Twin Research and Human Genetics

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Establishing a Twin Register: An Invaluable Resource for (Behavior) Genetic, Epidemiological, Biomarker, and 'Omics' Studies

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Twin registers are wonderful research resources for research applications in medical and behavioral genetics, epidemiology, psychology, molecular genetics, and other areas of research. New registers continue to be launched all over the world as researchers from different disciplines recognize the potential to boost and widen their research agenda. In this article, we discuss multiple aspects that need to be taken into account when initiating a register, from its preliminary sketch to its actual development. This encompasses aspects related to the strategic planning and key elements of research designs, promotion and management of a twin register, including recruitment and retaining of twins and family members of twins, phenotyping, database organization, and collaborations between registers. We also present information on questions unique to twin registers and twin-biobanks, such as the assessment of zygosity by SNP arrays, the design of (biomarker) studies involving related participants, and the analyses of clustered data. Altogether, we provide a number of basic guidelines and recommendations for reflection when planning a twin register.

■ **Keywords:** twin register, twin research, recruitment, zygosity determination

Since the earliest times, it has been intuited that twins provide a window into the human condition, with numerous references in philosophical and literary texts, notably by Augustine of Hippo (400 AD), Shakespeare, and others. The formalization of this intuition towards scientific study may best be attributed to Francis Galton in the late 19th century (Galton, 1875), although at that time it was not yet established that there were two discrete types of twins. Researchers in the early 1900s (Fisher, 1919; Thorndike, 1905) pointed to the scientific value of twin studies (Rende et al., 1990), but in 1919 Sir Ronald Fisher still advocated that there was only one type of twins, stating that '... twins ordinarily share the hereditary nature of one gamete but not of the other' (Fisher, 1919).

The first half of the 20th century saw a slow but steady development of twin research through the pioneering work of researchers, such as Poll (1914), Merriman (1924), Siemens (1924), and Holzinger (1929) (see Mayo (2009) for a detailed account of this development). In Russia, twin studies were initiated as early as in 1900, with the first focus on psychological disturbances (see Box 1). Many twin studies were then undertaken and were essential in understanding the etiology of disorders. In France in the 1950s, Lejeune et al. (1959), for example, became puzzled by the high concordance seen for Down syndrome in identical twins in comparison to the extremely low concordance in non-identical twins. This concordance pattern was inconsistent with single gene inheritance and was one of the observations that led to the discovery of trisomy-21 as the cause of Down syndrome. The history of twin studies,

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Box 1: Twin Research in Russian Science

Reviews on the history of twin research tend to focus on developments in Western European countries and the United States. However, early references to twin studies are also to be found in Russian science. Psychiatrists Sergey Sukhanov and Tihon Yudin studied the similarity of psychosis in twins from 1900 (Sukhanov, 1900), and several small twin research of morphological, physiological, and psychological characteristics were conducted from 1900 to 1929 (Yudin, 1907). The Russian Medical and Biological Institute, which was created in 1929 and continued as the Medical and Genetic Institute from 1935 onwards, conducted systematic and large-scale twin research where more than 700 twin pairs were studied. The research was conducted by medical doctors, psychologists, and pedagogues under the guidance of Solomon Levit. A special kindergarten for twins was created in the institute, in which motor functions, different forms of memory, level of psychic development, attention, and intellect features were studied. The method of twin-control design was used to study effectiveness of pedagogic, medical, and psychological interventions (Levit, 1935). Unfortunately, these studies were limited and prohibited at the end of 1930s, and were restarted in Russia only in the 1970s in the laboratory of genetic psychophysiology created by Irina Ravich-Shcherbo (Grigorenko & Ravich-Shcherbo, 1997; Malykh et al., 1998).

including the basic methodological insights and developments, has been described in multiple papers (Boomsma et al., 2002; Martin et al., 1997; Mayo, 2009; Rende et al., 1990). In the classical twin design, which includes mono and dizygotic (MZ and DZ) twin pairs reared together, the resemblance for one or more human traits is compared between MZ and DZ twins to obtain estimates of uni and multivariate heritability. A larger resemblance in MZ twins is consistent with genetic influences on the trait under study. In the classical twin design, the statistical power is largest for detecting additive genetic influences (A). The two other variance components that contribute to resemblance of relatives, common or shared environmental variance (C), and dominance or non-additive genetic variance (D) require larger samples or the inclusion of additional family members to achieve reasonable power. Explorations of power of the classical twin study, first by simulation and later by direct analysis, showed that many thousands of pairs would be needed to separate these sources of variance (Martin et al., 1978; Posthuma & Boomsma, 2000). This became the justification for the founding of large twin registries in a number of European countries, in the Scandinavian countries, the Netherlands, the United Kingdom, as well as in the United States and Australia. Many other countries in different parts of the world have followed suit. Compilations of twin registers across the world have been carried out periodically and published in the journal *Twin Research and Human Genetics* (2002, 2006, and 2013) and elsewhere (van Dongen et al., 2012). Many of these twin registries are longitudinal, population based, and sufficiently large for epidemiological studies.

