapping. Four brain features emerged as most relevant for PD vs. HC classification (**Figure_4/Table_S4**). Consistent positive signs of weights were found for CT in the left middle temporal gyrus and right rostral anterior cingulate cortex, which would imply a tendency of the SVM for pushing the classification *towards the PD class* given increased CT in these regions. Consistent negative signs of weights were found for CSA of the left superior frontal gyrus and SCV of the left pallidum, which would imply the opposite tendency of the SVM to assign participants to *HC class* given increased SVC/CSA in these regions. Note that the derived sign consistency p -values do not correspond to a univariate group comparison, but represent the most important features of the multivariate pattern used by the SVM classifier, and should therefore be interpreted with caution (details in **Supplementary_Methods**).

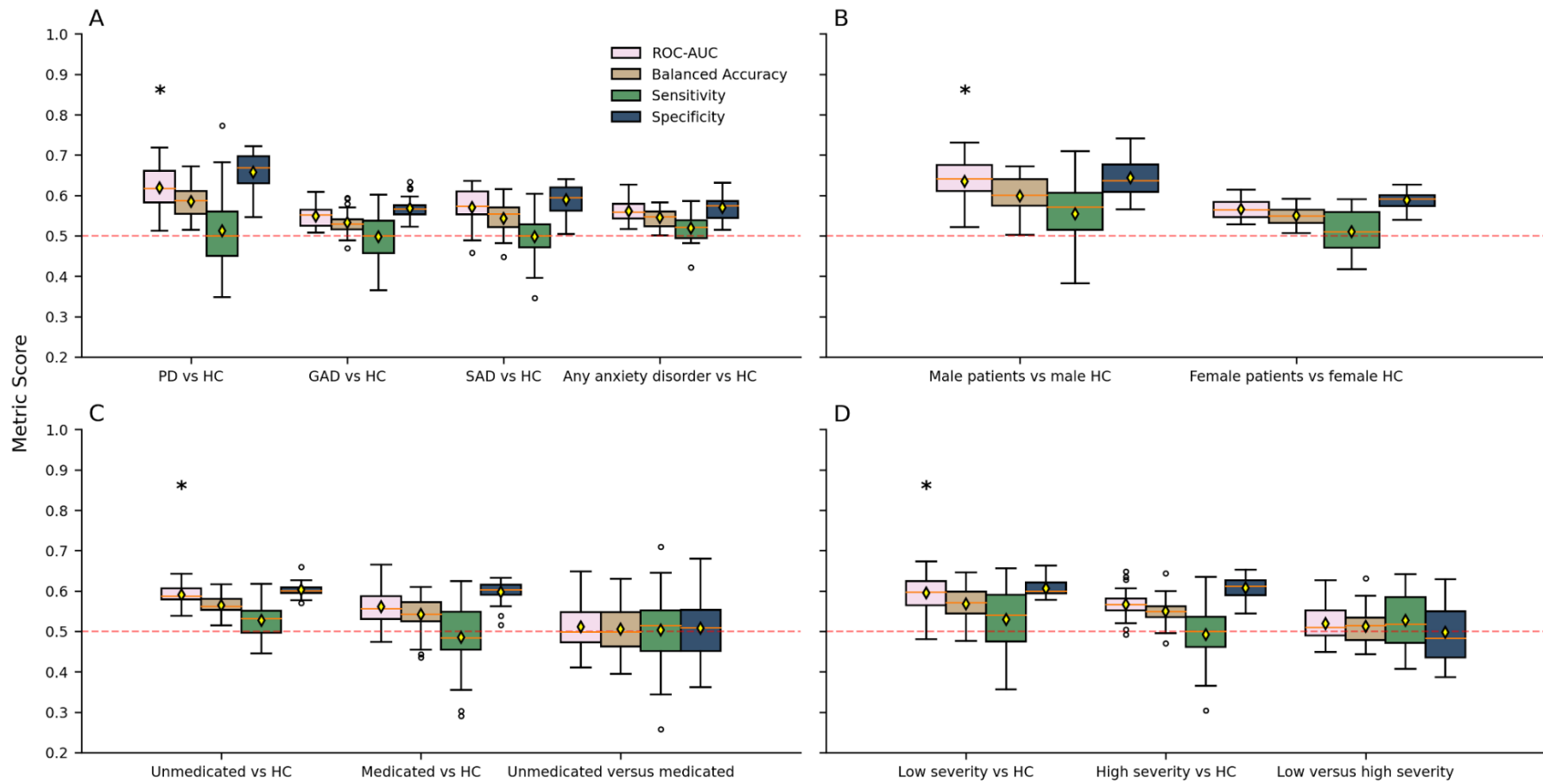


