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Cohort profile: DBDS Genomic Cohort, a prospective and comprehensive resource for integrative and temporal analysis of genetic, environmental, and lifestyle factors affecting health of blood donors.

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Abstract

Purpose: To establish a cohort that enables identification of genomic factors that influence human health and empower increased blood donor health and safe blood transfusions.

Human health is complex and involves several factors, a major one being the genomic aspect. The genomic era has resulted in many consortia encompassing large samples sizes, which has proven successful for identifying genetic factors associated with specific traits. However, it remains a big challenge to establish large cohorts that facilitate studies of the interaction between genetic factors, environmental and life-style factors as these change over the course of life. A major obstacle to such endeavors is that it is difficult to revisit participants to retrieve additional information and obtain longitudinal, consecutive measurements.

Participants: Blood donors (n=110,000) have given consent to participate in the Danish Blood Donor Study. The study utilizes the infrastructure of the Danish blood banks.

Findings to date: The cohort comprises extensive phenotype data and whole genome genotyping data. Further, it is possible to retrieve additional phenotype data from national registries as well as from the donors at future visits, including consecutive measurements.

Future plans: To provide new knowledge on factors influencing our health. Thus, provide a platform for studying the influence of genomic factors on human health, in particular the interaction between environmental and genetic factors.

INTRODUCTION

A person's health is determined by complex interactions between genetic, environmental, and lifestyle factors. Analyzing these factors collectively and prospectively is preferable. However, this is usually only possible using birth-cohorts and large population-based cohorts, and due to the extensive effort involved in establishing such cohorts, they are rare. The Danish Blood Donor Study (DBDS; dbds.dk) is a large prospective cohort of blood donors aiming at identifying predictors of healthy donors. As part of this cohort, we have now established DBDS Genomic Cohort assessing common SNPs in 110,000 donors. Thus, the DBDS Genomic Cohort provides a comprehensive catalog for large-scale genetic analyses in relation to numerous environmental and lifestyle factors affecting donor's health.

The evaluation of blood donor's health is important for several reasons. It is crucial for both the donor and the blood recipient that a healthy donor population is maintained with a high donation rate and a low dropout rate, thereby ensuring a steady blood supply. Evidence-based guidelines for donor recruitment, care, and retention are needed to ensure that donor recruitment can focus on individuals who are likely to remain healthy and donate frequently in the long term. One obvious relevant influential parameter relates to iron metabolism: We know that hundreds of genes impact the generation and regulation of blood cells[1] and also influence phenotype variations of iron absorption and metabolism[2-4]. Genome-wide SNP information is expected to provide knowledge enabling us to evaluate to whom donating blood will be unproblematic, thus facilitating retention of a stable blood donor population. Another parameter is altruism. Altruism as part of a prosocial behavior, the selfless concern for the welfare of others[5], is generally considered a typical blood donor characteristic[6]. However, altruism in the context of voluntary blood donation has also been shown to be a very complex phenotype[6]. In a previous study, we found a substantially larger genetic influence on blood donor behavior compared to most previous twin studies on altruism, which further highlights the heterogeneity of the blood donor personality[7]. We expect that genome-wide SNP information will

provide knowledge that can aid in the identification of long-term and steady donors. Further, we will test for association between genotype SNP information and prodromal symptoms of somatic and psychiatric disease or illnesses.

The DBDS Genomic Cohort offers the possibility to assess the impact of heterogeneous exposures in a broad range of phenotypes, such as mental state, risk-taking behavior, and characterization of blood components, and immune defense. The setup of the study allows for researchers to assess the genetic association in a) cross sectional studies to investigate e.g. the variation of phenotypic characteristic, clinical, and biochemical measurements, b) retrospective studies of e.g. rehabilitation capacity, and c) prospective studies e.g. analyzing the variation of phenotypes and clinical measurements over time and even identify prodromal symptoms.

COHORT DESCRIPTION

Population: The nation-wide blood donor population in Denmark consists of more than 230,000 donors giving more than 300,000 blood donations annually (www.bloddonor.dk). Blood donation in Denmark is voluntary and unpaid. This means that the donation is based on the desire to help others, who need it and not the desire of an economic benefit. Blood donors must be physically well, aged 17 and 67 years, and weigh more than 50 kilos. Individuals in chronic medical treatment or frequent travelers to countries with high prevalence of blood disease are not allowed to participate. The deferral rules can be seen at www.bloddonor.dk. Blood donors from foreign countries must have lived in Denmark for a minimum of one year, have a Danish social security number and have learned the Danish language to prevent misunderstandings between the donor and the blood bank professionals.

The nationwide Danish blood bank is an integrated part of the Danish healthcare system financed by local and state taxes. The Danish healthcare system is administrated by democratically elected assemblies from national state institutions, regions and municipalities. The Danish blood banks are

non-profit organizations owned and operated by each of the five regions in Denmark. The blood banks have a national board to structure collaboration across regions on recruiting donors, processing and distributing the blood for the Danish population. The DBDS is building upon the structured Danish blood bank system in the regions responsible for administrating donation sites at 27 hospitals in addition to five mobile donation units using 180 selected sites nationally (e.g., large companies, sports centers and universities)

At the blood donation centers and attached laboratories, the entire necessary infrastructure needed for the collection of biological samples and structured data is in place. Both blood plasma and whole blood for DNA extraction are available from all donors. The blood bank infrastructure already has laboratory facilities with educated staff (nurses, technicians, IT specialists and physicians). In addition, the blood bank professionals facilitate the testing of the blood for a variety of biomarkers and holds expertise in large scale storage of biological material.

Contact to participants at the blood banks is fundamental to our study. Blood donors are asked to participate and sign an informed consent when they visit the blood bank to donate blood. This consent allows us to use the blood samples from their past and future donations to study the impact of genetic and immunological factors on current and future health and disease. The inclusion and exclusion criteria for blood donation and participation in DBDS are the same with 95% of the blood donors who are invited agrees to participate in DBDS[8].

Questionnaires: From March 2010 until July 2015, all participating donors had to complete a four-page paper-based questionnaire with questions of self-experienced physical and mental health including the 12-item short form (SF-12®) standardized health survey, smoking habits, alcohol intake, exercise, food intake, supplemental iron intake, height, weight, and waist circumference. 85,000 individuals filled out the paper questionnaire. As a follow-up to the initial paper-based

questionnaire, we have developed and implemented a digital and flexible tablet-based questionnaire platform, using the open source survey software tool LimeSurvey[9]. This enables a rapid, easy, and cost-effective procedure to collect self-reported data on health traits from the participating donors at the donor sites at multiple time-points. The first digital questionnaire was implemented and used from July 2015 until May 2018. The questionnaire was focused the following research questions: allergy, ADHD, migraine, hidradenitis, depression, and Restless Legs Syndrome. It also contains questions from the paper-based questionnaire; SF-12, smoking habits, alcohol intake, height and weight. In total, 48,000 DBDS participants completed the first digital questionnaire. The second digital questionnaire started June 2018. It includes questions on: Sleep patterns, anxiety, migraine, stress, skin diseases, endometriosis, pain, learning difficulties, SF-12, smoking habits, alcohol intake, height and weight.

DBDS Organization: The DBDS itself is described in detail by Pedersen et al.[8]. Briefly, DBDS is governed by a steering committee with a scientific advisory board. All projects are managed by the DBDS steering committee. Genetic projects involving genetic data in DBDS are run in collaboration between DBDS Genomic Consortium that consists of the DBDS steering committee, deCODE Genetics®, and scientific collaborators.

Genotyping: DNA is purified from whole blood and subsequent stored at -20°C. All samples are then genotyped in two batches at deCODE genetics using the Global Screening Array (GSA) by Illumina (batch 1 n=85,000 and batch 2 n=25,000). The array has a very rich up-to-date content of >650.000 SNPs with custom chip content optimized for comparison with the Illumina Omni Express chip. All genotype data are processed simultaneously for genotype calling, quality control and imputation. Initially, individuals or SNPs with more than 10% missing data are excluded, as are individuals

deviating more than 3 standard deviations from the population heterozygosity (correcting for individuals carrying large copy number variations (>100Kbp)).

Imputation

The genotyping data is imputed using a reference panel backbone consisting of 1) UK 1KG phase 3 and HapMap reference to predict non-genotyped SNPs with MAF>1%, and 2) an in-house dataset consisting of n>6,000 Danish whole genome sequences to improve the prediction of variations with a MAF down to around 0.01%. Variants listed in the American College of Medical Genetics and Genomics guidelines are not predicted[10].

Copy number variations

Using the genotype of b-allele count and log Ratio, copy number variations (CNVs) are called using pennCNV[11]. CNVs called using <20 SNPs are excluded and the remaining CNVs are visually inspected to exclude false positives.

Statistical design: All data are stored and analyzed on a specialized, secure section of the 16,000 core Danish National Supercomputer for Life Sciences - Computerome (www.computerome.dtu.dk). Data storage and computational analysis is performed on a protected, private cloud environment built using industry-standard components. The environment has been designed to allow administrators to control user access down to a very granular (conditional per column) level. The data security and resilience are handled by an enterprise-grade HIPAA-compliant storage system configured to perform automatic hourly backups. The analysis environment is capable of dynamic scaling and has been successfully tested in a composition of over 100 servers totaling more than 300 CPUs, over 13TB of RAM and has access to up to 5.7PB (5,700TB) of disk space. The cluster comes with a preconfigured queueing system, possibility to run Virtual Machines and containers (e.g. Docker, Singularity), a set of over 900 preinstalled tools and packages and a possibility to add GPU servers optimized for Machine Learning and specialized big memory systems (1-8TB of RAM). In terms of physical

security, the Computerome site has a guard station with 24/7 active security with thermal imaging cameras and a direct connection to law enforcement including K-9 units. All access in and out of the site is strictly regulated including requirement of presenting a photo IDs and having a pre-approved supervisor on site at all times.

For each hypothesis tested, a synopsis is provided including a detailed analysis plan. Information on each synopsis will be published either as link to published articles describing the results or as summary statistic on the study website: www.dbds.dk.