Twin registries have been a resource for thousands of studies, estimating the relative impact of genetic and environmental factors on trait variation across a wide range of biomedical and social science disciplines (Ayorech et al., 2016; Polderman et al., 2015). Their potential, however, for disentangling the role of genetics in human traits goes much further, through different designs and investigative methods, from genetic epidemiology to molecular approaches (Bell & Spector, 2011; Groen-Blokhuis et al., 2011; Kaprio & Silventoinen, 2011; Knopick et al., 2017). In many of the large registers, data collection is undertaken by mailed questionnaire or by telephone interview, and more recently, by online survey, and record linkage. Clinical twin studies were of necessity often smaller, requiring twins to visit research or medical facilities, or researchers visiting twins at home. In anticipation of the age of molecular genetics, many registers started DNA collections in the early 1980s, and these samples have become a highly valuable and much used resource for zygosity assessment, genetic linkage, and association studies. Collecting multiple sources of biological material has enabled twin studies of epigenetics, transcriptomics, metabolomics, proteomics, microbiome, and a wealth of biomarkers.

This article aims to discuss several aspects in establishing a twin register. We will attempt to cover what is important and why, and how to develop such a scientific resource. In this endeavor, we will also refer to when to start and who should participate. Our aim is not to offer a checklist or a complete step-by-step technical guide, but rather to discuss the issues that, in our experience, should be addressed at the launch and in the management of a twin register.

The First Steps

The first question that should be addressed is: 'Do we really need to start a new twin register?' Establishing a twin register is a huge and long-lasting effort and, although it pays back in the long run, it is costly, both in terms of economic and personal investments. Hence, research objectives must be clearly defined. Working with twins has many compensations that go even beyond the classical methodological advantages (see Box 2), and establishing a register may appear to be the best choice. However, it may not be the only option. Already established twin registries with data, or willing to collect new information, may be open to collaboration. In fact, nearly always a twin researcher can be found with an interest in collaboration and in replication of results. Using this option will not only result in economy of

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Box 2: Do It With Twins

In 1982, David Lykken listed in his presidential address to the Society for Psychophysiological Research (Lykken, 1982) several compelling reasons for doing research with twins that are 'in addition to' the genetic analyses that the classical twin design allows:

- Twins are plentiful and easily recruited as experimental subjects.
- Twins are probably more representative of the general population than any other group.
- This representativeness is even more true of the families of twins.
- Twin data are invaluable to explore issues measurement. Any measure that shows high within-pair correlation among MZ twins deserves to be treated with respect.
- The method of co-twin control provides enhanced experimental power. Using one twin from each pair for the experimental group and the other for the control group provides a test of one's hypothesis that is as powerful as an experiment employing twice as many pairs of singletons.
- If one treats one's subjects properly, and keeps in touch, then it will be possible to bring many of them back repeatedly over the years to participate in additional experiments. This is useful not only for longitudinal research but also as a method of enhancing each subsequent experiment with the information previously gathered on these same individuals.

Box 3: How Many Twins?

For most of the 20th century, until about 1970, there were only vague notions of how big twin studies needed to be to provide useful estimates of the degree of genetic influence (heritability), and many of the early studies, including small numbers, gave highly inconsistent results when complex human traits were analyzed. In retrospect, we can see this was mainly due to studies being underpowered, although inaccurate zygosity diagnosis also played a role. 'Is there a genetic contribution to scholastic performance?' was the motivating question behind the first Australian twin study on school examination results from 1967 (Martin, 1975; Martin & Martin 1975). While the study of 150 twin pairs was fairly large by the standards of that time, it soon became apparent that it was far too small to reliably estimate all genetic and environmental sources of variation, specifically the separation of additive genetic (A) and common or shared environmental variance (C). Multiple analytical and simulation studies now provide detailed tables with the required numbers of twin pairs for continuous and categorical traits, often distinguishing between uni and multivariate designs (Martin et al., 1978; Neale et al., 1994; Posthuma & Boomsma, 2000).

effort, avoiding duplication, but the proposed project may also benefit from the experience of other twin researchers.

Still, there may be many good reasons to start a twin register, for instance, in specific countries or populations. In that case, some other focal questions arise, starting with the question of initial funding. This is, of course, a relevant question, and the available options will depend on many, often local, factors. When applying for initial funds, it may be practical to adapt the objectives to limited resources by focusing on a specific research topic rather than putting in a more general appeal to establish a research infrastructure. It is often wise to refine the research agenda in order to meet two complementary objectives: obtaining meaningful results in the short term and looking for synergy with other research groups. Planning a long and complex research question will delay results that will be needed, since future funding will probably depend on early success. Hence, it is important to select a main phenotype to study based on its originality, the interest in the question within the scientific community, the uniqueness of the sample, or the available resources for data collection (see Box 3). Complementarily, it is important to seek out researchers not only within other twin registers around the world, but also outside the twin community, who have an interest in the selected phenotype and/or have relevant data. Collaboration with experienced researchers in the field is of value for a new project, while researchers from different disciplines may be interested in the possibilities that collaboration with a twin cohort offers. Identifying possible topics of common interest to the newly starting twin register and existing groups, which can contribute specific knowledge or techniques, may open new perspectives and facilitate trade-offs.