Figure 3. Classification performances for each working group separately and for transdiagnostic classification(s) across working groups. Boxplots summarize scores obtained across the repeated stratified K-Fold cross-validation folds; *yellow diamonds* indicate mean scores, *circles* indicate outliers, *asterisks* depict significance as assessed by label permutation tests and the dashed line represents chance-level performance. PD = Panic Disorder, GAD = General Anxiety Disorder, SAD = Social Anxiety Disorder, HC = Healthy Control.

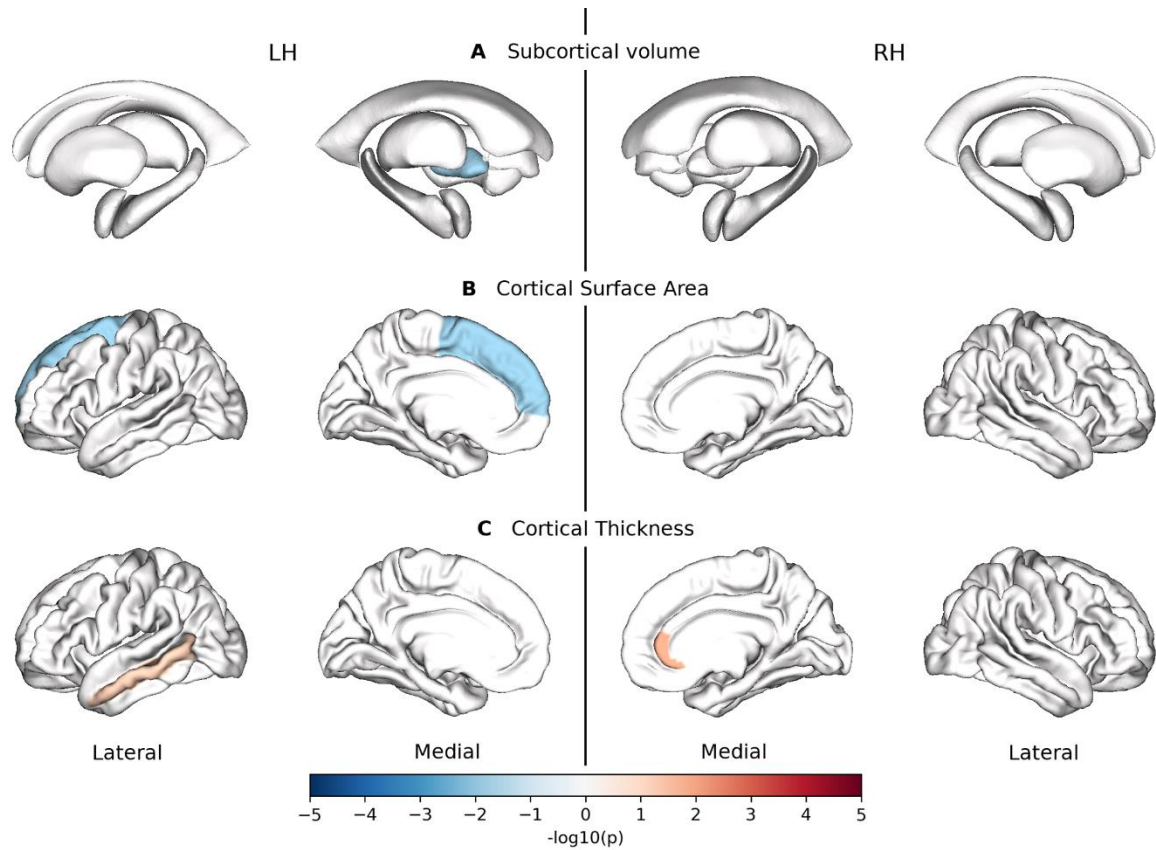


Figure 4. The $-\log(p)$ value maps characterizing significant brain regions contributing the most to panic disorder patients versus healthy controls classification as determined by sign-based consistency mapping (40). Hot colors indicate consistently assigned positive weights by the SVM that drive classification towards the patients class, and cold colors indicate negative weights that drive classification towards controls. The figure was made with the ENIGMA Toolbox package (<https://enigma-toolbox.readthedocs.io/en/latest/>). Full region names may be found in **Supplementary Table S2**. LH = Left Hemisphere, RH = Right Hemisphere.