Samples: During each visit to a blood donor facility (up to four times per year for whole blood donors or up to ten times if plasma donors), every participant donates one EDTA plasma sample. At inclusion in DBDS one whole blood sample is also taken. Plasma samples taken prior to the inclusion date are stored for quality assessments and will also be accessible for future analyses. All samples are frozen within 6 hours of donation and stored in the primary collection tubes until processing.

Routine blood measurements including e.g. blood group, red and white blood cell counts, hemoglobin concentration, and hematocrit are obtained at each donation. Besides routine measures, project-related measurements are available e.g. subgroups of patients are assessed for ferritin levels, infection status (*Cytomegalovirus*, *Toxoplasma gondii*, and *Herpes Simplex Virus*), HLA-typing, and other selected markers of infection (circulating cytokines, C-reactive protein, etc.).

General Data Protection and Ethical Issues and Principles: DBDS has secured necessary permissions and approval from the Danish Data Protection Agency (2007-58-0015) and the Scientific Ethical Committee system (M-20090237). New projects within the DBDS Genomic consortium will require additional approval by the National Committee on Health Research Ethics. DBDS will be responsible for the continued contact with, and securing future permissions from, relevant Danish authorities regarding research on DBDS samples.

The study will adhere to the FAIR (http://datafairport.org/: Findable, Accessible, Interoperable and Reusable) concepts. Within this legal framework DBDS Genomic Consortium Board can thus decide how and under which conditions the data can be shared. Generally, relevant summary data will be publicly available via repositories 3 months after acceptance for publication (H2020 open-access policy).

FINDINGS TO DATE

Initial quality measures that have been assessed:

Kinship

As described, giving blood often runs in families and the heritability has been estimated to be >53%[7]. It is clear from the estimated kinship (Figure 1, Table 1), that there is a considerable 1st, 2nd and 3rd degree relatives among the participants in the DBDS Genomic consortium.

Ethnicity

Based on ~15K overlapping SNPs from the genotyped data and the 1000 Genomes samples, we confirm the expected population structure of the DBDS cohort; most participants are of European ancestry (99%) and the following two ethnicity groups are of South Asian (0.4%) and East Asian (0.2%) ancestry, respectively. The proportion of participants with recent African ancestry is extremely low (0.002%) which is expected given the strict donor travel quarantine rules. Ethnicity was evaluated using FlashPCA2[12].

Minor allele frequencies

The distribution of the minor allele frequency (MAF), shown that majority of SNPs (84%) is above 1% (Figure 2) as expected, which provides solid basis for genotype imputation.

STRENGTH AND LIMITATIONS

Consecutive measurements: A unique feature of this large blood donor cohort is the ability to do consecutive assessments. In standard settings, participants are typically recruited at a baseline time-point and are invited for follow-up studies once or twice in the following years. The blood bank represents an advantage because most donors have a long-term committed relationship for blood donation and are seen one to four times annually[13]. It is therefore possible to collect several yearly and consecutive biological samples and questionnaire information over decades for a large number of participants. Again, subgroups and samples from specific time-points can be used in a retrospective manner.

National-based registries: Denmark has several comprehensive national registries, which include both health information and socio-demographical measures on an individual level. The informed consent allows for combining information obtained from the DBDS participants and the national registries; the Danish National Patient Registry (since 1977), the Danish Cancer (since 1943) and Diabetes Registries (since 1992-2012), the Danish Registry of Medicinal Product Statistics (since 1994), the Civil Registration Registry (since 1968 vital status, number of children, birthplace, address, relocation and more), the Integrated Database for Labor Market Research (since 1982, e.g. educational level, occupation status, income, social status, and other related parameters). This facilitates retrospective, cross-sectional, or prospective studies using registry-based measurements in combination with questionnaire-derived data.

Donor selection: Although, DBDS participants resemble the Danish population a few limitations in the study design may affect the generalizability of the results[14]. The blood donor exclusion criteria dismiss individuals with: infections or diseases that are transmittable through blood, weight below 50 kg, hemoglobin (Hb) levels below 12.9 g/dL in males and 12.0 g/dL in females, and curious behaviors: individuals with high travel rates to countries with high risk of hepatitis and HIV, men who have sex with men, individuals who have previously worked as prostitutes, those who have used

intravenous substances, and pregnant women. Comparing socio-demographical parameters of blood donors with that of the total Danish population, we know that very low- and high-income individuals are underrepresented among blood donors[14]. In this respect, we acknowledge that DBDS lacks coverage of certain parts of the general population in contrast to traditional population-based studies. Similarly, the population based UK Biobank study have also reported a "healthy volunteer" bias[15].

DISCUSSION

The extension of the DBDS with a genomic cohort will profoundly impact the usability and empower studies on genetic, environmental, and lifestyle factors that influence blood donor health. Furthermore, the study provides a unique platform that facilitates analysis of common phenotypes not otherwise found in the national health registries, disease resilience factors, and interactions between genes and environment. At finally, such a large healthy cohort holds a huge potential for providing crucial information for future precision medicine initiatives and similar efforts have been started, e.g. "All of US" (by NIH, US). In Denmark we have the advantage of a collection of extensive, national health registries that facilitate epidemiological studies on specific diseases/outcomes in such a large cohort. For phenotypes and symptoms not monitored systematically in these health registries; e.g. lifestyle factors such as smoking habits, sleep patterns, and self-perceived health; large epidemiological studies are needed but typical difficult to conduct. The DBDS Genomic Cohort can facilitate such studies. As described above, the DBDS Genomic Cohort exploits an existing blood donor platform with an extremely high participation rate (>95%), which facilitates a straightforward evaluation of donor health in large epidemiological studies. Furthermore, the electronic questionnaire platform allows for easy and fast implementation of new, targeted investigations in subgroups of the donor population. Together with outcomes from the Danish health registries and the millions of retrospective plasma samples stored in easily accessible freezers, DBDS represents a solid phenotyping platform that can be used for both cross-sectional epidemiological

studies and for retrospective biomarker studies. We believe that these strengths make the DBDS Genomic Cohort a strong competitive player in the field of precision medicine.

The DBDS Genomic Cohort allows us to study gene-environment interaction that are otherwise difficult to study: testing disease development hypotheses; e.g. a) cognitive performance in interaction with genetic factors and the risk of dementia, and b) determining the contribution of genetic and environmental factors to a phenotype such as sleep pattern. The DBDS Genomic Cohort is particularly valuable for studying disease resistance in individuals exposed to one or more known disease risk factors and yet do not proceed to develop the disease. One such example could be participants carrying a high load of a highly inheritable trait like psychiatric illness in the family or known genetic risk factors, who do not have psychiatric illness themselves. Lastly, DBDS also provides sequential storage of plasma samples, which allows for sequential blood measurements. Such measurements could be used to investigate health markers like suPAR (soluble urokinase plasminogen activating receptor) and the variation associated with the donor's general health[16].

In short, the DBDS genomic cohort facilitates the investigation of the impact of genomic factors on health traits and states.

Integrative analysis of different 'omics, i.e. multi-omics analysis will be possible in the large DBDS genomic cohort, which adds tremendously to its value as a resource for studying the health of blood donors and correlation between blood related traits and states. We expect that the DBDS genomic cohort will contribute to discovery and validation of prodromal symptoms and biomarkers of disease, thus providing a better understanding of the disease pathologies and suggesting new drug targets.

COLLABORATION

We encourage scientific collaborations using the data generated in the DBDS Genetic Consortium. Published summarized data is available om request. Otherwise request of data necessitate first

approval by the DBDS steering committee and if the request is considered outside the aim of DBDS, application to the national scientific ethical committee is obligatory. Additionally, material transfer and data protection agreement need to be acquired. Please visit www.dbds.dk.

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Contributorship statement

TH, KB, and KSB conceived and planned the experiments. KB carried out the analyses. OBP, HH, HMP, KRN, CE,HU, PJJ, TW, JO, GBJ, MN, SA, PIJ,ES and XX contributed to cohort and research design. DW, PJC, KB and SB led to data infrastructure design. CE, KSB, MHL, ES and MSP contributed to data capture. TH, KB and KSB contributed to the interpretation of the results. TH, KB, and KSB took the lead in writing the manuscript. All authors provided critical feedback and helped shape the analysis and manuscript and approaved the final version.

Competing interests

The authors declare no competing interest

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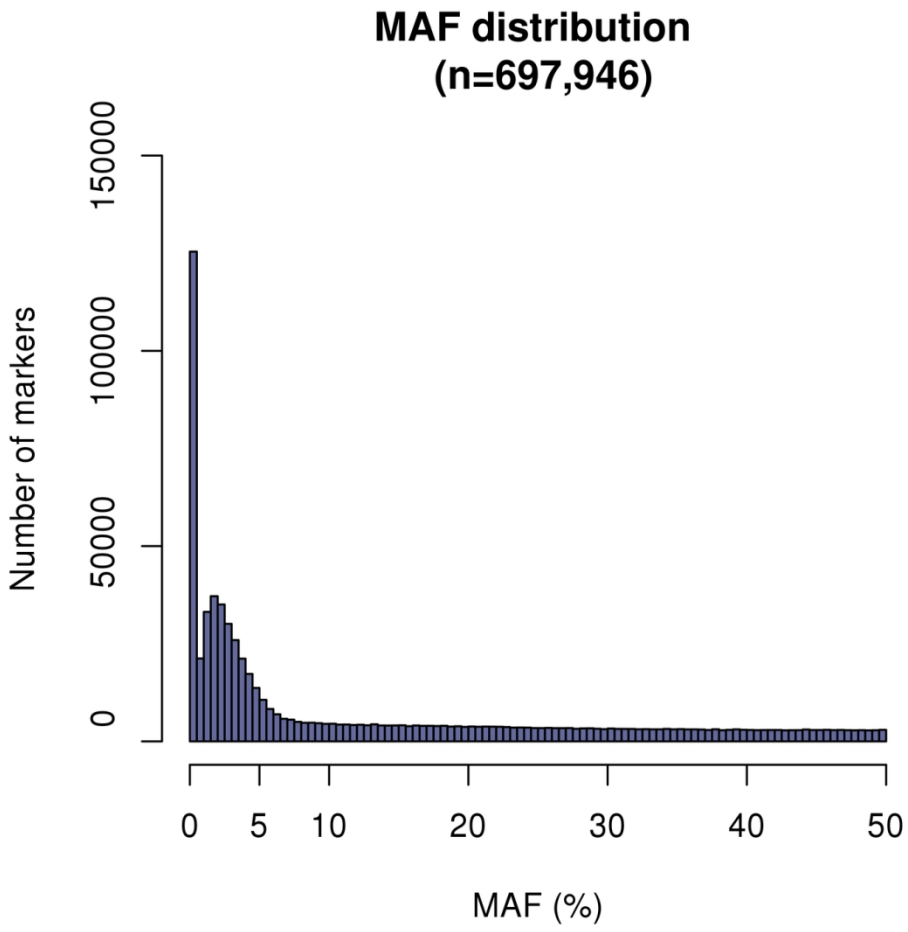
Figure 1. Relationships for DBDS donors genotyped as part of the first batch(n=85,000). Each point represents a pair of related individuals and the colors indicate the degree of relatedness (InfType): monozygotic twins(MZ) and technical duplicates(DUP) in green (in the upper left corner), 1st degree relatives as parent-child pairs (PO, dark blue) and full siblings (FS, blue), 2nd and 3rd degree relatives in blue, red and light red, respectively. The y-axis shows the estimated kinship coefficient, defined as the probability that two alleles sampled at random (one from each individual) are identical by descent. The x-axis shows the proportion of zero identity-by-state (IBS0), defined as the proportion of SNPs at which two samples share no alleles. KING's criteria[17] was used to estimate the degree of relatedness.