Strategic Planning

A twin register ideally is a longitudinal resource and, therefore, the first steps should be considered as the basis of a long-term effort. Decisions made during the first steps should facilitate the strategic planning of the register as a long-lasting organization. This involves setting the main goals and selecting the activities, in accordance to the available resources, which need to be undertaken to achieve the established objectives. Here, we first discuss human resources and adaptability to changing conditions. Human resources are, obviously, a core element. A group of highly motivated and coordinated researchers is needed to start and develop a twin register. Thus, the question of identifying who may provide valuable help and be willing to participate in the endeavor becomes crucial. Two different kinds of human resources should be contemplated:

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established researchers, from inside and outside the twin research community, who can contribute expertise, advice, and logistics in their respective fields; and researchers or support personnel, who will be in charge of developing and maintaining the register. While the former are important in providing support and visibility, the latter are essential, since they will take care of the multiple tasks involved in the daily running of the register, i.e., from planning and conducting data collection or updating contacts to analyzing data or writing papers. Therefore, human resources management (including selection, training, and career development) with the objective of forming a reliable and enduring core group is paramount if the register is to go ahead. Flexibility, adaptability, and keeping an eye on opportunities are also relevant issues. In a changing environment, where critical aspects such as funding or collaborations may change constantly, it seems wise to contemplate different horizons and be able to quickly adapt research objectives to different scenarios. This implies to the capacity to keep going with limited resources while being prepared for incoming opportunities. Focusing only on long-term and complex research objectives may represent a handicap for register development in case of funding shortage or operational obstacles. Keeping in mind and planning parallel sets of objectives adapted to different conditions may help to overcome temporary difficulties.

Basic Elements

There are several key elements that are at the core of the development of a twin register and that will determine its endurance and scientific success.

Recruitment Methods

One of the foremost questions of every researcher willing to start a twin register relates to which are the best practices for optimum recruitment and retention methods. There is not one clear answer and there may be as many methods as established registers. Recruitment strategies depend on a research protocol that can specify, for example, age at recruitment, inclusion and exclusion criteria, recruitment group (e.g., parents of young twins, adolescent, and adult twins), and possibilities for the research team, which may be affiliated with an academic institution or a medical infrastructure.

Table 1 summarizes, in a non-exhaustive manner, some of the possibilities for recruitment of participants. They can be divided into four major groups: (1) existing databases managed by public (e.g., city council, educational, or health systems) or private (e.g., hospitals or insurance companies) stakeholders; (2) institutions or organizations that have access to twins; (3) participants recruited through media, advertisements, and social events; (4) word-of-mouth and recruitment through enrolled participants of the register. There are many ways to find and enlist twins and, within these categories, researchers should be creative in finding ways to invite participants to a register. Different citizen registers or records can provide information about twins (e.g., birth or military records). In some countries, sampling twin pairs is based on computerized population registers, either from direct information on multiple births or from applying algorithms based on sharing of date of birth, family name at birth, place of birth, and so on. A request to provide addresses of persons born from the same mother with an identical date of birth can be done by municipalities. In all cases, 'real' twins have to be distinguished from a larger subset of 'possible' or 'administrative' twins, as sharing the name of the mother and date of birth might occur by chance (Goldberg et al., 1997). Next, parents of twins or twins need to be contacted, with an invitation to participate in the register. Population samples can also be obtained through collaboration with hospitals and schools. Records can be available at maternity hospitals, which may give an opportunity of direct recruitment of study participants. The recruitment through schools gives possibilities to obtain information on school achievements from teachers. Many registries collaborate with twins or parents of twins associations.

TABLE 1

Recruitment Methods of Twin Registries

Using existing databases with information on twins managed publicly or privately
Previous twin studies
Population registries
Birth records
Immunization registries
Different patient registries
Voter records
Military records
Collaboration with institutions and organizations
State public health resources (e.g., healthcare departments)
Hospitals, maternal hospitals, and outpatient clinics
Insurance companies
Schools
Orphanages and adoption agencies
Multiple birth associations
Twin clubs and associations
Recruitment through media and social events
Media, newspapers, TV, and radio
Advertisement
Information brochures
Website
Social media
Scientific and social events (e.g., twin festivals and annual gatherings)
Word-of-mouth and through enrolled participants in register

Note: The table does not attempt to be exhaustive.

Other twin collections are gathered independently of centralized records or institutions and may depend more on the motivation of the twins or their parents. Recruitment through advertising has been used, as well as through mass media articles on twins and twin research in which information on major achievements is combined with continuing studies and contact information. Such approaches can be effective, and the possible effects of bias in non-randomly ascertained samples can be dealt with by statistical methods (Bechger et al., 2002; Neale & Eaves, 1993).

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Twin pairs can be registered via completion of a registration form online by either the twins or their parents if they are under the age of legal consent. Other avenues of recruitment include offering booklets to parents who expect twins. The exposure of twin research findings in general media also attracts new participants. Some registries organize social events (e.g., twin festivals, a range of exhibitions about twins, including photos and pictures). Common meetings of enrolled and new participants can benefit the realization of a register and contribute to the strengthening of the role and value awareness in participants. A useful practice can be when participants give presentations about their own experience during meetings or on social media or websites.

Informed Consent

Twin registers are set up with the aim of conducting multiple studies across a long period of time and generally collect a wide variety of data in their participants. While participants upon registration may agree at the start of the study to the general aims provided in information brochures and will consent to be approached in years to come, the initial consent will not cover all the data to be collected in the future. Participants should be kept informed of the ways in which their data are used and be provided with the option of withdrawal at any stage of the research. Researchers need to establish how they will meet the participants' rights to know and to withdraw. Although the way this is laid down in law will not be the same across all countries, it is always part of good scientific conduct. In the past, technology was not sufficient to provide individual feedback, and information on the use of collected data was often provided in a general manner via websites or mass mailing of newsletters. As a result of technological advances, it is now possible to build portals or apps to provide much more personalized information, showing a person for which purpose his/her data were used, and allowing participants to indicate whether they want to participate in specific projects or withdraw from the ongoing study. Such personalized platforms may require additional information from participants such as an email address or phone number for verification purposes and log-in information. The extent to which active informed consent requiring a handwritten signature is needed or is sufficient to inform the participant and have an opt-out procedure needs to be discussed with an ethical committee any time new data collection takes place. Thinking of the different kinds of projects that will take place and the way information will be shared with participants and getting the tools ready before the start of the twin register will not only save valuable resources later on, but it may also show the participants you will protect their rights, leading to increased trust in the twin register.