3.2 Sex, Medication, Severity Classifiers

The male patients vs. male HC transdiagnostic classification comprised seven significant features: positive signs of weights that pushed the classification *towards the patient class* were located in CT of right lateral occipital cortex and right superior frontal gyrus, and consistent negative signs of weights that pushed classification *towards HC* were found in both CT and CSA of the right superior temporal gyrus, and CSA in the *pars orbitalis*, parahippocampal gyrus and cuneus cortex in the right hemisphere (**Figure_5/Table_S5**).

Unmedicated patient vs. HC transdiagnostic classification comprised 21 significant features (**Figure_6/Table_S6**). These included consistent positive signs of weights for the right caudate SCV, CT in the right cuneus, left lingual and left superior temporal gyrus, and CSA in the left middle temporal gyrus, left entorhinal, right superior parietal and right posterior-cingulate cortex (classification towards the patient class given increased CT/CSA). Negative signs of weights were found for the SCV of the left caudate and amygdala, CT in the left inferior parietal cortex and right middle temporal gyrus, and CSA in right inferior temporal, supramarginal and left fusiform gyrus, bilateral temporal poles, right banks superior temporal sulcus, right paracentral lobule and left medial orbital frontal and inferior parietal cortex (classification towards HC class given increased CT/CSA/SCV).

Low severity patients vs. HC transdiagnostic classification comprised six significant features (**Figure_7/Table_S7**). Positive signs of weights were found for CT in the right inferior parietal cortex and CSA in the right rostral anterior cingulate and left entorhinal cortex (classification towards patient class given increased CT/CSA). Consistent negative signs of weights were found for CT and CSA in the right inferior temporal gyrus, and CSA in the right *pars triangularis* (classification towards HC class given increased CT/CSA).