Figure 2. Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

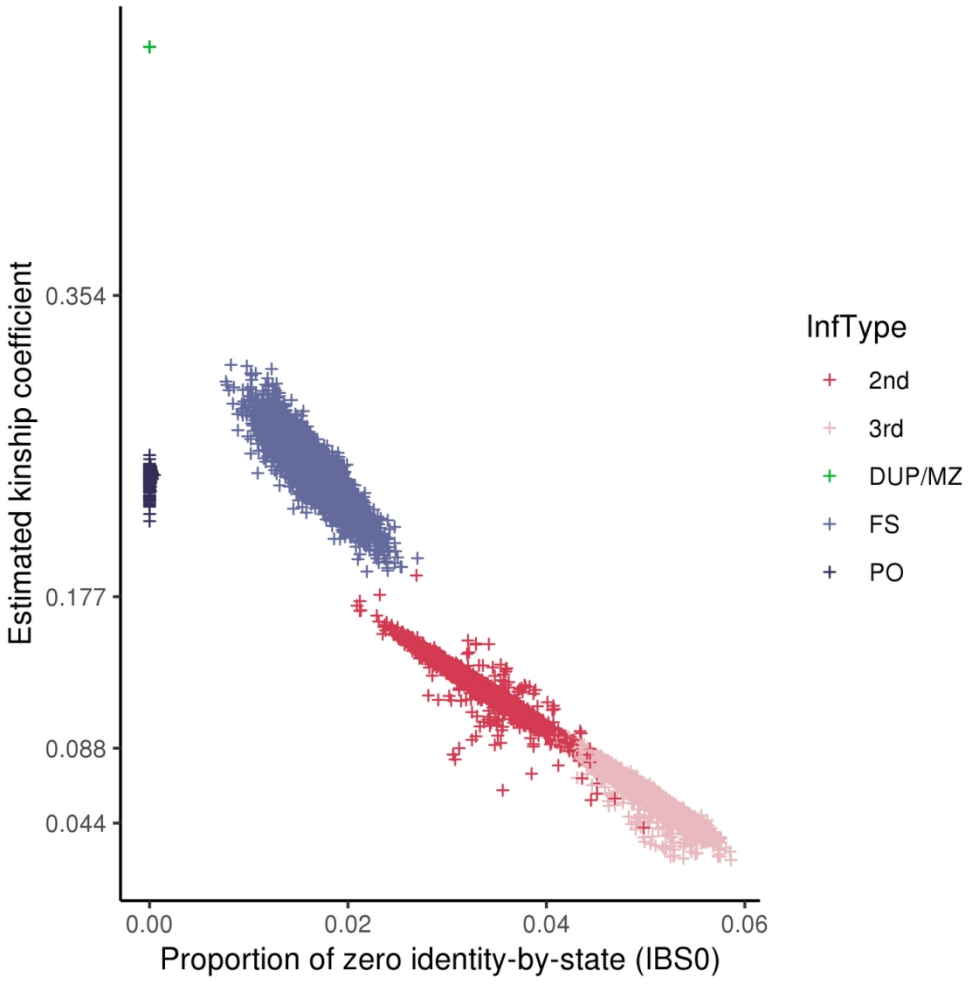
Table 1. Related pairs (3rd degree or closer) for batch 1 DBDS donors genotyped (N=85,000). Counts are derived from the relationship information presented in Figure 1.

Relationship	*Monozygotic twins*	*Parent-offspring*	*Full siblings*	*2nd degree*	*3rd degree*
Pairs	51	4,246	3,309	4,375	11,433



Caption : Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

127x127mm (300 x 300 DPI)



Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

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Cohort profile: DBDS Genomic Cohort, a prospective and comprehensive resource for integrative and temporal analysis of genetic, environmental, and lifestyle factors affecting health of blood donors.

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Abstract

Purpose: To establish a cohort that enables identification of genomic factors that influence human health and empower increased blood donor health and safe blood transfusions.

Human health is complex and involves several factors, a major one being the genomic aspect. The genomic era has resulted in many consortia encompassing large samples sizes, which has proven successful for identifying genetic factors associated with specific traits. However, it remains a big challenge to establish large cohorts that facilitate studies of the interaction between genetic factors, environmental and life-style factors as these change over the course of life. A major obstacle to such endeavors is that it is difficult to revisit participants to retrieve additional information and obtain longitudinal, consecutive measurements.

Participants: Blood donors (n=110,000) have given consent to participate in the Danish Blood Donor Study. The study utilizes the infrastructure of the Danish blood banks.

Findings to date: The cohort comprises extensive phenotype data and whole genome genotyping data. Further, it is possible to retrieve additional phenotype data from national registries as well as from the donors at future visits, including consecutive measurements.

Future plans: To provide new knowledge on factors influencing our health. Thus, provide a platform for studying the influence of genomic factors on human health, in particular the interaction between environmental and genetic factors.

INTRODUCTION

A person's health is determined by complex interactions between genetic, environmental, and lifestyle factors. Analyzing these factors collectively and prospectively is preferable. However, this is usually only possible using birth-cohorts and large population-based cohorts, and due to the extensive effort involved in establishing such cohorts, they are rare. The Danish Blood Donor Study (DBDS; dbds.dk) is a large prospective cohort of blood donors aiming at identifying predictors of healthy donors. As part of this cohort, we have now established DBDS Genomic Cohort assessing common single nucleotide polymorphisms (SNPs) in 110,000 donors. Thus, the DBDS Genomic Cohort provides a comprehensive catalog for large-scale genetic analyses in relation to numerous environmental and lifestyle factors affecting donor's health. A description of summary statistics on phenotypes and data coverage are provided in Table 1. A detailed sociodemographic description of Danish blood donors, including sex and age distribution, ethnicity, education, employment and level of urbanization are found in Burgdorf et.al 2017[1].

The evaluation of blood donor's health is important for several reasons. It is crucial for both the donor and the blood recipient that a healthy donor population is maintained with a high donation rate and a low dropout rate, thereby ensuring a steady blood supply. Evidence-based guidelines for donor recruitment, care, and retention are needed to ensure that donor recruitment can focus on individuals who are likely to remain healthy and donate frequently in the long term. One obvious relevant influential parameter relates to iron metabolism: We know that hundreds of genes impact the generation and regulation of blood cells[2] and also influence phenotype variations of iron absorption and metabolism[3-5]. Genome-wide SNP information is expected to provide knowledge enabling us to evaluate to whom donating blood will be unproblematic, thus facilitating retention of a stable blood donor population. Another parameter is altruism. Altruism as part of a prosocial behavior, the selfless concern for the welfare of others[6], is generally considered a typical blood donor characteristic[7].

However, altruism in the context of voluntary blood donation has also been shown to be a very complex phenotype[7]. In a previous study, we found a substantially larger genetic influence on blood donor behavior compared to most previous twin studies on altruism, which further highlights the heterogeneity of the blood donor personality[8]. The considerable amount of kinship (twins and siblings) in the cohort in combination with socioeconomic variables from the Danish registries will enable us to further differentiate between genetic impact and social basis of altruism in blood donation. The genome-wide SNP information will provide knowledge that can aid in the identification of long-term and steady donors. Further, we will test for association between genotype SNP information and prodromal symptoms of somatic and psychiatric disease or illnesses.

The DBDS Genomic Cohort offers the possibility to assess the impact of heterogeneous exposures in a broad range of phenotypes, such as mental state, risk-taking behavior, and characterization of blood components, and immune defense. The setup of the study allows for researchers to assess the genetic association in a) cross sectional studies to investigate e.g. the variation of phenotypic characteristic, clinical, and biochemical measurements, b) retrospective studies of e.g. rehabilitation capacity, and c) prospective studies e.g. analyzing the variation of phenotypes and clinical measurements over time and even identify prodromal symptoms.

COHORT DESCRIPTION

Population: The nation-wide blood donor population in Denmark consists of more than 230,000 donors giving more than 300,000 blood donations annually (www.bloddonor.dk). Blood donation in Denmark is voluntary and unpaid. This means that the donation is based on the desire to help others, who need it and not the desire of an economic benefit. Blood donors must be physically well, aged 17 and 67 years, and weigh more than 50 kilos. Individuals in chronic medical treatment or frequent travelers to countries with high prevalence of blood disease are not allowed to participate. The deferral

rules can be seen at www.bloddonor.dk. Blood donors from foreign countries must have lived in Denmark for a minimum of one year, have a Danish social security number and have learned the Danish language to prevent misunderstandings between the donor and the blood bank professionals.

