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Title page

Hyperhidrosis and human leucocyte antigens in the Danish Blood Donor Study

Running title: Hyperhidrosis and human leucocyte antigens

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Abbreviations

BMI, Body mass index

CIs, Confidence intervals

DBDS, Danish Blood Donor Study

DNPR, Danish National Patient Registry

GWAS, Genome-wide association study

HH, Hyperhidrosis

HLA, Human leucocyte antigen

ICD-10, International classification of disease 10th edition

IQR, Interquartile range

ORs, Odds ratio

PC, Principal component

SNP, Single nucleotide polymorphisms

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Abstract

Familial clustering of the skin disease primary hyperhidrosis suggests a genetic component to the disease. The human leucocyte antigen (HLA) is implicated in a range of diseases, including many comorbidities to hyperhidrosis. No study has investigated whether the HLA genes are involved in the pathogenesis of hyperhidrosis. We, therefore, compared HLA alleles in individuals with and without hyperhidrosis in this study of 65,000 blood donors. In this retrospective cohort study, we retrieved information on individuals with and without hyperhidrosis using self-reported questionnaires, the Danish National Patient Registry and the Danish National Prescription Registry on participants recruited to the Danish Blood Donor Study between 2010 and 2019. Association tests using logistic regression were conducted for each HLA allele corrected for sex, age, body mass index, smoking and principal components. Overall, 145 of 65,795 (0.2%) participants had hospital diagnosed hyperhidrosis. Similarly, 1,379 of 15,530 (8.9%) participants had moderate-severe self-reported hyperhidrosis, of whom 447 (2.9%) had severe self-reported hyperhidrosis. Altogether 28 participants had both hospital diagnosed and moderate-severe self-reported hyperhidrosis. Severe self-reported hyperhidrosis was associated with HLA-A*80:01 (adjusted odds ratio 26.97; 95% confidence interval 5.32–136.70; n=7; $p<0.001$). Moderate-severe self-reported hyperhidrosis and hospital diagnosed hyperhidrosis were not associated with any HLA. The association between hyperhidrosis and HLA-A*80:01 was based on a very small number of cases and not replicated in other patient subsets, and therefore likely a chance finding. Thus, this study suggests that genes other than the HLA are involved in the pathogenesis of hyperhidrosis.

Keywords

Blood Donors; HLA Antigens; Hyperhidrosis

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Conflict of interest statement

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Main text

Introduction

Hyperhidrosis (HH) is a skin disease defined as pathologically excessive sweating without an apparent cause, and it has a reported prevalence of 4-5%[1-3]. HH has a substantial negative impact with recurrent skin infections and psychiatric comorbidity, including depression, anxiety, stress and quality of life impairments[4-8]. Additionally, individuals with HH have a reduced socioeconomic status with lower education and income compared to others[9]. Etiological classifications divide HH into a primary and a secondary form[3]. Although the exact cause of primary HH is not completely understood, research has indicated a genetic predisposition of the disease[10]. A genetic linkage analyses have identified potential loci on chromosomes 1q41-1q42.3, 2p14-2p13.3, 2q21.2-2q23.3, 2q31.1, 14q11.2-q13 and 15q26.3[11-13]. A genome-wide association study (GWAS) on 3,293 Japanese women with the phenotype self-evaluated increased sweating, which not was validated to diagnose HH, and 1,245 control individuals identified associations with single nucleotide polymorphisms (SNPs) on chromosomes 2 and 16[14].

The human leucocyte antigen (HLA) is a set of cell surface proteins present on all nucleated cells[15]. The biological function of the HLA is to present antigens to T-cells[15, 16]. The HLA is highly polymorphic, which means that it can be encoded by a large diversity of different gene variants (i.e. alleles)[15]. Previous research has found that HLA alleles are associated with different comorbidities to HH, such as anxiety and skin infections, but no study has specifically investigated whether the HLA genes are involved in the pathogenesis of HH[17-19]. Therefore, in the hitherto largest study on the genetics of HH, we compared the HLA alleles in 65,000 blood donors with and without HH.

Materials and Methods

Study design

The study was conducted as a retrospective cohort study based on registry and questionnaire data.

