BRIEF REPORT

Genetic Risk Factors in Isolated Dystonia Escape Genome-Wide Association Studies

Björn-Hergen Laabs, PhD,[1] Katja Lohmann, PhD,[2] Eva-Juliane Vollstedt, MD,[2] Tobias Reinberger, PhD,[3] Lisa-Marie Nuxoll, MSc,[1] Gamze Kilic-Berkmen, PhD,[4] Joel S. Perlmutter, MD,[5] Sebastian Loens, MD,[6] Carlos Cruchaga, PhD,[7] Andre Franke, PhD,[8] Valerija Dobricic, PhD,[9] Frauke Hinrichs,[2] Anne Grözinger, BSc,[2] Eckart Altenmüller, MD,[10] Steven Bellows, MD,[11] Sylvia Boesch, MD, MSc,[12] Susan B. Bressman, MD,[13,14] Kevin R. Duque, MD,[15] Alberto J. Espay, MD,[15] Andreas Ferbert, MD,[16] Jeanne S. Feuerstein, MD,[17] Samuel Frank, MD,[18] Thomas Gasser, MD,[19,20] Bernhard Haslinger, MD,[21] Robert Jech, MD, PhD,[22] Frank Kaiser, PhD,[23,24] Christoph Kamm, MD,[25] Katja Kollewe, MD,[26] Andrea A. Kühn, MD,[27] Mark S. LeDoux, MD, PhD,[28,29] Ebba Lohmann, MD,[19,20] Abhimanyu Mahajan, MD, MHS,[30] Alexander Münchau, MD,[6] Trisha Multhaupt-Buell, MS, CGC,[31] Alexander Pantelyat, MD,[32] Sarah E. Pirio Richardson, MD,[33] Deborah Raymond, MS, CGC,[13] Stephen G. Reich, MD,[34] Rachel Saunders Pullman, MD, MPH, MSc,[13,14] Barbara Schormair, PhD,[35,36] Nutan Sharma, MD, PhD,[31] Azadeh Hamzehei Sichani, MA,[14,37] Kristina Simonyan, MD, PhD, DrMed,[14,30,31,37] Jens Volkmann, MD,[38] Aparna Wagle Shukla, MD,[39] Juliane Winkelmann, MD,[35,36,40] Laura J. Wright, MA,[41] Michael Zech, MD,[35,36] Kirsten E. Zeuner, MD,[42] Simone Zittel, MD,[43] Meike Kasten, MD,[2,44] Yan V. Sun, PhD,[45] Tobias Bäumer, MD,[6] Norbert Brüggemann, MD,[46] Laurie J. Ozelius, PhD,[31] Hyder A. Jinnah, MD, PhD,[4] Christine Klein, MD,[2*] and Inke R. König, PhD[1*]