Determination of Zygosity in Twin Registries

For a twin register, a critical measurement point is the zygosity status of a twin pair, that is, MZ or DZ, as it is the basis for subsequent research that will focus on heritability estimation and genetic covariance structure modeling. It is also one of the most frequently asked questions by the twins, as they are sometimes uncertain or misinformed about their zygosity status. Even when no genetic analyses are carried out and the large datasets are used for epidemiological studies, researchers may want to correct for clustering in the data, depending on zygosity status. Misclassification of zygosity status in MZ or DZ pairs generally results in the heritability estimate going down (Figure 1). In extreme cases, it may even result in wrong conclusions to be drawn from variance components modeling.

Zygosity can be determined according to simple rules (see Box 4), but DNA testing will give the most conclusive zygosity assessment. A recent development is to genotype both twins with single-nucleotide polymorphism (SNP) arrays such as the Illumina Infinium Global Screening Array (GSA) or the Affymetrix Axiom World Array (Ehli et al., 2017). These arrays allow for fast genotyping for over 600,000 SNPs, which is more than is required to determine twin zygosity. However, given the reductions in genotyping costs and the possibilities for future genetic association studies, a genome-wide array makes a good investment. Of course, both twins need to provide their DNA. This can be collected by available prefabricated DNA kits for collection of buccal or saliva DNA at home, or blood can be provided at the study site. Once in the lab, DNA needs to be extracted, purified if needed, and diluted to the right concentration. The subsequent steps might be more array specific, but involve the fragmentation of the DNA into smaller pieces, then precipitation, and then hybridization to the chosen array. Here, the sample fragments of DNA will 'connect' to the SNP alleles, variants of DNA sequence in humans, which are present on the array. This hybridization results in a fluorescent tag, which subsequently can be read from the array for all SNPs.

For zygosity assessment, the minimum number of typed SNPs needed is around 50; however, using between 20,000 and 30,000 typed SNPs is optimal. At the DNA level, MZ twins will share (close to) 100% of their alleles. DZ twins will share on average 50% of their alleles, similar to siblings. After using the factory standard tools for array genotyping (Beadstudio or APT-genotyper), a tool like Plink (Purcell et al., 2007) can be employed to quality control the DNA data, select an optimal number of SNPs, and determine the allele sharing in all pairs (genome option). This sharing is then given by the percentage of markers for which a pair shares no alleles (Z0), one allele (Z1), and two alleles (Z2). From these proportions, the overall sharing is calculated, by π (pi), which equals $0.5 \times Z1 + Z2$. Then, MZ pairs can be identified from the results by finding pairs with a $\pi > 0.90$. The DZ pairs can likewise be selected, by finding pairs that have a π between $\sim$0.30 and $\sim$0.70, and a similar value for sharing 1 allele (Z1) (Figure 2). For other values of π,

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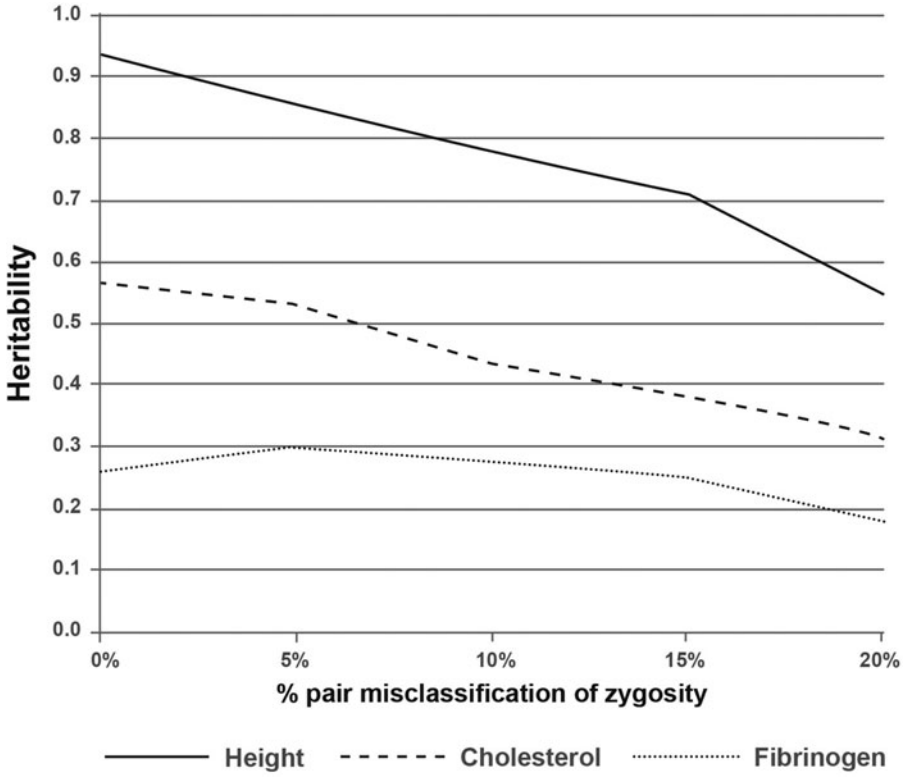

FIGURE 1

The effect of zygosity misclassification on the heritability estimates within a twin study.
In this figure, heritability estimates for height, total cholesterol, and fibrinogen are given on the *y*-axis. These estimates were calculated from the phenotypic correlations '*c*' between the two individuals of 391 Dutch DNA confirmed MZ and 391 DZ pairs, with the formula $(c_{MZ}-c_{DZ})/(1-c_{DZ})$. Subsequently, in 5, 10, 15, and 20% of these pairs, the zygosity status was flipped from MZ to DZ, and from DZ to MZ, introducing misclassification (*x*-axis). Then, the heritability was recalculated and plotted in the figure. Depending on how strong the heritability of the phenotype is, the misclassification in general reduces the overall heritability estimate.