Setting

Data was collected from the Danish Blood Donor Study (DBDS), which is a prospective Danish nationwide research cohort of voluntary blood donors initiated in 2010. The first 79,210 participants included in the DBDS have been genotyped and were the basis for this study[20]. By using the Danish Civil Registration number, which is assigned to all individuals living in Denmark, we linked data between the DBDS and the Danish National Patient Registry (DNPR) and the Danish National Prescription Registry[21].

Participants

Volunteer blood donors aged 18 to 67 years, with a bodyweight of at least 50 kg, who provided written informed consent between 2010 and 2019 were included in the DBDS. Upon DBDS inclusion, blood donors completed a DBDS study questionnaire with items on demographics, habits and comorbidities[22]. Three DBDS questionnaire iterations have been administered to blood donors. The first iteration was between March 2010 and April 2015, the second between May 2015 and May 2018 and the third between June 2018 and December 2019[22].

Variables

Outcomes – hospital diagnosed hyperhidrosis

Participants in the DBDS with the international classification of disease 10th edition (ICD-10) diagnosis R610 for localized HH were classified as having hospital diagnosed HH. Participants without ICD-10 diagnoses for HH, diabetes mellitus or thyroid disease were classified as comparators I. Data on ICD-10 diagnoses were collected from the DNPR, which contains hospital record data since 1977[23]. The ICD-10 diagnostic system was implemented in the Danish secondary health care sector in 1994[23]. The ICD-10 data of this study included primary, secondary and underlying medical condition diagnoses. Data on redeemed prescriptions were collected from the Danish National Prescription Registry, in which data on all sold prescriptions in Denmark have been recorded since 1994. In this study, we had access to data on prescriptions and ICD-10 codes between 1994 and 2019. The ICD-10 diagnoses and prescriptions used to define diabetes mellitus, thyroid disease and HH are presented in Supplementary Table 1.

Outcomes – self-reported hyperhidrosis

The DBDS participants were asked the HH screening question ‘Do you have troublesome sweating?’, which is validated to diagnose sweating by physical examination[24]. Those who answered ‘Yes, moderately’ or ‘Yes, severely’ were classified as having moderate-severe self-reported HH. Those who answered ‘Yes, severely’ were additionally classified as having severe self-reported HH. Participants who answered ‘No’ were classified as comparators II. The HH screening question was only part of the third iteration of the DBDS questionnaires. Participants with diabetes

mellitus or thyroid disease were excluded from moderate-severe self-reported HH, severe self-reported HH and comparators II. Participants with ICD-10 diagnoses for HH were excluded from comparators II.

Predictors – Human leucocyte antigen

Imputed HLA genotypes from participants in the DBDS were analyzed. The imputed HLA types included both alleles of HLA-A, HLA-B, HLA-C, HLA-DPB1, HLA-DQB1 and HLA-DRB1. From the imputation, the probability scores were as follows: HLA-A median 0.99 (interquartile range [IQR] 0.97–1.00), HLA-B median 0.99 (IQR 0.96–1.00), HLA-C median 1.00 (IQR 0.99–1.00), HLA-DPB1 median 0.96 (IQR 0.90–0.98), HLA-DQB1 median 0.99 (IQR 0.97–1.00) and HLA-DRB1 median 0.97 (IQR 0.81–0.99). The HLA alleles were coded as 0, 1 or 2.

Covariables

Sex was coded as female and male sex. Age was coded as a continuous variable. Self-reported height and weight were included as body mass index (BMI) in kg/m^2. Smoking was coded as a binary variable for current habitual or no habitual smoking, independent of the amount of tobacco consumed. Data on sex, age, height, weight and smoking were collected from the DBDS questionnaires.

Human leucocyte antigen

The study participants were genotyped using the Infinium Global Screening Array 2 by Illumina[20]. Then, the HLA genotypes were imputed using the R package HIBAG ver. HLA imputation using attribute BAGing[25], using SNP data and a Caucasian genomic reference[25]. The specificity of the imputed HLA was assessed using HLA from 8,965 blood donors who had been determined by clinical standards, and the agreement rates were 98.7% for HLA-A, 97.7% for HLA-B, 99.0% for HLA-C, 43.0% for HLA-DPB1, 91.8% for HLA-DQB1 and 96.0% for HLA-DRB1. Owing to the low agreement rate, HLA-DPB1 was not included in further analyses. A principal component (PC) analysis was also conducted, which allowed for the identification of substructures. The eigenvalues of the ten first principal components (PC) showed that more than five PC only led to marginal more variance explained (Supplementary Figures 1 and 2). First-degree relatives were excluded based on a PIHAT score of >0.2.