[1]*Institute of Medical Biometry and Statistics, University of Lübeck, Lübeck, Germany* [2]*Institute of Neurogenetics, University of Lübeck, Lübeck, Germany* [3]*Institute for Cardiogenetics, University of Lübeck, Lübeck, Germany* [4]*Department of Neurology, Emory University, Atlanta, Georgia, USA* [5]*Department of Neurology, Radiology and Neuroscience, Washington University School of Medicine, St. Louis, Missouri, USA* [6]*Institute of Systems Motor Science, CBBM, University of Lübeck, Lübeck, Germany* [7]*Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri, USA* [8]*Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany* [9]*Lübeck Interdisciplinary Platform for Genome Analysis, University of Lübeck, Lübeck, Germany* [10]*Institute of Music Physiology and Musician's Medicine, Hanover University of Music, Drama and Media, Hanover, Germany* [11]*Parkinson's Disease Center and Movement Disorder Clinic, Baylor College of Medicine, Houston, Texas, USA* [12]*Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria* [13]*Department of Neurology, Mount Sinai Beth Israel Medical Center, New York, New York, USA* [14]*Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, New York, USA* [15]*James J. and Joan A. Gardner Family Center for Parkinson's Disease and Movement Disorders Neurology and Rehabilitation Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA* [16]*Department of Neurology, Kassel School of Medicine, Klinikum Kassel, Kassel, Germany* [17]*Department of Neurology, School of Medicine, University of Colorado, Aurora, Colorado, USA* [18]*Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA* [19]*Department of Neurology, University of Tübingen, Tübingen, Germany* [20]*Hertie Institute for Clinical Brain Research and DZNE, University of Tübingen, Tübingen, Germany* [21]*Department of Neurology, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany* [22]*Department of Neurology, Charles University in Prague, 1st Faculty of Medicine and General University Hospital in Prague, Prague, Czech Republic* [23]*Institute of Human Genetics, University Hospital Essen, University Duisburg-Essen, Essen, Germany* [24]*Essener Zentrum für Seltene Erkrankungen, University Hospital Essen, Essen, Germany* [25]*Department of Neurology, University Medical Centre Rostock, Rostock, Germany* [26]*Clinic for Neurology, Hannover Medical School, Hannover, Germany* [27]*Department of Neurology and Experimental Neurology, Charité–University Medicine, Berlin, Germany* [28]*Veracity Neuroscience LLC, Memphis, Tennessee, USA* [29]*Department of Psychology, University of Memphis, Memphis, Tennessee, USA* [30]*Department of Neurological Sciences, RUSH University, Chicago, Illinois, USA* [31]*Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA* [32]*Department of Neurology, Johns Hopkins Medicine, Baltimore, Maryland, USA* [33]*Department of Neurology, University of New Mexico, Albuquerque, New Mexico, USA* [34]*University of Maryland School of Medicine, Baltimore, Maryland, USA* [35]*Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany* [36]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany* [37]*Department of Otolaryngology–Head and Neck Surgery, Massachusetts Eye and Ear and Harvard Medical School, Boston, Massachusetts, USA* [38]*Department of Neurology, University Hospital Würzburg, Würzburg, Germany* [39]*Department of Neurology, University of Florida, Gainesville, Florida, USA* [40]*Munich Cluster for Systems Neurology, SyNergy, Munich, Germany* [41]*Department of Neurology, Washington University School of Medicine, St. Louis, Missouri, USA* [42]*Clinic for Neurology, Christian-Albrechts-University, Kiel, Germany* [43]*Department of Neurology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany* [44]*Department of Psychiatry and Psychotherapy, University of Lübeck, Lübeck, Germany* [45]*Department of Epidemiology, Emory University Rollins School of Public Health, Emory University, Atlanta, Georgia, USA* [46]*Department of Neurology, University of Lübeck, Lübeck, Germany*

ABSTRACT: Background: Despite considerable heritability, previous smaller genome-wide association studies (GWASs) have not identified any robust genetic risk factors for isolated dystonia.

Objective: The objective of this study was to perform a large-scale GWAS in a well-characterized, multicenter

sample of >6000 individuals to identify genetic risk factors for isolated dystonia.

Methods: Array-based GWASs were performed on autosomes for 4303 dystonia participants and 2362 healthy control subjects of European ancestry with subgroup analysis based on age at onset, affected body regions, and a newly developed clinical score. Another 736 individuals were used for validation.

Results: This GWAS identified no common genome-wide significant loci that could be replicated despite sufficient power to detect meaningful effects. Power analyses imply that the effects of individual variants are likely very small.

Conclusions: Moderate single-nucleotide polymorphism–based heritability indicates that common variants do not contribute to isolated dystonia in this cohort. Sequence-based GWASs (eg, by whole-genome sequencing) might help to better understand the genetic basis. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: isolated dystonia; GWAS; age at onset; clinical score; case–control

Genetic factors contributing to isolated dystonia[1,2] are largely unknown. Notably, 25% of patients with various forms of isolated dystonia also have relatives with dystonia, suggesting a substantial genetic contribution.[3,4] Although several monogenic causes have been

*__Correspondence to:__ Prof. Dr. Christine Klein, Institute of Neurogenetics, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: christine.klein@uni-luebeck.deProf. Dr. Inke R. König, Institute of Medical Biometry and Statistics, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: inke.koenig@uni-luebeck.de

Björn-Hergen Laabs, Katja Lohmann, and Eva-Juliane Vollstedt contributed equally to this work.