Box 4: Basic Rules for Zygosity Determination

- Opposite-sex: DZ
- Different blood groups: DZ
- Large differences in eye, skin, and hair color: DZ
- One placenta: MZ (note that two placentas does not imply DZ)
- Alike as two peas in a pod; parents cannot tell the children apart: likely MZ
- Offspring and grandchildren cannot tell parents or grandparents and their twin apart: likely MZ
- Discordance for blood group or DNA markers: DZ

researchers need to recheck which DNA sample was typed for the twins.

This approach has several more advantages. There is a useful genotyped dataset that allows for checking additional issues like genetic relatedness among participants, gender, heterozygosity, and if the study population is ethnically heterogeneous. As next steps, SNP sets can be imputed to, for example, the 1,000 Genomes phase 3 or the Haplotype Reference Consortium (HRC) genome reference panels (McCarthy et al., 2016). These data can then be analyzed in genetic association studies and contribute through meta-analysis in consortia to localization of genes for complex traits, to polygenic risk scores analyses and estimation of SNP heritability, or to employing Mendelian randomization to find causative relations.

Phenotyping: From Survey to Record Linkage

Twin registers have obtained a wide variety of phenotype data through various methods. The basic measurement method is often the survey, with registers sending out surveys at regular intervals. When deciding on what to include in a survey, the purpose of the current survey and the long-term goals need to be taken into account. For instance, a funded study may focus on alcohol use, but a long-term goal may be to determine how genes and lifestyle contribute to depression, so it would make sense to include a depression scale in the survey. Also, it is important to take into account which data can still change over time and which data are fixed and do not need to be obtained again. This may of course be age specific. For instance, in a middle-aged

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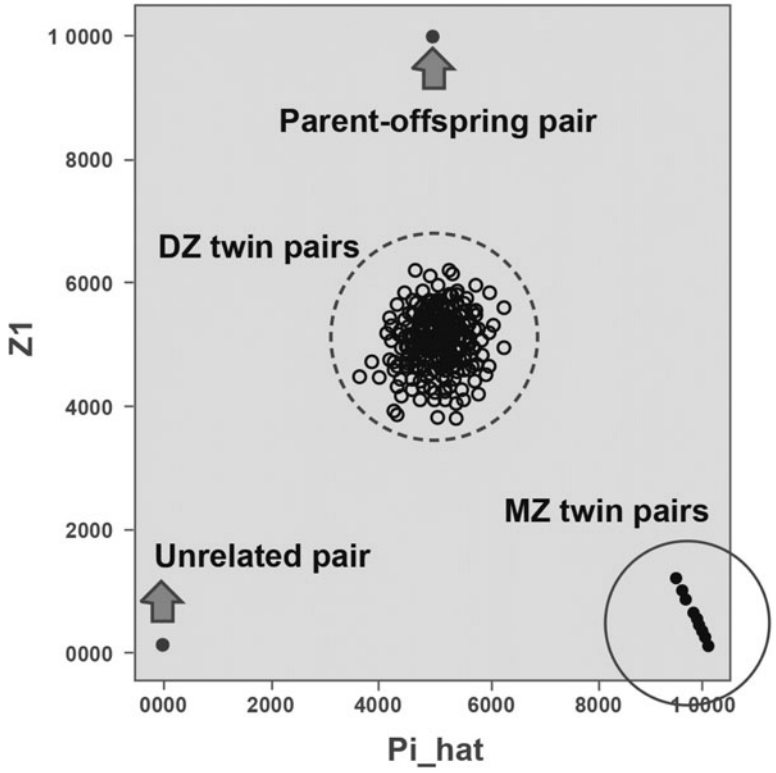

FIGURE 2

Allele sharing of various family pairs plotting the sharing of one allele versus Pi-hat to identify monozygotic and dizygotic twin pairs.

group, questions regarding educational level may not need to be repeated. Questionnaires also need to keep a balance between the quantity of information gathered and the participants' needs, since they should not be burdened with too many questions, risking attrition, or incorrect/missing data. While devising the first survey may seem daunting, many twin registers will be happy to share information to help the new register use well-established procedures and avoid pitfalls in survey set-up. While survey data can be obtained in all or at least large groups of participants, some data can only be collected in limited numbers. Laboratory procedures or specific phenotypes needing complex settings, long assessment times, or expensive equipment are not easily applicable to large samples. Examples would be studies on brain imaging or extensive cognitive testing. In these cases, participants may be invited based on specific inclusion criteria. New developments taking advantage of information technologies are modifying data collection procedures in epidemiological research and are also applied in twin studies. This includes computer-assisted surveys, and ambulatory assessment of objective (e.g., actimetry) and self-reported (e.g., mood and exercise) phenotypes through web or mobile applications.