Limiting bias

The inclusion of blood donors in the DBDS cohort was subject to Danish blood donor inclusion and exclusion criteria[26]. These criteria are expected to affect HH cases and controls equally as a non-differential bias. Recall bias was minimized as the outcome hospital diagnosed HH was determined by hospital physicians and the predictor HLA genes were imputed from blood samples. Other outcomes were the self-reported moderate-severe and severe HH, in which moderate-severe HH has been validated to diagnose sweating by physical examination[24].

Statistics

Descriptive statistics

The variables sex and smoking were presented in frequency distribution with percentages. The variables age and BMI were assessed for normality using histograms and then presented as mean with standard deviation or median with IQR depending on normality. Differences between binary variables were determined using chi-square or Fisher's exact test. Differences in distribution between HH cases and comparators were determined using Student T-test for normally distributed continuous data and Mann Whitney U for non-normally distributed continuous data. Collinearity was assessed for the continuous variables age and BMI with scatterplots and Spearman's or Pearson's correlation. Age was centered on its mean and BMI on 25 as it is the cutoff between normal weight and overweight. Descriptive statistics based on 1 to 4 participants were presented as <5 with a corresponding percentage, to avoid unintended identification.

Analytical statistics

Univariable associations between each outcome and each covariable (i.e. sex, age, BMI and smoking) were determined using logistic regression. The covariables in the regression model were selected because previous research has shown them to associate with hyperhidrosis[9]. The five first PCs were retained in all models for the reasons described above. Then, in an additive model, association tests based on logistic regression, as part of the R package HIBAG, were conducted. Estimates from the logistic regression were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Observations with missing data for the variables sex, age, BMI and smoking were excluded from the statistical analyses. There were no missing observations for the HLA allele data or PCs. The number of missing observations with percentages is presented in Table 1. The level of nominal statistical significance was set to <0.05. All p-values were Bonferroni corrected based on the number of tests. Analyses were conducted in R version 4.0.4 and R Studio version 3.6.3[27-30].

Ethics statement

All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Regional Health Research Ethics Committees in Central Denmark Region and Zealand Region (M-20090237 and SJ-740, respectively). The Danish Data Protection Agency approved the study (general approval number P-2019-99) in the Capital Region and Zealand Region data are handled under the same approval. All participants provided written informed consent before study participation.

Availability of data

Research data are not shared.

The inclusion of participants

The genetic dataset used for analysis consisted of 65,795 individuals. Of these, 145 (0.2% of the 65,795) participants in the genetic dataset had the ICD-10 diagnosis R610 for localized HH and were thus classified as having hospital

diagnosed HH. After applying the in- and exclusion criteria, 61,867 (94.0%) were classified as comparators I (Figure 1). Likewise, 15,530 participants had answered the HH screening question, as described in Figure 2. After applying in- and exclusion criteria, 1,379 (8.9% of the 15,530) participants were classified as having moderate-severe self-reported HH, 447 (2.9%) as having severe self-reported HH and 10,173 (65.5%) as comparators II (Figure 2). Altogether 28 participants were included in both hospital diagnosed HH and moderate-severe self-reported HH.

Results

Descriptive statistics

Participants with hospital diagnosed HH and both moderate-severe and severe self-reported HH were significantly younger than their respective comparator groups (Table 1). Participants with moderate-severe and severe self-reported HH smoked more than their comparators. Likewise, participants with hospital diagnosed HH had a significantly lower BMI than comparators I, while participants with moderate-severe HH and severe self-reported HH had a significantly higher BMI than comparators II (Table 1). The number of different alleles per HLA gene is presented in Table 1.

Analytical statistics

Severe self-reported HH was associated with HLA-A*80:01 (adjusted OR 26.97 [95% CI 5.32–136.70]; $p<0.001$). Moderate-severe self-reported HH and hospital diagnosed HH were not associated with any HLA-alleles after Bonferroni correction, see Tables 2-4. Several HLA genes were nominally (i.e. non-Bonferroni corrected) associated with hospital diagnosed HH, moderate-severe self-reported HH and severe self-reported HH.