Christine Klein and Inke R. König are cosenior authors and contributed equally to this work.

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identified in dystonia,[5] they explain the molecular pathogenesis in only a minority of patients, mostly those with early onset and additional clinical features.[6,7] In contrast, genetic risk factors that are thought to contribute to the disease in the remainder of patients are largely unknown. Candidate-based association studies in dystonia were not insightful.[8] Genome-wide association studies (GWASs) in large patient–control samples are considered effective for the hypothesis-free identification of genetic risk variants. Because dystonia is a relatively rare disorder, only three rather small GWASs have been reported to date, including 158, 212, and 919 patients,[9-11] and focused on either patients with cervical[9,10] or musicians' dystonia.[11] Potential candidates could not be confirmed unequivocally.[10,12,13] Given these overall inconclusive results and limitations, we performed a larger GWAS in different types of isolated dystonia and carried out subgroup analyses.

Participants and Methods

Study Participants

We included 4303 patients with dystonia and 2362 healthy controls. Samples were mainly recruited in the United States and Germany (Supporting Information Tables S1 and S2). All samples were of European ancestry, as confirmed by principal-component analysis against 1000Genomes. All participants gave written informed consent and underwent a standardized neurological examination by a movement disorder specialist. For the replication of potential hits, we used existing genotype data from 736 German dystonia patients (Affymetrix Axiom or Illumina Global Screening Array arrays). Patients with secondary causes of dystonia or monogenic dystonia were excluded. The study was approved by the Ethics Committees of all participating clinical centers.

First, we performed a case–control association study. Next, we carried out subgroup analyses in patients with available information including age at onset (AAO; as a continuous variable) and a newly developed clinical score scaling the degree of presumed genetic burden[6] with a maximum of 6 points as follows: family history (yes: 2 points; no: 0 points), AAO (<21 years: 2 points; 21–50 years: 1 point; >50 years: 0 points), and distribution of dystonic features (generalized: 2 points; segmental/multifocal: 1 point; focal: 0 points).

Further, we analyzed association with AAO and the clinical score in two patient subgroups based on the site of onset (Table S2), that is, craniocervical onset and onset in the upper extremities. Final sample sizes and demographic information for each analysis are given in Table 1, and a flow chart depicting sample exclusion is provided in Supporting Information Figure S9.

Genetic and Statistical Analyses

For the GWAS, blood-derived DNA was analyzed by genome-wide SNP (single-nucleotide polymorphism) genotyping using the Infinium Global Screening Array (Illumina Inc.) for all but the US control subjects, who were genotyped using the Infinium Global Diversity Array (GDA; Illumina Inc.). After quality control and imputation (for details, see Supporting Information Methods in Data S1), we applied SNPTEST v.2.5.1[14] for analysis of each cohort (German, US) separately, using logistic regression models for the overall affected status and linear regression models for the patient-only analyses. Details on the methods and quality control are given in the Supporting Information.

To identify variants with similar effects in the German and US cohorts, we combined both results in respective meta-analyses (META v1.7[14]) using an inverse-variance method based on a fixed-effects model.

All variants with $P < 5 \times 10^{-8}$ were considered genome-wide significant to account for multiple testing based on a Bonferroni correction per GWAS. A potentially meaningful GWAS signal was defined to require at least five SNPs with $P < 5 \times 10^{-5}$.

In addition, we conducted a power estimation for the case–control, the AAO, and the clinical score GWAS.

Finally, we used GCTA[15] to estimate SNP-based heritability in our datasets. For heritability estimation of case–control status, we assumed a prevalence of idiopathic dystonia of 16.4/100,000.[16] Heritability was estimated in each cohort separately and combined using the inverse variance–weighted method for meta-analyses.