The nationwide Danish blood bank is an integrated part of the Danish healthcare system financed by local and state taxes. The Danish healthcare system is administrated by democratically elected assemblies from national state institutions, regions and municipalities. The Danish blood banks are non-profit organizations owned and operated by each of the five regions in Denmark. The blood banks have a national board to structure collaboration across regions on recruiting donors, processing and distributing the blood for the Danish population. The DBDS is building upon the structured Danish blood bank system in the regions responsible for administrating donation sites at 27 hospitals in addition to five mobile donation units using 180 selected sites nationally (e.g., large companies, sports centers and universities)

At the blood donation centers and attached laboratories, the entire necessary infrastructure needed for the collection of biological samples and structured data is in place. Both blood plasma and whole blood for DNA extraction are available from all donors. The blood bank infrastructure already has laboratory facilities with educated staff (nurses, technicians, IT specialists and physicians). In addition, the blood bank professionals facilitate the testing of the blood for a variety of biomarkers and holds expertise in large scale storage of biological material.

Contact to participants at the blood banks is fundamental to our study. Blood donors are asked to participate and sign an informed consent when they visit the blood bank to donate blood. This consent allows us to use the blood samples from their past and future donations to study the impact of genetic and immunological factors on current and future health and disease. The inclusion and exclusion

criteria for blood donation and participation in DBDS are the same with 95% of the blood donors who are invited agrees to participate in DBDS[9].

Questionnaires: From March 2010 until July 2015, all participating donors had to complete a four-page paper-based questionnaire with questions of self-experienced physical and mental health including the 12-item short form (SF-12®) standardized health survey, smoking habits, alcohol intake, exercise, food intake, supplemental iron intake, height, weight, and waist circumference. 85,000 individuals filled out the paper questionnaire. As a follow-up to the initial paper-based questionnaire, we have developed and implemented a digital and flexible tablet-based questionnaire platform, using the open source survey software tool LimeSurvey[10]. This enables a rapid, easy, and cost-effective procedure to collect self-reported data on health traits from the participating donors at the donor sites at multiple time-points. The first digital questionnaire was implemented and used from July 2015 until May 2018. The questionnaire was focused the following research questions: allergy, ADHD, migraine, hidradenitis, depression, and Restless Legs Syndrome. It also contains questions from the paper-based questionnaire; SF-12, smoking habits, alcohol intake, height and weight. In total, 48,000 DBDS participants completed the first digital questionnaire. The second digital questionnaire started June 2018. It includes questions on: Sleep patterns, anxiety, migraine, stress, skin diseases, endometriosis, pain, learning difficulties, SF-12, smoking habits, alcohol intake, height and weight. Using the questionnaire data, several studies have already assessed the factors describing the general health of blood donors, e.g. hidradentis[11], risk of infection[12], migraine, restless legs, and depression[13 14]. Also, by revisiting samples it has been possible to assess wether infections correlates with obesity[15] and how iron deficiency and hemoglobin levels affect donor health in general[16 17].

Registry-based data: There is and has always been equal access to the healthcare system in Denmark, hence, the centralized national civil and health registries are unbiased and comprehensive resources of healthcare data. The registries include the National Patient Registry containing all hospital-

registered diagnoses since 1975[18], as well as other specialized registries, e.g. the Danish Medical Birth Register, the Danish Register of Causes of Death, and Statistics Denmark monitoring e.g. socio-economic data. We have already used the DBDS cohort in epidemiological studies assessing e.g. the mortality [19] of donors and the effect of blood donation on offspring birth weight[20].

DBDS Organization: The DBDS itself is described in detail by Pedersen et al.[9]. Briefly, DBDS is governed by a steering committee with a scientific advisory board. All projects are managed by the DBDS steering committee. Genetic projects involving genetic data in DBDS are run in collaboration between DBDS Genomic Consortium that consists of the DBDS steering committee, deCODE Genetics®, and scientific collaborators.

Genotyping: DNA is purified from whole blood and subsequent stored at -20°C. All samples are then genotyped in two batches at deCODE genetics using the Global Screening Array (GSA) by Illumina (batch 1 n=85,000 and batch 2 n=25,000). The array has a very rich up-to-date content of >650.000 SNPs with custom chip content optimized for comparison with the Illumina Omni Express chip. All genotype data are processed simultaneously for genotype calling, quality control and imputation. Initially, individuals or SNPs with more than 10% missing data are excluded, as are individuals deviating more than 3 standard deviations from the population heterozygosity (correcting for individuals carrying large copy number variations (>100Kbp)).

Imputation

The genotyping data is imputed using a reference panel backbone consisting of 1) UK 1KG phase 3 and HapMap reference to predict non-genotyped SNPs with minor allele frequency (MAF)>1%, and 2) an in-house dataset consisting of n>6,000 Danish whole genome sequences to improve the prediction of variations with a MAF down to around 0.01%[21]. Variants listed in the American College of Medical Genetics and Genomics guidelines are currently not predicted, due to restrictions from the

Danish National Ethics Committee [22]. For future collaborative studies the Ethics Committee will approve analysis of these variants on a case by case basis.

Copy number variations

Using the genotype of b-allele count and log Ratio, copy number variations (CNVs) are called using pennCNV[23]. CNVs called using <20 SNPs are excluded and the remaining CNVs are visually inspected to exclude false positives.

Statistical design: All data are stored and analyzed on a specialized, secure section of the 16,000 core Danish National Supercomputer for Life Sciences - Computerome (www.computerome.dtu.dk). Data storage and computational analysis is performed on a protected, private cloud environment. The analysis environment is capable of dynamic scaling and has been successfully tested in a composition of over 100 servers totaling more than 300 CPUs, over 13TB of RAM and has access to up to 5.7PB (5,700TB) of disk space. The cluster comes with a preconfigured queueing system, possibility to run Virtual Machines and containers (e.g. Docker, Singularity), a set of over 900 preinstalled tools and packages and a possibility to add GPU servers optimized for Machine Learning and specialized big memory systems (1-8TB of RAM).

For each hypothesis tested, a synopsis is provided including a detailed analysis plan. Information on each synopsis will be published either as link to published articles describing the results or as summary statistic on the study website: www.dbds.dk.

Samples: During each visit to a blood donor facility (up to four times per year for whole blood donors or up to ten times if plasma donors), every participant donates one EDTA plasma sample. At inclusion in DBDS one whole blood sample is also taken. Plasma samples taken prior to the inclusion date are stored for quality assessments and will also be accessible for future analyses. All samples are frozen within 6 hours of donation and stored in the primary collection tubes until processing.

Routine blood measurements including e.g. blood group, red and white blood cell counts, hemoglobin concentration, and hematocrit are obtained at each donation. Besides routine measures, project-related measurements are available e.g. subgroups of patients are assessed for ferritin levels, infection status (*Cytomegalovirus*, *Toxoplasma gondii*, and *Herpes Simplex Virus*), HLA-typing, and other selected markers of infection (circulating cytokines, C-reactive protein, etc.).

General Data Protection and Ethical Issues and Principles: DBDS has secured necessary permissions and approval from the Danish Data Protection Agency (2007-58-0015) and the Scientific Ethical Committee system (M-20090237). New projects within the DBDS Genomic consortium will require additional approval by the National Committee on Health Research Ethics. DBDS will be responsible for the continued contact with, and securing future permissions from, relevant Danish authorities regarding research on DBDS samples.

The study will adhere to the FAIR (http://datafairport.org/: Findable, Accessible, Interoperable and Reusable) concepts. Within this legal framework DBDS Genomic Consortium Board can thus decide how and under which conditions the data can be shared. Generally, relevant summary data will be publicly available via repositories 3 months after acceptance for publication (H2020 open-access policy).

Patient and Public Involvement: Patients and public were not involved in the design of this study.

Data availability statement: No additional data available

FINDINGS TO DATE

Initial quality measures that have been assessed:

Kinship

As described, giving blood often runs in families and the heritability has been estimated to be >53%[8]. It is clear from the estimated kinship based on the first batch of participants (n=85.000) (Figure 1, Table 2), that there is a considerable 1st, 2nd and 3rd degree relatives among the participants in the DBDS Genomic cohort.

Ethnicity

Based on ~15K overlapping SNPs from the genotyped data and the 1000 Genomes samples, we confirm the expected population structure of the DBDS cohort; most participants are of European ancestry (99%) and the following two ethnicity groups are of South Asian (0.4%) and East Asian (0.2%) ancestry, respectively. The proportion of participants with recent African ancestry is extremely low (0.002%) which is expected given the strict donor travel quarantine rules. Ethnicity was evaluated using FlashPCA2[24].

Minor allele frequencies

The distribution of the MAF, shown that majority of SNPs (84%) is above 1% (Figure 2) as expected, which provides solid basis for genotype imputation.

STRENGTH AND LIMITATIONS

Consecutive measurements: A unique feature of this large blood donor cohort is the ability to do consecutive assessments. In standard settings, participants are typically recruited at a baseline time-point and are invited for follow-up studies once or twice in the following years. The blood bank represents an advantage because most donors have a long-term committed relationship for blood donation and are seen one to four times annually[25]. It is therefore possible to collect several yearly and consecutive biological samples and questionnaire information over decades for a large number of participants. Again, subgroups and samples from specific time-points can be used in a retrospective manner.

National-based registries: Denmark has several comprehensive national registries, which include both health information and socio-demographical measures on an individual level. The informed consent allows for combining information obtained from the DBDS participants and the national registries; the Danish National Patient Registry (since 1977), the Danish Cancer (since 1943) and Diabetes Registries (since 1992-2012), the Danish Registry of Medicinal Product Statistics (since 1994), the Civil Registration Registry (since 1968 vital status, number of children, birthplace, address, relocation and more), the Integrated Database for Labor Market Research (since 1982, e.g. educational level, occupation status, income, social status, and other related parameters). This facilitates retrospective, cross-sectional, or prospective studies using registry-based measurements in combination with questionnaire-derived data.

Donor selection: Although, DBDS participants resemble the Danish population a few limitations in the study design may affect the generalizability of the results[1]. The blood donor exclusion criteria dismiss individuals with: infections or diseases that are transmittable through blood, weight below 50 kg, hemoglobin (Hb) levels below 12.9 g/dL in males and 12.0 g/dL in females, and curious behaviors: individuals with high travel rates to countries with high risk of hepatitis and HIV, men who have sex with men, individuals who have previously worked as prostitutes, those who have used intravenous substances, and pregnant women. Comparing socio-demographical parameters of blood donors with that of the total Danish population, we know that very low- and high-income individuals are underrepresented among blood donors[1]. In this respect, we acknowledge that DBDS lacks coverage of certain parts of the general population in contrast to traditional population-based studies. Similarly, the population based UK Biobank study have also reported a "healthy volunteer" bias[26].