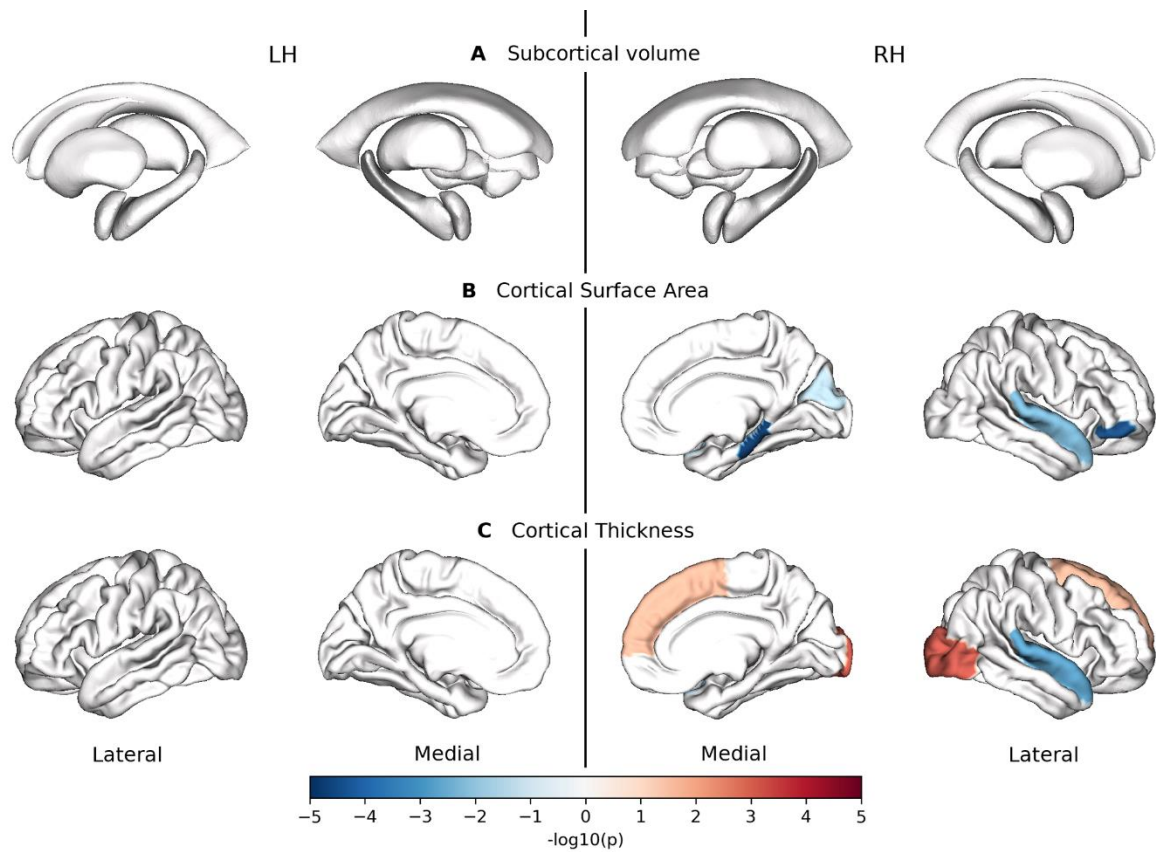


Figure 5. The $-\log(p)$ value maps characterizing significant brain regions contributing the most to male patients versus male healthy controls classification across working groups as determined by sign-based consistency mapping (40). Hot colors indicate consistently assigned positive weights by the SVM that drive classification towards the patients class, and cold colors indicate negative weights that drive classification towards controls. The figure was made with the ENIGMA Toolbox package (<https://enigma-toolbox.readthedocs.io/en/latest/>). Full region names may be found in **Supplementary Table S3. LH = Left Hemisphere, RH = Right Hemisphere.**