Discussion

HLA may be associated with a given disease either directly because antigen presentation is part of the pathogenesis, or indirectly because it is part of the pathogenesis of a closely co-segregating comorbidity. In this retrospective cohort study, we compared HLA alleles in Danish blood donors with and without HH. The identified particularly strong association between severe self-reported HH and HLA-A*80:01 is most likely a chance finding because only a minute number of participants with severe self-reported HH (n<5) and comparators (n<5) had this allele. Therefore, the results of this analysis of 65,000 blood donors robustly indicate that HLA genes are not implicated in the development of moderate to severe HH. As previous studies clearly have indicated a genetic component in the transmission of HH, the findings of this paper suggest that genes other than the HLA are involved in the pathogenesis of HH[11-14]. Most obvious, the genes involved are, as previously suggested, genes located on chromosomes 1, 2, 14, 15 and 16[11-14]. Additionally, as anxiety and skin infections have previously been linked to HLA alleles, the association between these co-morbidities and HH is unlikely mediated by HLA genes. The association is more likely mediated through either other genes or environmental factors.

Limitations

Firstly, blood donors are healthy with few potential comorbidities, which limited the risk of confounding. Additionally, we adjusted the statistical analyses for known confounders and PCs. However, there is still a risk of residual confounding from exposures we were unaware of. Secondly, the phenotype severe self-reported HH is only validated together with moderate self-reported HH[24]. Nevertheless, to identify a homogenous group with the worst degree of HH, we included this phenotype as an outcome. Consequently, the interpretation of the associations with severe self-reported HH should be done cautiously. Thirdly, attributed to blood donation criteria that must be met by blood donors, the study participants were healthy without severe diseases or under medication. Accordingly, HLA-genes implicated in the development of severe and chronic diseases may be underrepresented in this study population, which may have reduced the chance of detecting associations between such HLA-genes and HH. Similarly, study exclusion criteria eliminated blood donors with diabetes mellitus or thyroid disease from self-reported HH, comparators I and comparators II as they can mimic and conceal HH. This can have reduced the chances of detecting associations between HLA-alleles and said diseases in self-reported HH. Owing to the low agreement rate of HLA-DPB1, we did not conduct analyses using this allele, as the results would be non-reliable. Therefore, this paper cannot evaluate potential associations between HH and HLA-DPB1. Only 28 individuals had both hospital diagnosed HH and moderate-severe self-reported HH, which likely is because only a small portion of patients with HH are treated in the secondary healthcare sector, where ICD-10 codes are used[9].

Generalizability

HLA-gene data of this study were exclusively collected from Danish blood donors. This can hamper the generalizability of the results to other populations due to inherent haplotype variations in different populations and that individuals with chronic diseases are not allowed to donate blood and were thus excluded from the study cohort. However, regarding primary HH, the DBDS is an ideal cohort to study this because the likelihood of secondary HH is very low.

In conclusion, this study of 65,000 blood donors with few comorbidity confounders robustly indicates that the HLA is unlikely implicated in the pathogenesis of primary HH. Considering the evidence for genetic transmission, this paper indicates that genes other than the HLA are involved in the pathogenesis of primary HH. Therefore, additional genetic research is necessary to uncover the etiology of primary HH. Using the same cohort of blood donors, additional genetic research may identify genes implicated in the pathogenesis of PHH.

Ethics statement

All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Regional Health Research Ethics Committees in Central Denmark Region and Zealand Region (M-20090237 and SJ-740, respectively). The Danish Data Protection Agency approved the study (general approval number P-2019-99) in the Capital Region and Zealand Region data are handled under the same approval. All participants provided written informed consent before study participation.