DISCUSSION

The extension of the DBDS with a genomic cohort will profoundly impact the usability and empower studies on genetic, environmental, and lifestyle factors that influence blood donor health. Furthermore, the study provides a unique platform that facilitates analysis of common phenotypes not otherwise found in the national health registries, disease resilience factors, and interactions between genes and environment. At finally, such a large healthy cohort holds a huge potential for providing crucial information for future precision medicine initiatives and similar efforts have been started, e.g. “All of US” (by NIH, US). In Denmark we have the advantage of a collection of extensive, national health registries that facilitate epidemiological studies on specific diseases/outcomes in such a large cohort. For phenotypes and symptoms not monitored systematically in these health registries; e.g. lifestyle factors such as smoking habits, sleep patterns, and self-perceived health; large epidemiological studies are needed but typical difficult to conduct. The DBDS Genomic Cohort can facilitate such studies. As described above, the DBDS Genomic Cohort exploits an existing blood donor platform with an extremely high participation rate (>95%), which facilitates a straightforward evaluation of donor health in large epidemiological studies. Furthermore, the electronic questionnaire platform allows for easy and fast implementation of new, targeted investigations in subgroups of the donor population. Together with outcomes from the Danish health registries and the millions of retrospective plasma samples stored in easily accessible freezers, DBDS represents a solid phenotyping platform that can be used for both cross-sectional epidemiological studies and for retrospective biomarker studies. We believe that these strengths make the DBDS Genomic Cohort a strong competitive player in the field of precision medicine.

The DBDS Genomic Cohort allows us to study gene-environment interaction that are otherwise difficult to study: testing disease development hypotheses; e.g. a) cognitive performance in interaction with genetic factors and the risk of dementia, and b) determining the contribution of genetic and environmental factors to a phenotype such as sleep pattern. One way to study this is by

using multivariate regression models. As an example, Fan *et al*. have recently portrayed an advanced example of gene-environment interaction analysis incorporating temporal and spatial considerations[27], an analysis based on data from the Danish civil registries. The DBDS Genomic Cohort is particularly valuable for studying disease resistance in individuals exposed to one or more known disease risk factors and yet do not proceed to develop the disease. One such example could be participants carrying a high load of a highly inheritable trait like psychiatric illness in the family or known genetic risk factors, who do not have psychiatric illness themselves. Lastly, DBDS also provides sequential storage of plasma samples, which allows for sequential blood measurements. Such measurements could be used to investigate health markers like suPAR (soluble urokinase plasminogen activating receptor) and the variation associated with the donor's general health[28].

In short, the DBDS genomic cohort facilitates the investigation of the impact of genomic factors on health traits and states.

Integrative analysis of different 'omics, i.e. multi-omics analysis will be possible in the large DBDS genomic cohort, which adds tremendously to its value as a resource for studying the health of blood donors and correlation between blood related traits and states. We expect that the DBDS genomic cohort will contribute to discovery and validation of prodromal symptoms and biomarkers of disease, thus providing a better understanding of the disease pathologies and suggesting new drug targets.

COLLABORATION

We encourage scientific collaborations using the data generated in the DBDS Genetic Consortium. Published summarized data is available om request. Otherwise request of data necessitate first approval by the DBDS steering committee and if the request is considered outside the aim of DBDS, application to the national scientific ethical committee is obligatory. Additionally, material transfer and data protection agreement need to be acquired. Please visit www.dbds.dk.

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Contributorship statement

TH, KB, and KSB conceived and planned the experiments. KB carried out the analyses. OBP, HH, HMP, KRN, CE,HU, PJJ, TW, JO, GBJ, MN, SA, PIJ,ES and LWT contributed to cohort and research design. DW, PJC, KB and SB led to data infrastructure design. CE, KSB, MHL, ES and MSP contributed to data capture. TH, KB and KSB contributed to the interpretation of the results. TH, KB, and KSB took the lead in writing the manuscript. All authors provided critical feedback and helped shape the analysis and manuscript and approaved the final version.

Competing interests

The authors declare no competing interest

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Figure 1. Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

Figure 2. Relationships for DBDS donors genotyped as part of the first batch(n=85,000). Each point represents a pair of related individuals and the colors indicate the degree of relatedness ("InfType" in KING IBD segment inference): monozygotic twins and technical duplicates(MZ/DUP) in pink (in the upper left corner), 1st degree relatives as parent-offspring pairs (PO, dark green) and full siblings (FS, light green), 2nd and 3rd degree relatives in dark and light purple, respectively. The y-axis shows the estimated kinship coefficient, defined as the probability that two alleles sampled at random (one from each individual) are identical by descent. The x-axis shows the proportion of zero identity-by-state (IBS0), defined as the proportion of SNPs at which two samples share no alleles. KING's criteria was used to estimate the degree of relatedness (--related command in KING). We used a set of independent high-quality markers (excluding palindromic and non-autosomal markers, markers with MAF<1%, low call-rate (<99%) and markers in regions with high Linkage Disequilibrium) for the relatedness calculation.

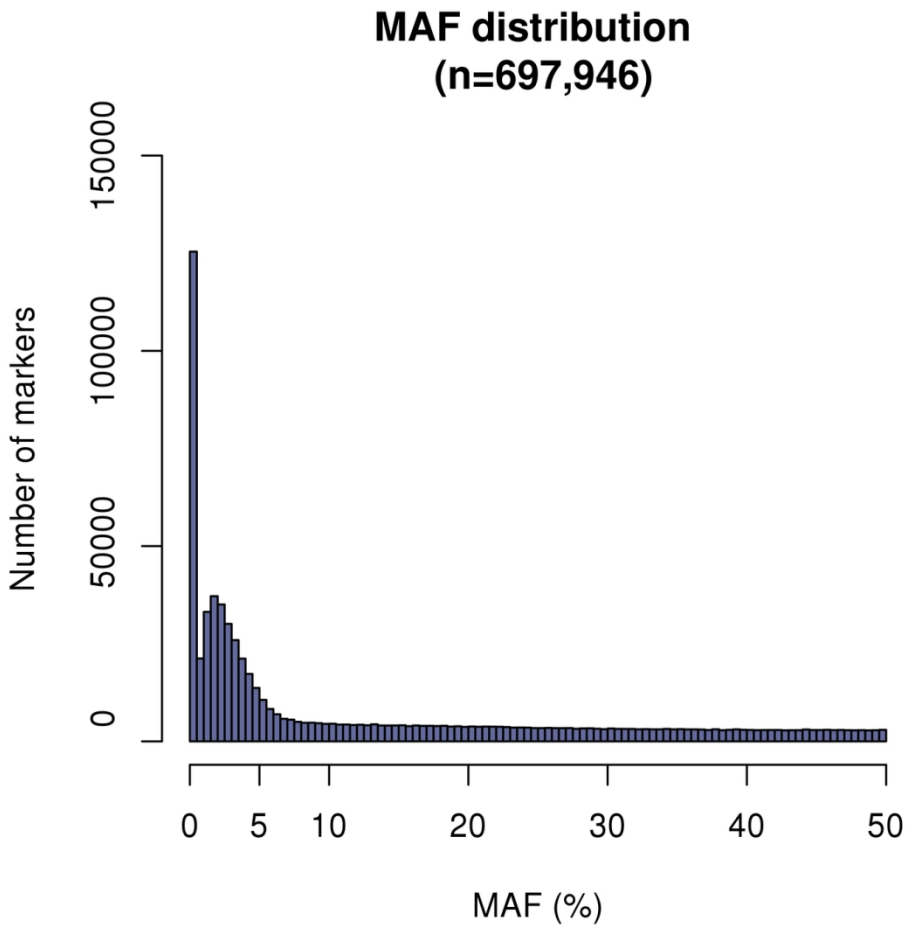
Table 1. Prevalence and missingness of enviroenmental variables

	Participants	*Missingness*
Men	48.8 %	0%
Women	51.2 %	0%
*Age**	41 (29-50) *	0%
*Height (cm) **	175.6 (169-182)	0.53%
*Weight (kg) **	78.1 (67-87)	0.82%
BMI	25.3 (22-27)	1.2%
Smoking	11.9%	1.3%
Alcohol consumption (daily)	3.0%	4.7%
Questionnaire 1	87.000	
Questionnaire 2	50.000	

* Mean (Quartiles).

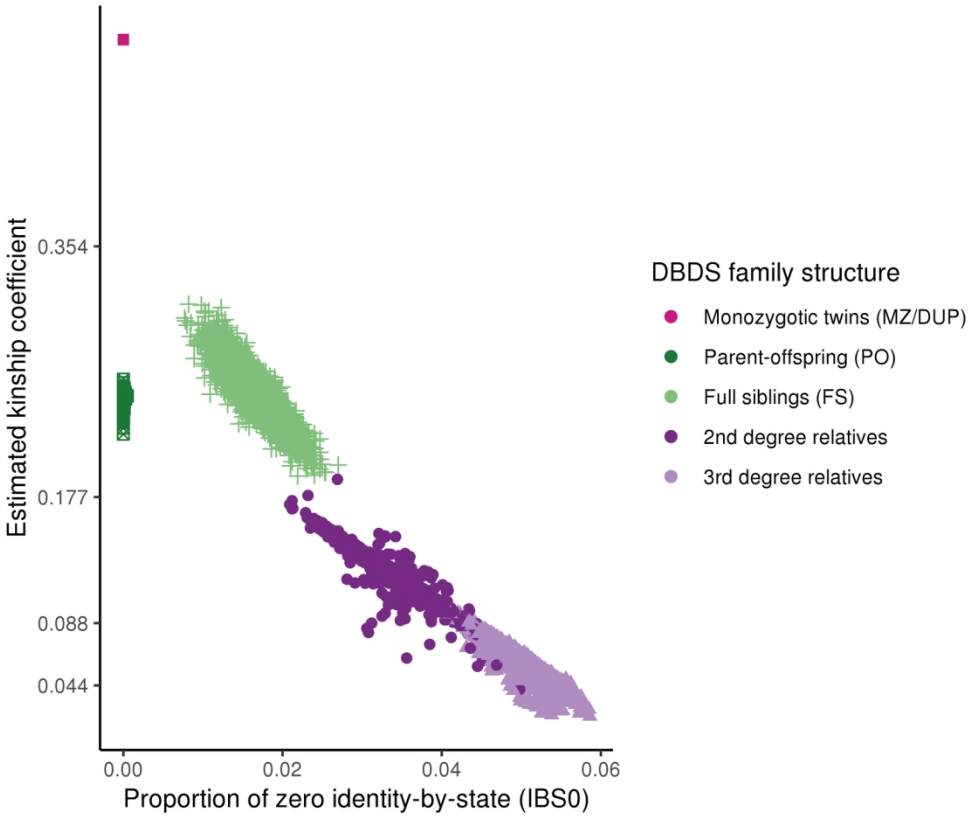
Table 2. Related pairs (3rd degree or closer) for batch 1 DBDS donors genotyped (N=85,000).