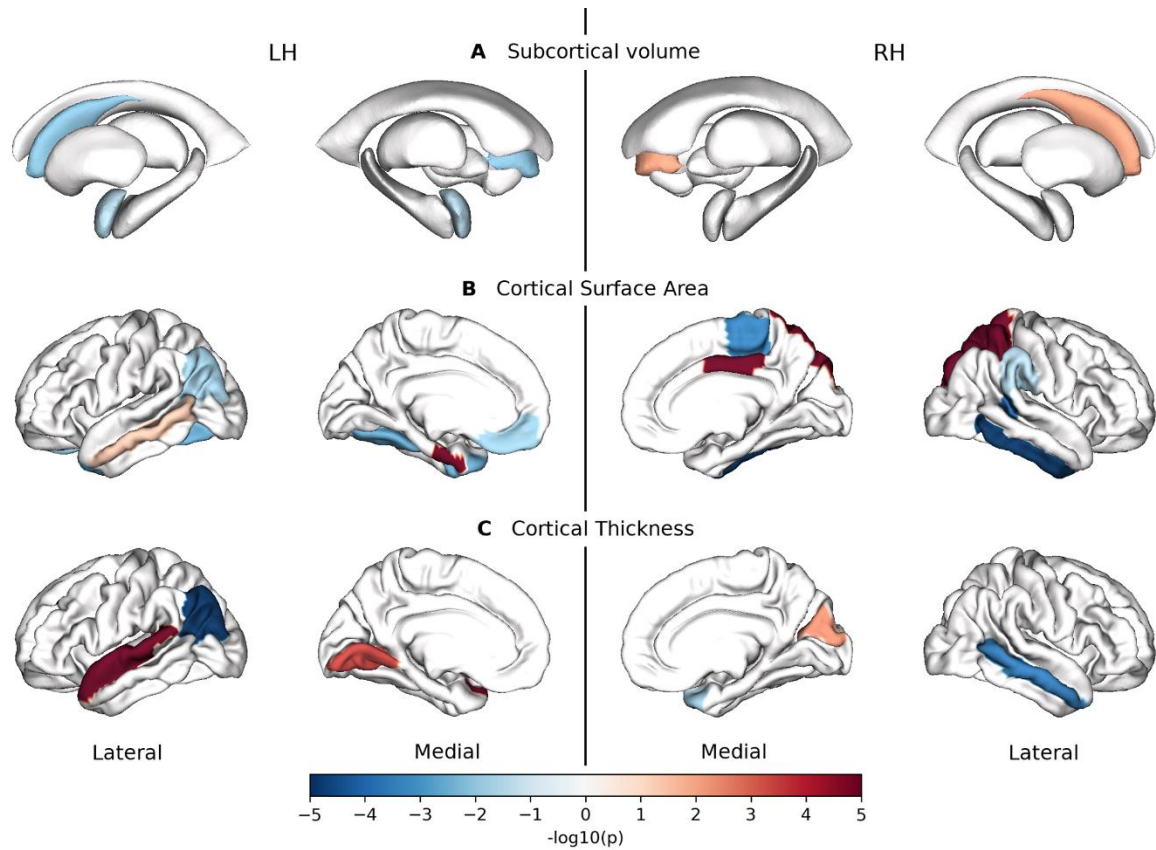


Figure 6. The $-\log(p)$ value maps characterizing significant brain regions contributing the most to unmedicated patients versus healthy controls classification across working groups as determined by sign-based consistency mapping (40). Hot colors indicate consistently assigned positive weights by the SVM that drive classification towards the patients class, and cold colors indicate negative weights that drive classification towards controls. The figure was made with the ENIGMA Toolbox package (<https://enigma-toolbox.readthedocs.io/en/latest/>). Full region names may be found in **Supplementary Table S4**. LH = Left Hemisphere, RH = Right Hemisphere.