Replication Analyses

We tested single SNPs with genome-wide significance in the case-only analyses from regions with at least five SNPs with $P < 5 \times 10^{-5}$ in the meta-analyses using a previously genotyped European replication sample with 736 dystonia cases. The same models were applied as before, except for the adjustment for principal components.

Results

Genome-Wide Association Analyses

In the Aiation studies, there were no strong genome-wide significantly associated signals (Fig. 1, Supporting Information Figs. S1–S3). All suggestive signals are listed in Table S3. Noteworthy hits, also from a functional point of view, that is, predicted gene function overlapping with pathways known to be altered in dystonia,[17] included rs77507424 (minor allele frequency [MAF] = 0.05; Beta = 4.39 years; $P = 2.55 \times 10^{-7}$) on chromosome 5 and rs2536490 (MAF = 0.09; Beta = 3.85 years; $P = 5.79 \times 10^{-7}$) on chromosome 7 for the AAO association study. The former signal harbors *PDE6A*, encoding a phosphodiesterase. Phosphodiesterases have been shown

TABLE 1 *Sample sizes and demographic characteristics per analysis after quality control*

	Origin			
	Germany		USA (with European Ancestry)	
	Cases	Controls	Cases	Controls
Case–control status				
No. of samples	1424	1345	1113	933
Age (mean, SD), y	42.8 (16.3)	55.5 (14.3)	44.8 (15.4)	66.7 (8.9)
Sex				
f	817	707	762	501
m	581	638	351	432
u	26	0	0	0
Age at onset (mean, SD), y	42.8 (16.3)	–	44.8 (15.3)	–
No. of samples	1277	–	1253	–
Sex				
f	761	–	848	–
m	515	–	405	–
u	1	–	0	–
Age at craniocervical onset (mean, SD), y	46.3 (15.3)	–	45.2 (14.6)	–
No. of samples	612	–	1006	–
Sex				
f	413	–	747	–
m	199	–	259	–
Age at upper extremities onset (mean, SD), y	36.4 (17.4)	–	36.1 (17.7)	–
No. of samples	139	–	187	–
Sex				
f	72	–	108	–
m	67	–	79	–
Clinical score (mean, SD)	1.34 (1.13)	–	1.28 (1.10)	–
No. of samples	1313	–	1080	–
Age (mean, SD), y	56.7 (14.6)	–	59.8 (12.9)	–
Sex				
f	787	–	737	–
m	525	–	343	–
u	1	–	0	–
Clinical score (craniocervical onset) (mean, SD)	1.37 (1.14)	–	1.27 (1.08)	–
No. of samples	576	–	847	–
Age (mean, SD), y	60.0 (12.6)	–	61.0 (11.5)	–
Sex				
f	387	–	637	–
m	189	–	210	–

(Continues)

TABLE 1 *Continued*

	Origin			
	Germany		USA (with European Ancestry)	
	Cases	Controls	Cases	Controls
Clinical score (upper extremities onset) (mean, SD)	1.54 (1.18)	–	1.83 (1.34)	–
No. of samples	142	–	169	–
Age (mean, SD), y	54.1 (15.0)	–	55.3 (14.6)	–
Sex				
f	73	–	99	–
m	69	–	70	–

Abbreviations: SD, standard deviation; f, female; m, male; u, unknown.