Relationship	*Monozygotic twins*	*Parent-offspring*	*Full siblings*	*2nd degree*	*3rd degree*
Pairs	51	4,246	3,309	4,375	11,433



Caption : Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

127x127mm (300 x 300 DPI)



Relationships for DBDS donors genotyped as part of the first batch(n=85,000). Each point represents a pair of related individuals and the colors indicate the degree of relatedness ("InfType" in KING IBD segment inference): monozygotic twins and technical duplicates(MZ/DUP) in pink (in the upper left corner), 1st degree relatives as parent-offspring pairs (PO, dark green) and full siblings (FS, light green), 2nd and 3rd degree relatives in dark and light purple, respectively. The y-axis shows the estimated kinship coefficient, defined as the probability that two alleles sampled at random (one from each individual) are identical by descent. The x-axis shows the proportion of zero identity-by-state (IBS0), defined as the proportion of SNPs at which two samples share no alleles. KING's criteria was used to estimate the degree of relatedness (--related command in KING). We used a set of independent high-quality markers (excluding palindromic and non-autosomal markers, markers with MAF<1%, low call-rate (<99%) and markers in regions with high Linkage Disequilibrium) for the relatedness calculation.

152x127mm (300 x 300 DPI)

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Cohort profile: DBDS Genomic Cohort, a prospective and comprehensive resource for integrative and temporal analysis of genetic, environmental, and lifestyle factors affecting health of blood donors.

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Cohort profile: DBDS Genomic Cohort, a prospective and comprehensive resource for integrative and temporal analysis of genetic, environmental, and lifestyle factors affecting health of blood donors.

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Abstract

Purpose: To establish a cohort that enables identification of genomic factors that influence human health and empower increased blood donor health and safe blood transfusions.

Human health is complex and involves several factors, a major one being the genomic aspect. The genomic era has resulted in many consortia encompassing large samples sizes, which has proven successful for identifying genetic factors associated with specific traits. However, it remains a big challenge to establish large cohorts that facilitate studies of the interaction between genetic factors, environmental and life-style factors as these change over the course of life. A major obstacle to such endeavors is that it is difficult to revisit participants to retrieve additional information and obtain longitudinal, consecutive measurements.

Participants: Blood donors (n=110,000) have given consent to participate in the Danish Blood Donor Study. The study utilizes the infrastructure of the Danish blood banks.

Findings to date: The cohort comprises extensive phenotype data and whole genome genotyping data. Further, it is possible to retrieve additional phenotype data from national registries as well as from the donors at future visits, including consecutive measurements.

Future plans: To provide new knowledge on factors influencing our health. Thus, provide a platform for studying the influence of genomic factors on human health, in particular the interaction between environmental and genetic factors.

INTRODUCTION

A person's health is determined by complex interactions between genetic, environmental, and lifestyle factors. Analyzing these factors collectively and prospectively is preferable. However, this is usually only possible using birth-cohorts and large population-based cohorts, and due to the extensive effort involved in establishing such cohorts, they are rare. The Danish Blood Donor Study (DBDS; dbds.dk) is a large prospective cohort of blood donors aiming at identifying predictors of healthy donors. As part of this cohort, we have now established DBDS Genomic Cohort assessing common single nucleotide polymorphisms (SNPs) in 110,000 donors. Thus, the DBDS Genomic Cohort provides a comprehensive catalog for large-scale genetic analyses in relation to numerous environmental and lifestyle factors affecting donor's health. A description of summary statistics on phenotypes and data coverage are provided in Table 1. A detailed sociodemographic description of Danish blood donors, including sex and age distribution, ethnicity, education, employment and level of urbanization are found in Burgdorf et.al 2017[1].

The evaluation of blood donor's health is important for several reasons. It is crucial for both the donor and the blood recipient that a healthy donor population is maintained with a high donation rate and a low dropout rate, thereby ensuring a steady blood supply. Evidence-based guidelines for donor recruitment, care, and retention are needed to ensure that donor recruitment can focus on individuals who are likely to remain healthy and donate frequently in the long term. One obvious relevant influential parameter relates to iron metabolism: We know that hundreds of genes impact the generation and regulation of blood cells[2] and also influence phenotype variations of iron absorption and metabolism[3-5]. Genome-wide SNP information is expected to provide knowledge enabling us to evaluate to whom donating blood will be unproblematic, thus facilitating retention of a stable blood donor population. Another parameter is altruism. Altruism as part of a prosocial behavior, the selfless concern for the welfare of others[6], is generally considered a typical blood donor characteristic[7].

However, altruism in the context of voluntary blood donation has also been shown to be a very complex phenotype[7]. In a previous study, we found a substantially larger genetic influence on blood donor behavior compared to most previous twin studies on altruism, which further highlights the heterogeneity of the blood donor personality[8]. The considerable amount of kinship (twins and siblings) in the cohort in combination with socioeconomic variables from the Danish registries will enable us to further differentiate between genetic impact and social basis of altruism in blood donation. The genome-wide SNP information will provide knowledge that can aid in the identification of long-term and steady donors. Further, we will test for association between genotype SNP information and prodromal symptoms of somatic and psychiatric disease or illnesses.

The DBDS Genomic Cohort offers the possibility to assess the impact of heterogeneous exposures in a broad range of phenotypes, such as mental state, risk-taking behavior, and characterization of blood components, and immune defense. The setup of the study allows for researchers to assess the genetic association in a) cross sectional studies to investigate e.g. the variation of phenotypic characteristic, clinical, and biochemical measurements, b) retrospective studies of e.g. rehabilitation capacity, and c) prospective studies e.g. analyzing the variation of phenotypes and clinical measurements over time and even identify prodromal symptoms.

COHORT DESCRIPTION

Population: The nation-wide blood donor population in Denmark consists of more than 230,000 donors giving more than 300,000 blood donations annually (www.bloddonor.dk). Blood donation in Denmark is voluntary and unpaid. This means that the donation is based on the desire to help others, who need it and not the desire of an economic benefit. Blood donors must be physically well, aged 17 and 67 years, and weigh more than 50 kilos. Individuals in chronic medical treatment or frequent travelers to countries with high prevalence of blood disease are not allowed to participate. The deferral

rules can be seen at www.bloddonor.dk. Blood donors from foreign countries must have lived in Denmark for a minimum of one year, have a Danish social security number and have learned the Danish language to prevent misunderstandings between the donor and the blood bank professionals.

The nationwide Danish blood bank is an integrated part of the Danish healthcare system financed by local and state taxes. The Danish healthcare system is administrated by democratically elected assemblies from national state institutions, regions and municipalities. The Danish blood banks are non-profit organizations owned and operated by each of the five regions in Denmark. The blood banks have a national board to structure collaboration across regions on recruiting donors, processing and distributing the blood for the Danish population. The DBDS is building upon the structured Danish blood bank system in the regions responsible for administrating donation sites at 27 hospitals in addition to five mobile donation units using 180 selected sites nationally (e.g., large companies, sports centers and universities)

At the blood donation centers and attached laboratories, the entire necessary infrastructure needed for the collection of biological samples and structured data is in place. Both blood plasma and whole blood for DNA extraction are available from all donors. The blood bank infrastructure already has laboratory facilities with educated staff (nurses, technicians, IT specialists and physicians). In addition, the blood bank professionals facilitate the testing of the blood for a variety of biomarkers and holds expertise in large scale storage of biological material.

Contact to participants at the blood banks is fundamental to our study. Blood donors are asked to participate and sign an informed consent when they visit the blood bank to donate blood. This consent allows us to use the blood samples from their past and future donations to study the impact of genetic and immunological factors on current and future health and disease. The inclusion and exclusion

criteria for blood donation and participation in DBDS are the same with 95% of the blood donors who are invited agrees to participate in DBDS[9].

Questionnaires: From March 2010 until July 2015, all participating donors had to complete a four-page paper-based questionnaire with questions of self-experienced physical and mental health including the 12-item short form (SF-12®) standardized health survey, smoking habits, alcohol intake, exercise, food intake, supplemental iron intake, height, weight, and waist circumference. 85,000 individuals filled out the paper questionnaire. As a follow-up to the initial paper-based questionnaire, we have developed and implemented a digital and flexible tablet-based questionnaire platform, using the open source survey software tool LimeSurvey[10]. This enables a rapid, easy, and cost-effective procedure to collect self-reported data on health traits from the participating donors at the donor sites at multiple time-points. The first digital questionnaire was implemented and used from July 2015 until May 2018. The questionnaire was focused the following research questions: allergy, ADHD, migraine, hidradenitis, depression, and Restless Legs Syndrome. It also contains questions from the paper-based questionnaire; SF-12, smoking habits, alcohol intake, height and weight. In total, 48,000 DBDS participants completed the first digital questionnaire. The second digital questionnaire started June 2018. It includes questions on: Sleep patterns, anxiety, migraine, stress, skin diseases, endometriosis, pain, learning difficulties, SF-12, smoking habits, alcohol intake, height and weight. Using the questionnaire data, several studies have already assessed the factors describing the general health of blood donors, e.g. hidradentis[11], risk of infection[12], migraine, restless legs, and depression[13 14]. Also, by revisiting samples it has been possible to assess wether infections correlates with obesity[15] and how iron deficiency and hemoglobin levels affect donor health in general[16 17].