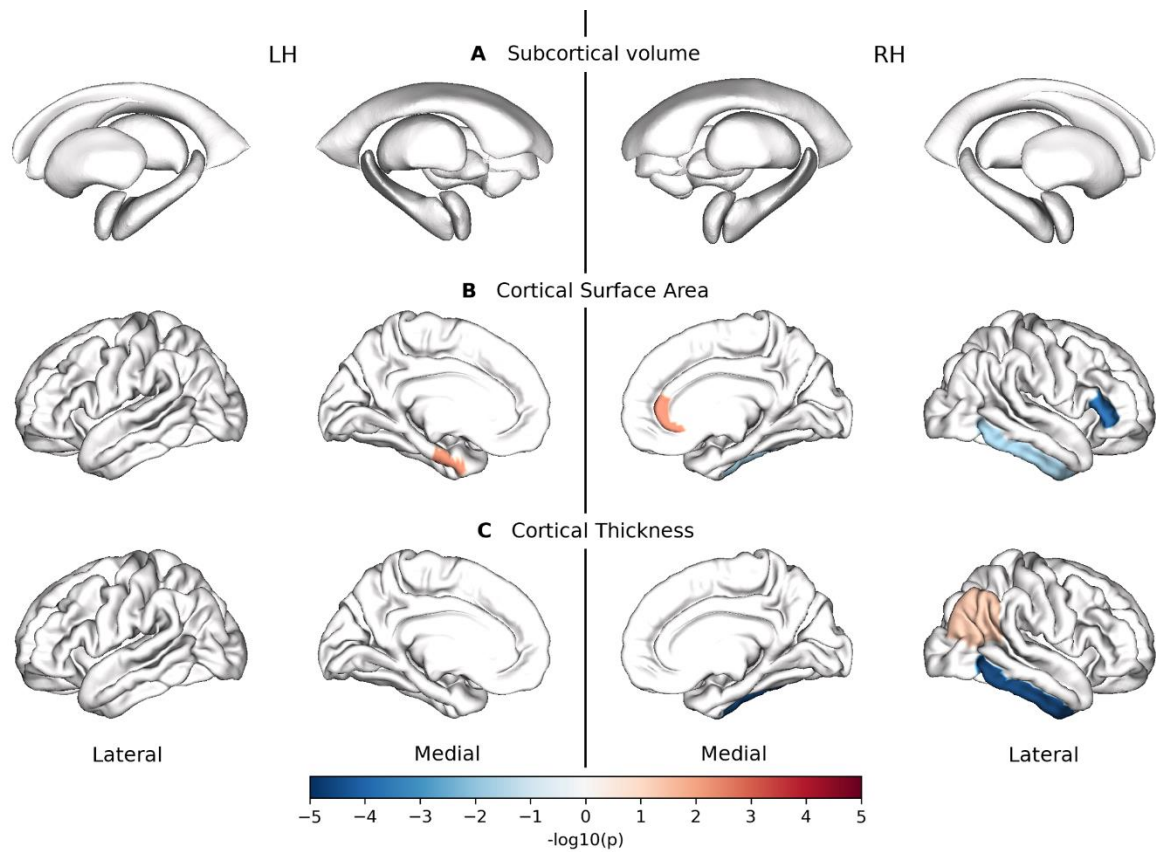


Figure 7. The $-\log(p)$ value maps characterizing significant brain regions contributing the most to low severity patients versus healthy controls classification across working groups as determined by sign-based consistency mapping (40). Hot colors indicate consistently assigned positive weights by the SVM that drive classification towards the patients class, and cold colors indicate negative weights that drive classification towards controls. The figure was made with the ENIGMA Toolbox package (<https://enigma-toolbox.readthedocs.io/en/latest/>). Full region names may be found in **Supplementary Table S5**. LH = Left Hemisphere, RH = Right Hemisphere.

Discussion

In this study we benchmarked classification of youth with anxiety disorders based on brain morphology, using structural MRI data from the large-scale multi-site ENIGMA-Anxiety Working Group, comprising a heterogeneous sample of 3,343 participants from 32 international sites. Modest, yet robust, classification performance was achieved for PD vs. HC (AUC=0.62), but classification performances of SAD, GAD, or any anxiety disorder (transdiagnostic) vs. HC failed to surpass chance-level significance. However, above chance-level transdiagnostic classification performances were obtained for particular subgroup analyses, namely, male patients vs. male HC, unmedicated patients vs. HC and low severity patients vs. HC, with AUCs ranging between 0.59-0.63. Of note, these above chance-level disorder-specific and transdiagnostic subgroup classifications were based on plausible and specific neuroanatomical features in fronto-striato-limbic and temporo-parietal regions. This study provides a realistic estimate of classification performance, which can be achieved in a large, ecologically valid, multi-site sample of young anxiety patients based on neurostructural data. While short of the clinically relevant AUC threshold of 0.80 (42), our results are on par with recent brain-based ML classifications of psychopathologies conducted in ENIGMA and other multi-site consortia.

PD Classifier

We identified several brain regions important for PD classification: CT of the left middle temporal gyrus and right rostral anterior cingulate cortex, along with the CSA of left superior frontal gyrus and SCV of the left pallidum. The middle temporal gyrus embedded in the temporal lobe is part of the limbic system, and involved in emotional responding, and thought to play an important role in PD pathophysiology (43,44). Perturbed temporal lobe GMV, specifically in the middle temporal gyrus and transverse temporal gyrus are reported in PD patients (44-46). The anterior cingulate cortex is another key region for PD, as it is implicated in the modulation of both normal and pathological anxiety, with VBM meta-analyses linking its disintegrity to PD diagnosis (45,47). Several studies also report alterations in task-based activation associated with treatment response towards cognitive-behavioral therapy (23,24). The superior frontal gyrus is involved in cognitive control and emotion regulatory processes and several studies link abnormal GMV in this region to PD pathophysiology (43,45,48). Finally, the pallidum is part of the lenticular nucleus residing in the basal ganglia, and is a convergence point for limbic reward signals and involved in diverse cognitive, affective and motor processes (49). Voxel-based morphometry (VBM) meta-analyses link gray matter volume (GMV) anomalies within the lenticular nucleus to clinical anxiety in general (47),

as well as PD specifically (45). Altogether, the aggregation of findings suggests that brain regions deemed important for PD classification are biologically plausible.

Sex, Medication, Severity Classifier

We investigated transdiagnostic classifications for subgroups with particular demographic or clinical characteristics, with the goal of reducing heterogeneity across sites and to potentially boost performance. Transdiagnostic case-control classifications were significantly above chance level for the specified subgroups (up to 0.63 AUC, $p < .05$) but not for the entire sample (AUC=0.56, $p = .093$). As depicted in **Figures 4-7**, the multivariate neurostructural pattern behind these transdiagnostic subgroup classifications comprised of fronto-parieto-limbic territories, previously linked to anxiety and its demographic (i.e., sex) and clinical (i.e., severity/medication use) and characteristics (6,7,35,50-53).