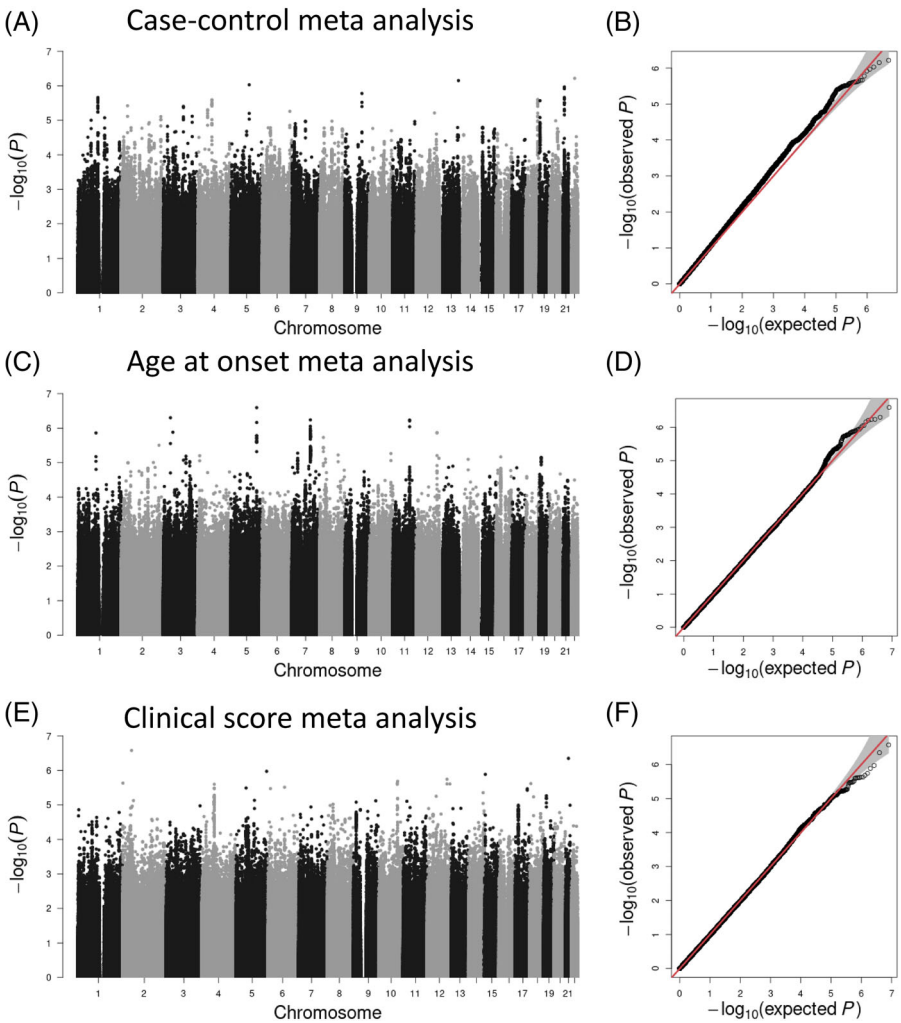

FIG. 1. Results of genome-wide association study for three different traits in patients with isolated dytonia. Manhattan plots for case–control status (**A**), age at onset (**C**), and a clinical score based on age at onset, symptom distribution, and family history (**E**), together with corresponding QQ plots of the *P* value distribution (**B**, **D**, **F**). All results are based on meta-analyses combining a US and a German cohort. [Color figure can be viewed at wileyonlinelibrary.com]

to regulate striatocortical basal ganglia circuitry and movement control via cyclic adenosine monophosphate (cAMP) signaling,[17,18] and pathogenic variants in *PDE10A* have been linked to dystonic symptoms. The signal on chromosome 7 is located in *PRKAR2B* that has also been linked to cAMP signaling.[19,20]

In patients with craniocervical onset (n = 1618), one variant (rs3010282) on chromosome 6 (MAF = 0.30; Beta = −4.19 years; $P = 2.47 \times 10^{-9}$) was genome-wide significantly associated with AAO (Supporting Information Fig. S4) and is located within a long non-coding RNA gene (ENSG00000226571). Two additional SNPs were considered candidates: rs9319387 (MAF = 0.49; Beta = −3.21 years; $P = 2.27 \times 10^{-7}$) on chromosome 13 and rs3744730 (MAF = 0.02; Beta = −7.86 years; $P = 3.06 \times 10^{-7}$) on chromosome 17 (Table S3). The nearest genes to the former signal are *POLR1D* (primarily expressed in skin[21]) and *NPM1P4*, a pseudogene. The signal on chromosome 17 is located in *VPS53*, encoding a vacuolar protein sorting protein involved in recycling endocytic vesicles.[22]