Registry-based data: There is and has always been equal access to the healthcare system in Denmark, hence, the centralized national civil and health registries are unbiased and comprehensive resources of healthcare data. The registries include the National Patient Registry containing all hospital-

registered diagnoses since 1975[18], as well as other specialized registries, e.g. the Danish Medical Birth Register, the Danish Register of Causes of Death, and Statistics Denmark monitoring e.g. socio-economic data. We have already used the DBDS cohort in epidemiological studies assessing e.g. the mortality [19] of donors and the effect of blood donation on offspring birth weight[20].

DBDS Organization: The DBDS itself is described in detail by Pedersen et al.[9]. Briefly, DBDS is governed by a steering committee with a scientific advisory board. All projects are managed by the DBDS steering committee. Genetic projects involving genetic data in DBDS are run in collaboration between DBDS Genomic Consortium that consists of the DBDS steering committee, deCODE Genetics®, and scientific collaborators.

Genotyping: DNA is purified from whole blood and subsequent stored at -20°C. All samples are then genotyped in two batches at deCODE genetics using the Global Screening Array (GSA) by Illumina (batch 1 n=85,000 and batch 2 n=25,000). The array has a very rich up-to-date content of >650.000 SNPs with custom chip content optimized for comparison with the Illumina Omni Express chip. All genotype data are processed simultaneously for genotype calling, quality control and imputation. Initially, individuals or SNPs with more than 10% missing data are excluded, as are individuals deviating more than 3 standard deviations from the population heterozygosity (correcting for individuals carrying large copy number variations (>100Kbp)).

Imputation

The genotyping data is imputed using a reference panel backbone consisting of 1) UK 1KG phase 3 and HapMap reference to predict non-genotyped SNPs with minor allele frequency (MAF)>1%, and 2) an in-house dataset consisting of n>6,000 Danish whole genome sequences to improve the prediction of variations with a MAF down to around 0.01%[21]. Variants listed in the American College of Medical Genetics and Genomics guidelines are currently not predicted, due to restrictions from the

Danish National Ethics Committee [22]. For future collaborative studies the Ethics Committee will approve analysis of these variants on a case by case basis.

Copy number variations

Using the genotype of b-allele count and log Ratio, copy number variations (CNVs) are called using pennCNV[23]. CNVs called using <20 SNPs are excluded and the remaining CNVs are visually inspected to exclude false positives.

Statistical design: All data are stored and analyzed on a specialized, secure section of the 16,000 core Danish National Supercomputer for Life Sciences - Computerome (www.computerome.dtu.dk). Data storage and computational analysis is performed on a protected, private cloud environment. The analysis environment is capable of dynamic scaling and has been successfully tested in a composition of over 100 servers totaling more than 300 CPUs, over 13TB of RAM and has access to up to 5.7PB (5,700TB) of disk space. The cluster comes with a preconfigured queueing system, possibility to run Virtual Machines and containers (e.g. Docker, Singularity), a set of over 900 preinstalled tools and packages and a possibility to add GPU servers optimized for Machine Learning and specialized big memory systems (1-8TB of RAM).

For each hypothesis tested, a synopsis is provided including a detailed analysis plan. Information on each synopsis will be published either as link to published articles describing the results or as summary statistic on the study website: www.dbds.dk.

Samples: During each visit to a blood donor facility (up to four times per year for whole blood donors or up to ten times if plasma donors), every participant donates one EDTA plasma sample. At inclusion in DBDS one whole blood sample is also taken. Plasma samples taken prior to the inclusion date are stored for quality assessments and will also be accessible for future analyses. All samples are frozen within 6 hours of donation and stored in the primary collection tubes until processing.

Routine blood measurements including e.g. blood group, red and white blood cell counts, hemoglobin concentration, and hematocrit are obtained at each donation. Besides routine measures, project-related measurements are available e.g. subgroups of patients are assessed for ferritin levels, infection status (*Cytomegalovirus*, *Toxoplasma gondii*, and *Herpes Simplex Virus*), HLA-typing, and other selected markers of infection (circulating cytokines, C-reactive protein, etc.).

General Data Protection and Ethical Issues and Principles: DBDS has secured necessary permissions and approval from the Danish Data Protection Agency (2007-58-0015) and the Scientific Ethical Committee system (M-20090237). New projects within the DBDS Genomic consortium will require additional approval by the National Committee on Health Research Ethics. DBDS will be responsible for the continued contact with, and securing future permissions from, relevant Danish authorities regarding research on DBDS samples.

Patient and Public Involvement: Patients and public were not involved in the design of this study.

Data availability statement: The study will adhere to the FAIR (http://datafairport.org/: Findable, Accessible, Interoperable and Reusable) concepts. Within this legal framework DBDS Genomic Consortium Board can thus decide how and under which conditions the data can be shared. Generally, relevant summary data will be publicly available via repositories 3 months after acceptance for publication (H2020 open-access policy).

FINDINGS TO DATE

Initial quality measures that have been assessed:

Kinship

As described, giving blood often runs in families and the heritability has been estimated to be >53%[8]. It is clear from the estimated kinship based on the first batch of participants (n=85.000) (Figure 1, Table 2), that there is a considerable 1st, 2nd and 3rd degree relatives among the participants in the DBDS Genomic cohort.

Ethnicity

Based on ~15K overlapping SNPs from the genotyped data and the 1000 Genomes samples, we confirm the expected population structure of the DBDS cohort; most participants are of European ancestry (99%) and the following two ethnicity groups are of South Asian (0.4%) and East Asian (0.2%) ancestry, respectively. The proportion of participants with recent African ancestry is extremely low (0.002%) which is expected given the strict donor travel quarantine rules. Ethnicity was evaluated using FlashPCA2[24].

Minor allele frequencies

The distribution of the MAF, shown that majority of SNPs (84%) is above 1% (Figure 2) as expected, which provides solid basis for genotype imputation.

STRENGTH AND LIMITATIONS

Consecutive measurements: A unique feature of this large blood donor cohort is the ability to do consecutive assessments. In standard settings, participants are typically recruited at a baseline time-point and are invited for follow-up studies once or twice in the following years. The blood bank represents an advantage because most donors have a long-term committed relationship for blood donation and are seen one to four times annually[25]. It is therefore possible to collect several yearly and consecutive biological samples and questionnaire information over decades for a large number of participants. Again, subgroups and samples from specific time-points can be used in a retrospective manner.

National-based registries: Denmark has several comprehensive national registries, which include both health information and socio-demographical measures on an individual level. The informed consent allows for combining information obtained from the DBDS participants and the national registries; the Danish National Patient Registry (since 1977), the Danish Cancer (since 1943) and Diabetes Registries (since 1992-2012), the Danish Registry of Medicinal Product Statistics (since 1994), the Civil Registration Registry (since 1968 vital status, number of children, birthplace, address, relocation and more), the Integrated Database for Labor Market Research (since 1982, e.g. educational level, occupation status, income, social status, and other related parameters). This facilitates retrospective, cross-sectional, or prospective studies using registry-based measurements in combination with questionnaire-derived data.

Donor selection: Although, DBDS participants resemble the Danish population a few limitations in the study design may affect the generalizability of the results[1]. The blood donor exclusion criteria dismiss individuals with: infections or diseases that are transmittable through blood, weight below 50 kg, hemoglobin (Hb) levels below 12.9 g/dL in males and 12.0 g/dL in females, and curious behaviors: individuals with high travel rates to countries with high risk of hepatitis and HIV, men who have sex with men, individuals who have previously worked as prostitutes, those who have used intravenous substances, and pregnant women. Comparing socio-demographical parameters of blood donors with that of the total Danish population, we know that very low- and high-income individuals are underrepresented among blood donors[1]. In this respect, we acknowledge that DBDS lacks coverage of certain parts of the general population in contrast to traditional population-based studies. Similarly, the population based UK Biobank study have also reported a "healthy volunteer" bias[26].

DISCUSSION

The extension of the DBDS with a genomic cohort will profoundly impact the usability and empower studies on genetic, environmental, and lifestyle factors that influence blood donor health. Furthermore, the study provides a unique platform that facilitates analysis of common phenotypes not otherwise found in the national health registries, disease resilience factors, and interactions between genes and environment. At finally, such a large healthy cohort holds a huge potential for providing crucial information for future precision medicine initiatives and similar efforts have been started, e.g. “All of US” (by NIH, US). In Denmark we have the advantage of a collection of extensive, national health registries that facilitate epidemiological studies on specific diseases/outcomes in such a large cohort. For phenotypes and symptoms not monitored systematically in these health registries; e.g. lifestyle factors such as smoking habits, sleep patterns, and self-perceived health; large epidemiological studies are needed but typical difficult to conduct. The DBDS Genomic Cohort can facilitate such studies. As described above, the DBDS Genomic Cohort exploits an existing blood donor platform with an extremely high participation rate (>95%), which facilitates a straightforward evaluation of donor health in large epidemiological studies. Furthermore, the electronic questionnaire platform allows for easy and fast implementation of new, targeted investigations in subgroups of the donor population. Together with outcomes from the Danish health registries and the millions of retrospective plasma samples stored in easily accessible freezers, DBDS represents a solid phenotyping platform that can be used for both cross-sectional epidemiological studies and for retrospective biomarker studies. We believe that these strengths make the DBDS Genomic Cohort a strong competitive player in the field of precision medicine.

The DBDS Genomic Cohort allows us to study gene-environment interaction that are otherwise difficult to study: testing disease development hypotheses; e.g. a) cognitive performance in interaction with genetic factors and the risk of dementia, and b) determining the contribution of genetic and environmental factors to a phenotype such as sleep pattern. One way to study this is by

using multivariate regression models. As an example, Fan *et al*. have recently portrayed an advanced example of gene-environment interaction analysis incorporating temporal and spatial considerations[27], an analysis based on data from the Danish civil registries. The DBDS Genomic Cohort is particularly valuable for studying disease resistance in individuals exposed to one or more known disease risk factors and yet do not proceed to develop the disease. One such example could be participants carrying a high load of a highly inheritable trait like psychiatric illness in the family or known genetic risk factors, who do not have psychiatric illness themselves. Lastly, DBDS also provides sequential storage of plasma samples, which allows for sequential blood measurements. Such measurements could be used to investigate health markers like suPAR (soluble urokinase plasminogen activating receptor) and the variation associated with the donor's general health[28].