Authors contributions

Mattias Henning, Christoffer Egeberg Hother, Karina Banasik, Kristina S Ibler, Sisse R Ostrowski, Christian Erikstrup, Kaspar Nielsen, Henrik Ullum, Henrik Hjalgrim, Thomas Folkmann Hansen, Kathrine Agergård Kaspersen, Betina S Sørensen, Susanne Gjørup Sækmose, Gregor Borut Ernst Jemec and Ole Birger Pedersen contributed to the study conception, design, material preparation and data collection. Analysis was performed by Mattias Henning, Ole Pedersen and Gregor Jemec. The first draft of the manuscript was written by Mattias Henning, and Christoffer Egeberg Hother, Karina Banasik, Kristina S Ibler, Sisse R Ostrowski, Christian Erikstrup, Kaspar Nielsen, Henrik Ullum, Henrik Hjalgrim, Thomas Folkmann Hansen, Kathrine Agergård Kaspersen, Betina S Sørensen, Susanne Gjørup Sækmose, Gregor Borut Ernst Jemec and Ole Birger Pedersen commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

	Hospital diagnosed HH, n=145	Comparators I, n=61,867	p-value†	Moderate-severe self-reported HH, n=1,379	Severe self-reported HH, n, n=447	Comparators II, n=10,173	p-value‡	p-value§
Female, n (%)	98 (67.6)	29,132 (47.1)		640 (46.4)	211	4,261 (41.9)		
Male, n (%)	47 (32.4)	32,735 (52.9)		739 (53.6)	236	5,912 (58.1)		
Missing sex, n (%)	0 (0)	0 (0)		0 (0)		0 (0)		
Median age (IQR)	34.8 (10.3)	41.0 (13.0)	<0.001	43.5 (12.1)	40.8 (12.5)	45.3 (11.5)	<0.001	<0.001
Missing age, n (%)	0 (0)	0 (0)		0 (0)	0 (0)	(0)		
Median BMI (IQR)	24.5 (22.1–27.5)	24.8 (22.7–27.5)	<0.001	26.6 (24.1–29.8)	26.9 (24.4–30.3)	25.3 (23.3–28.1)	<0.001	<0.001
Missing BMI, n (%)	<5 (<3.4)	544 (0.9)		15 (1.1)	6 (1.3)	66 (0.6)		
Smoking, n (%)	29 (20.0)	9,190 (15.9)	0.11	197 (14.3)	70 (15.7)	1,183 (11.6)	0.010	0.012
Non-smoking, n (%)	115 (79.3)	52,131 (84.3)		1,182 (85.7)	377 (84.3)	8,985 (88.3)		
Missing smoking, n (%)	<5 (<3.4)	546 (0.9)		0 (0)		5 (0.05)		
	Hospital diagnosed HH, n=145	Comparators I, n=61,867	In total	Moderate-severe self-reported, n=1,379	Severe self-reported HH, n, n=447	Comparators II, n=10,173		In total
HLA-A alleles, n	19	29	29	25	22	27		27
HLA-B alleles, n	34	54	54	42	38	50		50
HLA-C alleles, n	18	28	28	21	21	26		26
HLA-DQB1 alleles, n	15	17	17	17	15	17		17

HLA-DRB1 alleles, n	27	40	40	31	27	35	36

Table 1. Descriptive statistics

BMI, Body mass index; HH, Hyperhidrosis; IQR, Interquartile range; n, Number

† Hospital diagnosed HH versus Comparators I

‡ Moderate-severe-self reported HH versus Comparators II

§ Severe self-reported HH versus Comparators II

Allele	Haplotypes not carrying the HLA-allele, n	Haplotypes carrying the HLA-allele, n	Haplotypes not carrying the HLA-allele with hospital diagnosed HH, %	Haplotypes carrying the HLA-allele with hospital diagnosed HH, %	OR (95% CI)†	p-value†
HLA-A						
30:02	123,329	695	0.2	0.7	3.24 (1.33–7.89)	0.0095
74:03	124,011	13	0.2	7.7	30.06 (3.77–239.62)	0.0013
HLA-DQB1						
03:19	123,960	64	0.2	1.6	8.62 (1.17–63.28)	0.034
06:04	116,953	7,071	0.2	0.1	0.23 (0.086–0.62)	0.0038
HLA-DRB1						
13:02	116,283	7,741	0.2	0.1	0.27 (0.11–0.65)	0.0034

Table 2. Nominally significant associations with hospital diagnosed hyperhidrosis

BMI, Body mass index; CI, Confidence interval; HH, hyperhidrosis; HLA, Human leucocyte antigen; N, number; OR, Odds ratio; Pc, principal component;