In patients with onset in the upper extremities (n = 326), we found one variant (rs7907011) on chromosome 10 that showed genome-wide significance (MAF = 0.29; Beta = −13.74 years; $P = 3.55 \times 10^{-8}$) (Fig. S4) within *LOXL4*, encoding a lysyl oxidase that is relevant to the extracellular matrix and development.[23]

Using the clinical score in cases with craniocervical onset (n = 1423), one genome-wide significant variant (rs3802288) was identified on chromosome 8 (MAF = 0.06; Beta = 0.66; $P = 1.19 \times 10^{-8}$) in *ASPH* (a gene primarily expressed in brain and retina[21]).

Finally, one variant (rs77695916; MAF = 0.01; Beta = 1.80; $P = 4.48 \times 10^{-8}$) on chromosome 5 was genome-wide significantly associated with the clinical score in patients with onset in the upper extremities (n = 311). The signal harbors *MFAP3*, which is primarily expressed in brain and skin.[21]

Replication Analyses

None of the 14 SNPs in the replication analysis was associated with the respective trait suggested in the initial GWAS phase at a significance level of $P < 0.05/14 = 0.0036$. Detailed results on replication can be found in Supporting Information Table S3.

Power Estimation

With the case–control meta-analyses, we could have detected common variants (MAF > 20%) with an odds ratio ≥1.5 with a power of >90%. The power was also >90% to detect common variant associations with an effect of 4 years on AAO or 0.4 point on the clinical score, respectively. Detailed results on statistical power are shown in Supporting Information Figure S8.

Heritability Analysis

Overall, we observed moderate heritability of 14% for case–control status, 24% for AAO, and 19% for the clinical score. This means that <25% of the variance in the three different traits can be explained by the genotyped and imputed SNPs.

Discussion

This study aimed to identify genetic risk factors in isolated dystonia. We included a large sample (4108 isolated dystonia patients and 2357 healthy control subjects) and conducted multiple GWASs. Despite being the largest GWAS study to date of isolated dystonia, no robust, replicable associations were found in the overall comparison of cases and control subjects, AAO, or clinical score. Although there was <60% availability of information in the patients to calculate the clinical score (AAO, family history, distribution of dystonia), the study had an overall high power to detect meaningful effects. Notably, associations from previous genome-wide analyses have not been replicated. Furthermore, analyses for AAO and clinical score in subgroups of patients stratified for site at onset yielded several candidate hits. They should be followed up in subsequent studies with larger samples.

Despite the strengths of our study (largest sample size, sufficient power, clinical score, analyses of subgroups), there were several limitations. First, the study included only individuals of European ancestry, limiting the generalizability of the results to other populations. Second, other possible contributing factors such as environmental exposures or epigenetic modifications were not assessed. Third, although 10 genetic principal components were used to decrease population stratification bias, they might have reduced the power to find significantly associated SNPs. Finally, different arrays were used in the US cohort for cases and control subjects, which could have led to batch effects. However, we applied very strict quality criteria to overcome potential biases.

Notably, this study showed two important lessons and helped develop a new hypothesis regarding the impact of genetic variants for isolated dystonia in Europeans. First, there is likely high polygenicity, that is, many risk variants, including rare variants, with relatively small effects contributing to the development of dystonia, as has been demonstrated for other diseases, such as schizophrenia.[24] Thus, a much larger sample size would be required to identify the impact of common variants. In addition, sequencing-based GWAS (eg, whole-genome sequencing–based GWAS) might identify risk factors by detecting rarer variants and other variant types such as copy number variants. Along these lines, the heritability estimate indicated that <25% of the variance addressed by our GWAS can be attributed to the analyzed common variants. Second, although we included only participants with isolated dystonia and performed subgroup analyses, the included participants may still be heterogeneous with distinct biological mechanisms underlying their dystonia,[25] involving different sets of risk identification. To define molecular-based subgroups, the identification of meaningful biomarkers, which are currently unavailable, would be a prerequisite.