In short, the DBDS genomic cohort facilitates the investigation of the impact of genomic factors on health traits and states.

Integrative analysis of different 'omics, i.e. multi-omics analysis will be possible in the large DBDS genomic cohort, which adds tremendously to its value as a resource for studying the health of blood donors and correlation between blood related traits and states. We expect that the DBDS genomic cohort will contribute to discovery and validation of prodromal symptoms and biomarkers of disease, thus providing a better understanding of the disease pathologies and suggesting new drug targets.

COLLABORATION

We encourage scientific collaborations using the data generated in the DBDS Genetic Consortium. Published summarized data is available om request. Otherwise request of data necessitate first approval by the DBDS steering committee and if the request is considered outside the aim of DBDS, application to the national scientific ethical committee is obligatory. Additionally, material transfer and data protection agreement need to be acquired. Please visit www.dbds.dk.

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Contributorship statement

TH, KB, and KSB conceived and planned the experiments. KB carried out the analyses. OBP, HH, HMP, KRN, CE,HU, PJJ, TW, JO, GBJ, MN, SA, PIJ,ES and LWT contributed to cohort and research design. DW, PJC, KB and SB led to data infrastructure design. CE, KSB, MHL, ES and MSP contributed to data capture. TH, KB and KSB contributed to the interpretation of the results. TH, KB, and KSB took the lead in writing the manuscript. All authors provided critical feedback and helped shape the analysis and manuscript and approaved the final version.

Competing interests

The authors declare no competing interest

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Figure 1. Relationships for DBDS donors genotyped as part of the first batch(n=85,000). Each point represents a pair of related individuals and the colors indicate the degree of relatedness ("InfType" in KING IBD segment inference): monozygotic twins and technical duplicates(MZ/DUP) in pink (in the upper left corner), 1st degree relatives as parent-offspring pairs (PO, dark green) and full siblings (FS, light green), 2nd and 3rd degree relatives in dark and light purple, respectively. The y-axis shows the estimated kinship coefficient, defined as the probability that two alleles sampled at random (one from each individual) are identical by descent. The x-axis shows the proportion of zero identity-by-state (IBS0), defined as the proportion of SNPs at which two samples share no alleles. KING's criteria was used to estimate the degree of relatedness (--related command in KING). We used a set of independent high-quality markers (excluding palindromic and non-autosomal markers, markers with MAF<1%, low call-rate (<99%) and markers in regions with high Linkage Disequilibrium) for the relatedness calculation.

Figure 2. Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

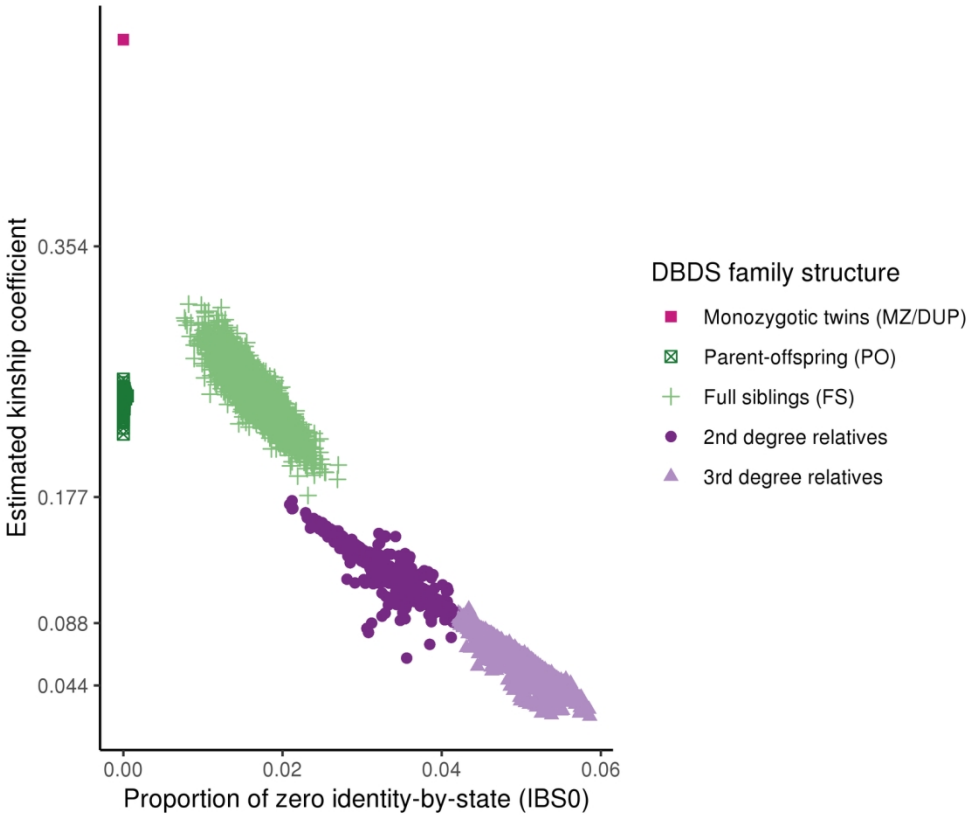
Table 1. Prevalence and missingness of enviroenmental variables

	Participants	*Missingness*
Men	48.8 %	0%
Women	51.2 %	0%
*Age**	41 (29-50) *	0%
*Height (cm) **	175.6 (169-182)	0.53%
*Weight (kg) **	78.1 (67-87)	0.82%
BMI	25.3 (22-27)	1.2%
Smoking	11.9%	1.3%
Alcohol consumption (daily)	3.0%	4.7%
Questionnaire 1	87.000	
Questionnaire 2	50.000	

* Mean (Quartiles).

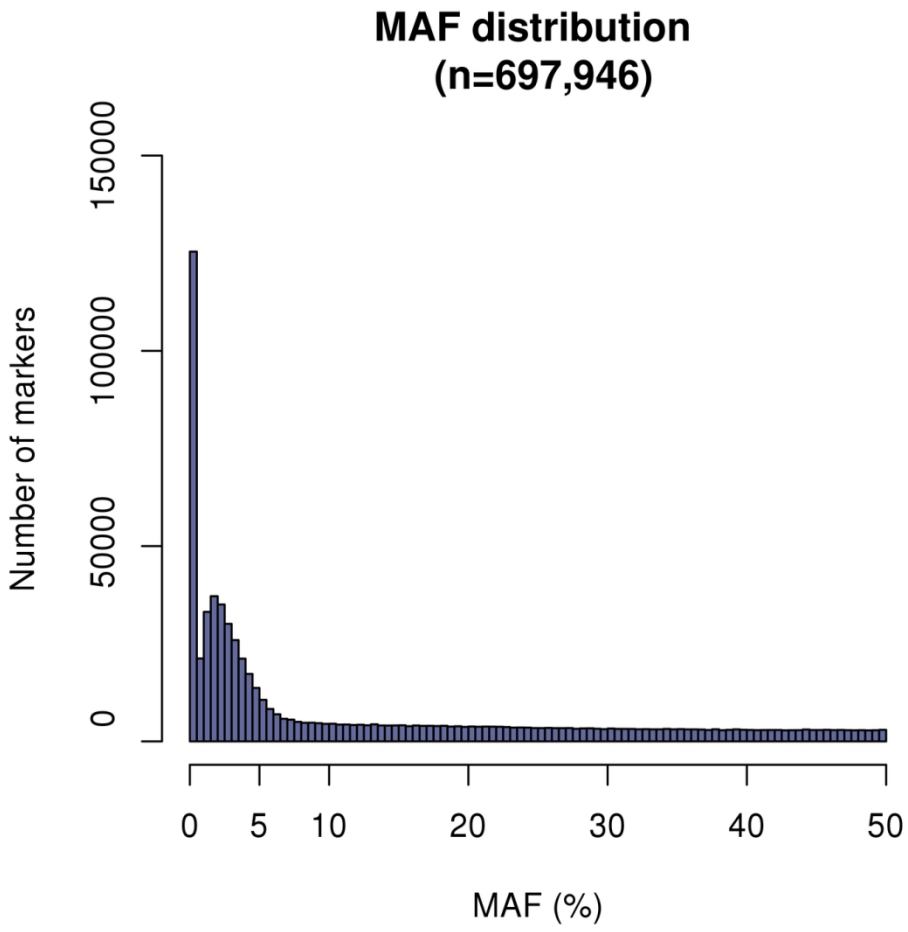
Table 2. Related pairs (3rd degree or closer) for batch 1 DBDS donors genotyped (N=85,000)..

Relationship	*Monozygotic twins*	*Parent-offspring*	*Full siblings*	*2nd degree*	*3rd degree*
Pairs	51	4,246	3,309	4,375	11,433



Relationships for DBDS donors genotyped as part of the first batch(n=85,000). Each point represents a pair of related individuals and the colors indicate the degree of relatedness ("InfType" in KING IBD segment inference): monozygotic twins and technical duplicates(MZ/DUP) in pink (in the upper left corner), 1st degree relatives as parent-offspring pairs (PO, dark green) and full siblings (FS, light green), 2nd and 3rd degree relatives in dark and light purple, respectively. The y-axis shows the estimated kinship coefficient, defined as the probability that two alleles sampled at random (one from each individual) are identical by descent. The x-axis shows the proportion of zero identity-by-state (IBS0), defined as the proportion of SNPs at which two samples share no alleles. KING's criteria was used to estimate the degree of relatedness (--related command in KING). We used a set of independent high-quality markers (excluding palindromic and non-autosomal markers, markers with MAF<1%, low call-rate (<99%) and markers in regions with high Linkage Disequilibrium) for the relatedness calculation

152x127mm (300 x 300 DPI)



Caption : Minor allele frequency (MAF) distribution of the genotyped SNPs prior to quality control.

127x127mm (300 x 300 DPI)