Whereas transdiagnostic classification of male anxiety patients vs. male HC could be reliably achieved (AUC=0.63, $p = .007$), this was not the case for females. Such male-specific effects interestingly also emerged in a recent ENIGMA-Anxiety GAD case-control group comparison (50), while increased variability in female brain structure due to menarche, menstrual cycle and hormonal contraceptives is also suggested (54). We also found that unmedicated patients could be distinguished from HC (AUC=0.59, $p = .013$), which was not the case for classification of medicated patients vs. HC or medicated vs. unmedicated patients. One may speculate that heterogeneity in medication type, dosage, or duration among medicated patients could differentially influence brain structure and render classification more complicated. Alternatively, psychotropic medication usage may normalize some neurostructural alterations in anxiety patients (4,6), a phenomenon also seen in other psychopathologies (55,56), and hence complicate classification from HC and their unmedicated anxious peers. Longitudinal studies that incorporate more detailed medication information may provide better insight into the short- and long-term effects of medication on brain structure in each specific disorder and across anxiety disorders.

Similarly, whereas low severity patients (as indexed by a below median STAI-trait score) could be distinguished from HC above chance-level (AUC=0.59, $p = .016$), this could not be achieved for classification of high severity patients vs. HC or high severity vs. low severity patients. While this result might be counterintuitive, it might also be attributed to the normalizing effects of medication in high severity patients, as severity and medication status were found to be positively correlated ($\Phi = 0.21$, $p < .001$). Alternatively, there might be more

divergent brain abnormalities in high severity PD/SAD/GAD patients (e.g., larger or smaller CT/CSA/SCV depending on the disorder), or larger variance in demographic and/or clinical characteristics (across disorders and sites) compared to low severity patients. Nonetheless, the significance (or lack of it, following permutation testing) for certain classifications could also reflect false positives or false negatives, and additional research is clearly needed to further explore, validate, and refine these subgroup findings. Ideally, we would have tested these subgroup classifications also for each anxiety disorder separately, but this was simply not feasible given the small number of patients who would have remained after filtering.

Classifier Performance and Site-Effects

Our results are on par with other ENIGMA studies (obsessive-compulsive disorder (OCD), bipolar disorder (BP), major depression disorder (MDD)) that used FreeSurfer data for case-control classification in a multi-site setting, with AUCs ranging between 0.62-0.72 (27-29). Similar to our results, none of the obtained performances reached the clinical threshold of $AUC > 0.80$ (42). These findings suggest that although there could be significant case-control differences in CT/CSA/SCV on the *group-level*, parcellated structural MRI data may at the moment not fully allow for clinically relevant case-control distinctions at the *individual-level*. Notably, the classification performances obtained here (up to 0.63 AUC) translate to effect sizes (Cohen's $d=0.47$; medium effect size, see (30) for AUC to effect size conversion) typically larger than those obtained in previous ENIGMA univariate case-control analyses of FreeSurfer data among psychiatric populations, such as OCD (max Cohen's $d=-0.33$ for CSA), BP (max Cohen's $d=-0.29$ for SVC), schizophrenia (SZC) (max Cohen's $d=-0.46$ for SVC), MDD (max Cohen's $d=-0.2$ for SCV, but up to -0.57 for CSA in adolescents vs matched controls), and attention deficit hyperactivity disorder (max Cohen's $d=-0.19$ for SVC) (26,57-61). However, one should be cautious to directly compare the above effect sizes, as our AUC-converted effect size was based on a multivariate method estimated through CV (out-of-sample) while the case-control effect sizes are in sample and univariate in nature. Future studies could apply more fine-grained neurostructural features (i.e., voxel-wise/vertex-wise maps), measures of brain connectivity and network function or multimodal data in combination with more sophisticated classification methods (i.e., Deep Neural Networks) (15,27). A combination of these options is postulated to improve classification performances compared to shallow ML algorithms applied to low-resolution data (15).