† Adjusted for Pc1, Pc2, Pc3, Pc4, Pc5, Sex, Age, BMI and Smoking

Allele	Haplotypes not carrying the HLA-allele, n	Haplotypes carrying the HLA-allele, n	Haplotypes not carrying the HLA-allele with moderate-severe self-reported HH, %	Haplotypes carrying the HLA-allele with moderate-severe self-reported HH, %	OR (95% CI)†	P value†
HLA-A						
02:06	29,935	35	11.9	25.0	2.56 (1.08–6.10)	0.034
25:01	29,291	679	11.8	15.7	1.45 (1.14–1.84)	0.0030
80:01	29,963	7	11.9	50.0	7.83 (1.56–39.14)	0.012
HLA-B						
35:01	28,471	1,499	12.0	10.2	0.82 (0.68–1.00)	0.049
40:02	29,611	359	12.0	8.3	0.65 (0.42–0.99)	0.046
44:02	27,213	2,757	11.8	13.1	1.15 (1.00–1.31)	0.046
56:01	29,772	198	12.0	5.7	0.44 (0.22–0.87)	0.018
HLA-C						
07:04	22,672	432	11.9	15.3	1.35 (1.03–1.76)	0.029
HLA-DRB1						
04:02	29,838	132	11.9	18.6	1.75 (1.04–2.96)	0.035

Table 3. Nominally significant associations with moderate-severe self-reported hyperhidrosis
BMI, Body mass index; CI, Confidence interval; HH, hyperhidrosis; HLA, Human leucocyte antigen; N, number; OR, Odds ratio; Pc, Principal component
† Adjusted for Pc1, Pc2, Pc3, Pc4, Pc5, Sex, Age, BMI and Smoking

Allele	Haplotypes not carrying the HLA-allele, n	Haplotypes carrying the HLA-allele, n	Haplotypes not carrying the HLA-allele with severe self-reported HH, %	Haplotypes carrying the HLA-allele with severe self-reported HH, %	OR (95% CI)†	p-value†
HLA-A						
25:01	29,291	679	4.2	6.2	1.61 (1.09–2.36)	0.015
26:01	29,641	629	4.3	2.2	0.50 (0.27–0.94)	0.033
30:02	29,809	161	4.2	9.1	2.17 (1.11–4.24)	0.024
80:01	29,963	7	4.2	50.0	26.97 (5.32–136.70)	<0.001*
HLA-DQB1						
02:02	27,808	2,162	4.3	3.1	0.73 (0.54–0.98)	0.035
HLA-DRB1						
07:01	18,975	2,265	4.3	3.4	0.78 (0.61-0.99)	0.041

Table 4. Nominally significant and Bonferroni corrected associations with severe self-reported hyperhidrosis

BMI, Body mass index; CI, Confidence interval; HH, hyperhidrosis; HLA, Human leucocyte antigen; N, number; OR, Odds ratio; Pc, principal component

*Significant after Bonferroni correction

† Adjusted for Pc1, Pc2, Pc3, Pc4, Pc5, Sex, Age, BMI, and Smoking

Figure legends

Figure 1 Flow chart of the inclusion of participants with hospital diagnosed hyperhidrosis

DBDS, Danish blood donor study; HH, Hyperhidrosis; ICD-10, International classification of disease 10th edition; N, number

Figure 2 Flow chart of the inclusion of participants with self-reported hyperhidrosis

DBDS, Danish blood donor study; HH, Hyperhidrosis; ICD-10, International classification of disease 10th edition; N, number