Data collection is not, of course, limited to surveys or laboratory assessment. The assessment of environmental exposures linked to address or workplace information and the development of exposome-wide association studies represent novel approaches to gathering information for research purposes that do not need the direct involvement of the register participant.

Record linkage to external databases (e.g., hospital, primary care, or insurance and education records) is also an invaluable source of information that has been used by registers in Scandinavian and other countries. For example, Van Beijsterveldt et al. (2016) linked phenotype information from the Netherlands Twin Register to the database of the Dutch pathological anatomy national automated archive. Record linkage was successful for over 9,000 twin pairs. The effect of chorion type was tested by comparing the within-pair similarity between monochorionic and dichorionic MZ twins on 66 traits. They concluded that the influence of the intra-uterine prenatal environment, as measured by sharing a chorion type, on MZ twin resemblance was small and limited to a few phenotypes, implying that the assumption of equal prenatal environment of mono and dichorionic MZ twins, which characterizes the classical twin design, is largely tenable.

Possibilities for Biobanking in Twin Registers

Many twin registries collect biological samples from their participants. Initially, the reason for collection of blood samples was often to have a reliable measure of zygosity

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based on blood group or DNA typing, but biological sample collection can also extend the research potential of genetic epidemiology into, for example, cardiovascular and late-life health and mortality, by allowing measurement of biomarkers. Combined with the twin design, this allows estimation of the contribution of genomic factors (genetic, epigenetic, and gene expression) and biochemical factors (metabolite and proteins) to intermediate phenotypes and risk factors of disease, such as lipid levels (Snieder et al., 1997). Designs involving MZ twin pairs allow discovery of variability genes, as demonstrated for lipid levels (Berg, 1988). The development of laboratory technologies has dramatically increased opportunities to study collections of bio-specimens and their related data. This allows for comprehensive studies of complex diseases and phenotypes, facilitates the identification of predisposing genes and epigenetic factors, and provides support for a better understanding of disease etiology.

The organization of biobanks becomes an important element with the increase of bio-specimens and the necessity to conserve them. For example, whereas germ-line variations in the DNA sequence of a person rarely depend on the age at which a sample was collected, this is different for somatic DNA variation, epigenetic, and telomeric variation, for which the subjects' age when the specimen was collected is an important determinant (Fraga et al., 2005; Slagboom et al., 1994; van Dongen et al., 2016). Other determinants of epigenetic profiles are tissue/cell type (Finnicum et al., 2017) and lifestyle factors such as smoking. Many types of samples (e.g., whole blood) contain a mixture of cell types with distinct epigenetic profiles. In epigenetic studies of such heterogeneous samples, assessment of cell counts allows to control for variation in cellular proportions between samples.

There are multiple strategies for collection, processing, and storage of biological samples. A wide variety of specimen types may be collected and in many molecular genetic studies more than one tissue is stored, including blood and blood fractions (plasma, serum, buffy coat, and red blood cells), RNA, saliva, buccal cells, urine, hair, fecal samples, or nails. Each of these specimen types needs to be collected, processed, and stored under conditions that preserve their stability with respect to the intended future analysis (Garcia-Closas et al., 2001; Holland et al., 2003; Tworoger & Hankinson, 2006; Vaught & Henderson, 2011).

Collection of blood specimens should be carried out by trained personnel. An evacuated tube system (vacutainers) or plastic tubes are commonly used to collect blood. Umbilical cord blood is a useful source for research purposes, since the method of collection is not invasive. It can be obtained either through venous puncture of the umbilical cord or direct drainage to a sterile container immediately after delivery (vaginal or cesarean). Blood is often fractionated in components (mononuclear leukocytes, neutrophils, erythrocytes, and plasma) before being analyzed or stored. When biobanking, blood should be aliquoted across series of tubes, as most assays use only a small amount of plasma or serum and this avoids thaw/refreeze. Serum or plasma allows for analyses of classical biomarker assays, antibodies, nutrients, lipids and lipoproteins, leptin, adiponectin, growth hormone axes, thyroid axis, inflammation, liver and kidney function, innate immunity, and metabolomic and proteomic analyses.

Metabolomics is the rapidly evolving field of the comprehensive measurement of ideally all endogenous metabolites in a biological fluid. The use of mass spectrometry and nuclear magnetic resonance spectroscopy provides novel biomarkers of metabolic health (Suhre et al., 2010). Depending on the biomarker of interest, it may be important to collect, and note, whether samples were taken after fasting, how long after a meal or on a particular day, and of the menstrual cycle in women.

Whole blood, saliva, or buccal cells are excellent sources of DNA. Self-collection of buccal cells is a safe, simple, and cheap method that can be used to reduce the cost of specimen collection and is often preferred over blood collection by participants. Several methods are used for collecting buccal cells, including swabs, cytobrushes, and a mouthwash protocol (Meulenbelt et al., 1995; Min et al., 2006; Vaught & Henderson, 2011). Other sources of DNA include, for example, toe nails (Hogervorst et al., 2014).

In contrast to DNA, RNA is very sensitive to degradation at room temperature. Transcriptomics studies require careful RNA collection, using the PAXgene Blood RNA System, which consists of a blood collection tube in which intracellular RNA is stabilized (PAXgene Blood RNA Tube) and can be isolated by using a nucleic acid purification kit (PAXgene Blood RNA Kit). Alternatively, the samples can also be snap frozen in liquid nitrogen, or RNA can be isolated from PBMCs using Histopaque density gradients. Total RNA, including miRNA, can be isolated simultaneously from different biological sources. Plasma (300 μL) and serum isolations can be performed using miRNeasy Serum/Plasma kit from Qiagen. For isolation, after homogenization, from tissue biopsies (e.g., cartilage or adipose tissue) the miRNeasy Mini Kit from Qiagen can be used.