This study, consistent with prior smaller GWASs, did not demonstrate robust genetic risk factors for isolated dystonia, suggesting that the risk factors for dystonia are likely more complex and may involve rare variants. Because it is not feasible to significantly enlarge the sample size, we need alternative approaches to identify further genetic contributions to dystonia, including testing other ethnicities, evaluating low-frequency variants with small effect sizes by genome sequencing, and aiming for a molecularly driven stratification of our patients.

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Data Availability Statement

The data that support the findings of this study are openly available in GWAS Catalog at https://www.ebi.ac.uk/gwas/home, reference number GCP000887.

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Supporting Data

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(1) Research project: A. Conception and design, B. Organization, C. Data acquisition and analysis; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript preparation: A. Drafting a significant portion of the manuscript and/or figures, B. Review and critique.

B.-H.L.: 1A, 1B, 2A, 2B, 3A
K.L.: 1A, 1B, 1C, 2C, 3A
E.-J.V.: 1A, 1B, 1C, 2C, 3B
T.R.: 1C, 2A, 2C, 3B
L.-M.N.: 1C, 2B, 3A, 3B
G.K.-B.: 1B, 1C, 2C, 3B
S.L.: 1B, 1C, 2C, 3B
C.C.: 1C, 2A, 2C, 3B
A. Franke: 1B, 1C, 2C, 3B
V.D.: 1B, 2C, 3B
F.H.: 1C, 2C, 3B
A.G.: 1C, 2C, 3B
E.A.: 1C, 2C, 3B
S. Bellows: 1C, 2C, 3B
S. Boesch: 1C, 2C, 3B
S.B.B.: 1C, 2C, 3B
K.R.D.: 1C, 2C, 3B
A.J.E.: 1C, 2C, 3B
A. Ferbert: 1C, 2C, 3B
J.S.F.: 1C, 2C, 3B
S.F.: 1C, 2C, 3B
T.G.: 1C, 2C, 3B
B.H.: 1C, 2C, 3B
R.J.: 1C, 2C, 3B
F.K.: 1B, 2C, 3B
C. Kamm: 1C, 2C, 3B
K.K.: 1C, 2C, 3B
A.A.K.: 1C, 2C, 3B
M.S.L.: 1C, 2C, 3B
E.L.: 1C, 2C, 3B
A. Mahajan: 1C, 2C, 3B
A. Münchau: 1C, 2C, 3B
T.M.-B.: 1C, 2C, 3B
A.P.: 1C, 2C, 3B
S.E.P.R.: 1C, 2C, 3B
D.R.: 1C, 2C, 3B
S.R.: 1C, 2C, 3B
R.S.P.: 1C, 2C, 3B
B.S.: 1C, 2C, 3B
N.S.: 1C, 2C, 3B
A.H.S.: 1C, 2C, 3B
K.S.: 1C, 2C, 3B
J.V.: 1C, 2C, 3B
A.W.S.: 1C, 2C, 3B
J.W.: 1C, 2C, 3B

L.J.W.: 1C, 2C, 3B
M.Z.: 1C, 2C, 3B
K.E.Z.: 1C, 2C, 3B
S.Z.: 1C, 2C, 3B
M.K.: 1B, 1C, 2C, 3B
Y.V.S.: 1C, 2A, 2C, 3B
T.B.: 1B, 1C, 2C, 3B
J.S.P.: 1B, 1C, 2C, 3B
N.B.: 1B, 1C, 2C, 3B
L.J.O.: 1B, 1C, 2C, 3B
H.A.J.: 1B, 1C, 2C, 3A
C. Klein: 1A, 1B, 1C, 2C, 3A
I.R.K.: 1A, 1B, 2A, 2C, 3A

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