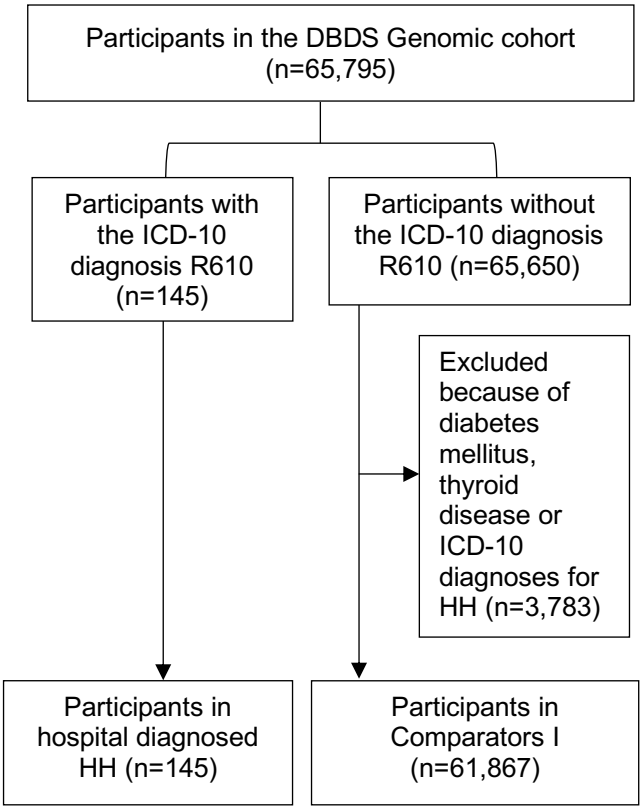

Figure 1 Flow chart of the inclusion of participants with hospital diagnosed hyperhidrosis

DBDS, Danish blood donor study; HH, Hyperhidrosis; ICD-10, International classification of disease 10th edition; N, number

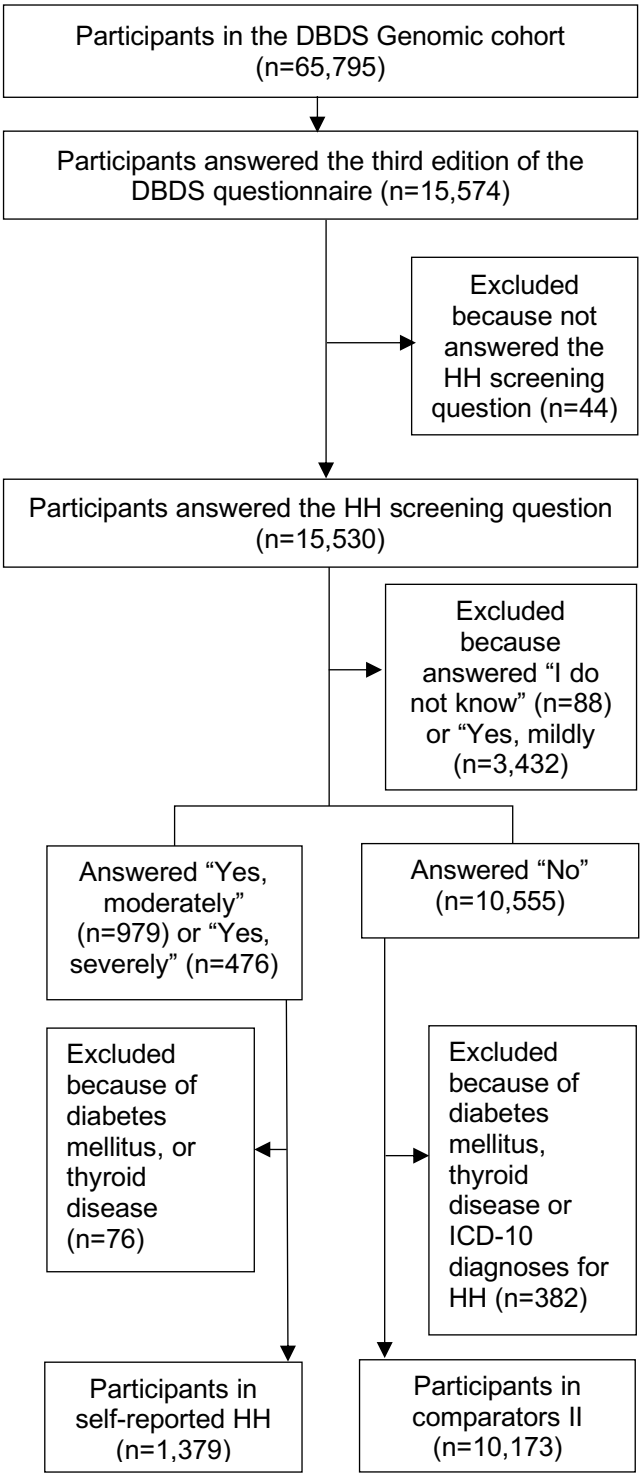

Figure 2 Flow chart of the inclusion of participants with self-reported hyperhidrosis

DBDS, Danish blood donor study; HH, Hyperhidrosis; ICD-10, International classification of disease 10th edition; N, number