To our knowledge, this is to date the largest, multi-site brain-based classification analysis of anxiety disorders among youth. Previous structural and functional MRI-based classification studies on anxiety disorders

have claimed to distinguish patients from HC, with AUCs reaching over 0.80 (17-24). However, these studies relied on small samples ($N < 100$) susceptible to unstable performance estimations during cross-validation (62,63), in which inflated performances are likely to be overrepresented through publication bias or insufficient testing (64-66). In addition, studies typically used data from a single research site, and performance typically drops when models are tested on unseen data from other sites. The large-scale multi-site ENIGMA database employed here provides ecologically valid and clinically representative information, which may be used by ML algorithms to identify multivariate patterns generalizable to patients across different sites (27-29). Having a large sample size and thus more data for model training and testing is typically beneficial for ML, and leads to more reliable performance estimates (36,67,68). However, pooling existing data in a multi-site context like ENIGMA may also reveal that methods have poorer true performance in reality, due to the realistic heterogeneity (in sample characteristics, hardware and methodology) that is actually represented (27,28,30). These global between-site differences, referred to as “site-effects”, can hamper the ability of the classifier to find common brain abnormalities between patients and HC across different sites. Site-effects can also leave confounds in the data that obscure interpretations, impair the generalizability and reproducibility of classification models, and lead to biased performance (27,37).

We addressed site-effects by standardizing neuroimaging data from each site according to their HC reference group. Without site-wise scaling, the SVM was able to accurately predict which site a given participant belongs to, but not when site-wise scaling was applied (**Table_S8**). Performances obtained for classifications using LOSO-CV were comparable to K-Fold CV, further indicating that our standardization approach effectively harmonized data across sites. We also accounted for site-effects in our permutation testing framework by restricting the exchangeability of class-labels to each site, so that remaining site-effects would be incorporated in the obtained null-distribution (see **Supplementary_Discussion**) (69). There are other methods available to handle site-effects, such as ComBat and normative modeling, but the assumptions for these techniques (i.e., $N > 25$ subjects per site, balanced samples and overlapping distribution of covariates across sites) are violated here, rendering them unsuitable for this study (37,70). Future classification studies on data from the ENIGMA-Anxiety Working Group using larger samples could investigate the feasibility of other site-harmonization techniques.

Strengths & Limitations

The key advantages of this study include the large worldwide sample (N=3,343 from 32 sites), access to *individual-level* data, and the conservative nature of the analyses wherein possible site-effects/data leakage/overfitting were stringently confronted. The international heterogeneity and multi-site nature allowed us to test ML classifications on a wide range of participants, more closely resembling the real-world clinical situation. Notwithstanding these strengths, there are several limitations to consider.

We used a global and retrospectively pooled data sample, where no harmonization of scanning acquisition or sample inclusion was performed. These sources of heterogeneity may have limiting effects on classification performance, but also provide an opportunity for a more realistic evaluation across samples that better represent the wide range of characteristics seen in the real-world population. This would be considered a strength, as it is necessary to evaluate whether results are reliable across a variety of hardware and protocols.

Since we specifically focused on youth (10-25 years), we had to exclude participants from each sample outside this age range and ended up with a relatively large number of sites with small sample sizes. This limited our options for site-effect harmonization, and led to insufficient sample sizes per site to investigate subgroup classifications for each anxiety disorder separately. Also, whereas developmentally-driven heterogeneity may have been at play, the sample size (especially patients) made it practically impossible to formally test this, as we lacked sufficient data for training/testing folds per meaningful age grouping. However, supplementary analyses were run to assess the link between age and classification performance within the transdiagnostic sample among different age groups (10-14 early adolescence; 15-17 middle adolescence; 18-25 late adolescence and young adults, (31,71,72). As seen in **Figure_S1**, there was no statistically significant association between the obtained performances and age groups ($r=-0.50$, $p=.667$), suggesting that the model performed equally well across the age spectrum investigated. Finally, we only had access to regional brain measures (FreeSurfer features), not raw/voxel-wise data or other brain-imaging modalities (i.e., function/connectivity). Incorporation of these additional data forms, and use of more sophisticated ML algorithms (i.e., deep learning) better apt in handling massively multi-modal data, might further improve classification performances reported here (15).

Conclusion

This study provides a realistic estimate of ML classification performance, which can be achieved in a large, ecologically valid, multi-site sample of youth with anxiety disorders based on neurostructural data. While short

of the clinically relevant AUC threshold of 0.80, our results are on par with recent brain-based ML classifications of psychopathologies conducted in ENIGMA and other multi-site consortia. This classification study sets a baseline for the ENIGMA-Anxiety Working Group and is an important step towards the development of models that could ultimately inform early detection, prevention, and care among clinically anxious youth. The added value compared to conventional diagnostic tools (e.g., structured interviews and questionnaires) needs to be further evaluated.

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