Many analytes, such as steroid hormones, pesticides, and a wide variety of drugs and their metabolites, can be measured in urine, making it a convenient specimen for a variety of studies. Urine collection can be performed under different conditions depending on the study goal: immediately upon rising in the morning, random urine specimens (for drug monitoring and cytology studies), fractional specimens after the last evening meal (to compare urine analyte levels with their concentration in blood), and timed urine collections (e.g., 12 and 24 hours to allow comparison of excretion patterns). Urine specimens should be maintained on ice or refrigerated for the duration of the collection. Collection vessels are generally larger than for other

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liquid specimens (from 50 to 3,000 mL). Due to the non-invasive method of collection and metabolic composition, urine is widely used in the research of metabolite biomarkers and a wide range of diseases (Duarte et al., 2014).

For microbiome investigation, fecal samples can be collected easily in a sealed container following simple instructions, and their processing can provide important information for classical twin analysis, such as in the studies estimating the heritability of gut microbiota (Goodrich et al., 2016), and related epidemiological and molecular approaches.

Databases for Twin Registers

Both administrative processes and scientific applications require database systems that recognize the clustered structure of data collected in twin families. Administrative processes may consist of importing new participants, who may or may not be related to existing participants, addressing management, documenting the participation status of individuals (moved, not willing to participate, ill, and deceased), and storing information on contacts and mailings with, for example, invitations to take part in particular studies, the responses to mailings and invitations, and outcomes of approaching non-responders. Any system that keeps track of personal information needs to adhere to guidelines concerning privacy. Identifying information, such as name, date of birth, and address, should be stored separately from other information collected on participants. Often, administrative and scientific processes will be supported by different database systems whose requirements depend on the dimensionality of the data. Phenotype data from surveys will require different systems than imputed genotype data that may contain as many as 50 million markers per person. Different databases may each work with separate anonymous IDs, and keys to link databases should be carefully kept.

Databases that contain multiple relatives should consider how to store information on family relations (Boomsma et al., 2008; Boomsma et al., 2018), especially when recruitment of participants not only involves twins, but also other relatives and multigeneration pedigrees — for example, parents or offspring of twins (see Box 5).

Data Analyses Issues in Twin Studies: Batch Effects and Family Clustering

Phenotyping in twins has often included biomarker assessments, such as lipids or hormone levels, and increasingly include assessments obtained by means of high-throughput technologies, such as genetic variants, gene expression data, and epigenetic modifications. These data are important to understand the nature of genetic variance components as established in twin and family studies (Schadt et al., 2005), and are themselves subject to such studies, for example, studies of the heritability of methylation and gene expression (Bell & Spector, 2012; McRae et al., 2014; Petronis, 2006, 2010; van Dongen et al., 2018; van Dongen et al., 2012; Wright et al., 2014).

Subtle differences in the processing of batches of biological samples are known to give rise to batch effect. The registration of information relevant to batch (batch number, analyst, time, date) provides the means to correct for such effects, and various methods have been developed to this end (Chen et al., 2011; Johnson et al., 2007; Lazar et al., 2013; Sun et al., 2011). Regardless of the methods to correct for batch effects, there is agreement that it is beneficial to randomize samples evenly over batches, and that this randomization should extend to case-control status and familial relatedness (Leek et al., 2010; Nygaard et al., 2016). Furthermore, sample size per batch is an important factor: the larger the sample size per batch, the more accurate the batch correction.

Batch assignment of samples collected in family members raises the question of whether samples of family members should be processed together in the same batch or should be distributed —as far as possible — over distinct batches. We examined this question in two small simulation studies (for details, see supplementary material). In the ideal situation of a balanced allocation design with relatively

Box 5: Twin Designs

The classical twin design encompasses MZ and DZ twin pairs but there are other designs. For instance, twin and adoption designs can be combined when twins reared apart are accessible. More often, twin registers may have the opportunity to incorporate other kind of relatives (extended twin family designs) that can be contemplated by a register even from the very beginning. These extended designs and possible combinations offer additional opportunities and statistical power to challenge research questions, such as the possibility of disentangling genetic from shared-environmental influences within family relationships (Keller et al., 2010; Knopick et al., 2017; Posthuma & Boomsma, 2000). The classical design may be enlarged around the twins by incorporating twins' parents (nuclear twin family design), twins' offspring (children-of-twins design), or parents, offspring, siblings, and spouses (Keller et al., 2009; Maes et al., 1997), according to available information, to finally incorporate all different kind of relationships that can be found within a register dataset. An example of such broadening of sample scope is provided by the Netherlands Twin Registry (Boomsma et al., 2018), which used an extended-twin pedigree, making use of all the relationship types available in their database (except teacher-student), to be able to estimate the contribution of shared household effects to neuroticism in the presence of non-additive genetic factors.

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large batch sample sizes, accurate correction of batch effects is feasible, as we established in a simulation study (see supplementary material). In the first simulation study, MZ twins were selected for concordance and discordance on phenotype X, which predicted phenotype Y, where Y (e.g., a biomarker) was subject to batch effects. Given the ideal scenario of random assignment and large batch sizes, we found that allocation regime (randomized as pairs or as individuals) had little effect on the results of either the regression of Y on X, or on the twin covariance matrix of Y conditional on X. The type of correction (random effects or fixed-effect correction for batch) had no bearing on these results.

In the second simulation study, we considered the decomposition of phenotypic variance into additive genetic and shared and unshared variance components (ACE model) using linear mixed modeling (McArdle & Prescott, 2005). The sample sizes (NMZ and NDZ) were relatively small: NMZ = NDZ = 200 (400 pairs) or NMZ = NDZ = 120 (240 pairs); the number of batches was 15 or 25. The batch assignment was random by pair (both twins share a batch) or by individual. Note that randomization by individual does not rule out batch sharing. Conditional on batch, the ACE components were 4 (A), 2 (C), and 4(E), and batch variance equaled 1 (i.e., 1/11 = 9.1% of the phenotypic variance). We conducted both one-step analyses and two-step analyses (correct for batch effects in step 1 and estimate variance components in step 2), and we treated the batch effects as fixed and random. The results suggested that in the one-step analyses, the estimates of the variance components were as good as those obtained in the standard ACE model (without batch effects). In the two-step analyses, we found that random assignment by individual resulted in slightly better estimates. Notably, the C variance components were underestimated following random assignment by pairs (see supplementary material for details).

Note that in the absence of batch effects, family clustering may still be an issue in statistical inference, based on the assumption that the data are independently and identically distributed. For instance, in genome-wide association studies (GWAS), family clustering violates the independence assumption. Happily, family clustering does not pose any statistical problems, as random effects modeling and generalized estimating equations can be used to either accommodate or to correct for the effects of family clustering, or more generally for genetic relatedness (Li et al., 2011; Lippert et al., 2011; Minica et al., 2015). Regardless of randomization scheme (or not), detailed information should be recorded on batch (date and time of processing), operator (technician), plate number, and position (row and column).

Retaining the Twins

To retain participants in a longitudinal study, it is important to remain in contact. Many twin registers have set up a website providing information of the latest study results, news on grants obtained, PhDs awarded, and more general information on twin meetings and such. However, these may not be the best ways to form an actual connection between the twin register and participants. Most twin registers therefore also contact their participants in a more personal manner, either by letter or e-mail, sending out a regular newsletter to make the participant aware that the register is still seeing them as a valuable contributor. A number of twin registers also organize events in which twins and their family members not only can meet each other but can also meet the researchers and ask any questions they may have in person. Worth mentioning here is the annual gathering of twins at the Twins Day Festival in Twinsburg Ohio, where researchers are welcome to recruit twins for specific studies. Unfortunately, financial limitations generally prevent the twin registers from organizing such large and regular gatherings, but when meetings are organized, they are generally judged as very valuable.

In addition to general information, personalized information may also be given out to participants. When participants take part in specific projects, information on test scores (e.g., the results of an IQ tests or the cholesterol levels obtained in a blood sample) may be returned to the participants, accompanied by an explanation of the results. However, often little feedback is provided to participants related to the surveys completed during the longitudinal follow-up, due to the material and personnel costs needed for sending personalized reports to the large number of participants generally included in a twin register. However, as technology advances, new ways emerge of providing personalized information. Participants' portals may provide individual reports without needing to write and post separate reports. At the Netherlands Twin Register, such an effort is now well underway, with participants obtaining information on the survey results via the MyNTR portal. As with the informed consent, it is important to consider the requirements of such a portal in advance. Constructing a participant panel even before starting the actual twin register that includes a number of twins who are willing to think about the various aspects involved in providing feedback would be helpful in setting this up in the best way possible for twins and the register support staff.

Conclusion

Twin registers have a long and successful history and a bright future as a research resource. The uniqueness of twin samples, the soundness and diversity of the methodological approaches, and the huge amount of data accumulated during the last decades characterize twin registers as valuable contributors to the advancement of science, including social science. Their versatility to adapt to multiple scenarios and their orientation to collaborative work will preserve their value in the future as priceless instruments for

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the expansion of knowledge in the complexities of human phenotypes.

Although the global research agenda in the coming decades is difficult to forecast, twin registers can contribute to our understanding in virtually all areas related to human health and behavior. Population-based registers, especially when representative of the general population, are still cohorts of enormous epidemiological interest. The unique characteristics of twin studies, including the ability to control both genetic and shared environmental background, allow for addressing questions that are not easily solved in any other research design. These capacities make them very useful for gene-environment transaction research or causal inference studies (Johnson et al., 2010; McGue, 2010). Twin pairs — in particular, those who are MZ — are remarkably informative in respect to variability of phenotypic expression, pathogenic mechanisms, epigenetics, and post-zygotic mutagenesis, and may serve as a model for research on genetic defects (Bell & Spector, 2011; Castillo-Fernandez et al., 2014; van Dongen et al., 2012; Zwijnenburg et al., 2010). Participation of twins in co-twin, control-designed, and randomized controlled trials is an informative, albeit infrequently used, design (Sumathipala et al., 2018). The use of twin studies has been advocated for guiding post-GWAS studies on the effects associated with genetic variants (Dick et al., 2018), enabling stronger tests of causal hypotheses (Iacono et al., 2017), formulating future strategies in pharmacogenomics research (Rahmioglu & Ahmadi, 2010), or refining phenotypic definitions and evaluating biomarkers for disease (van Dongen et al., 2012). Furthermore, due to their amenability to numerous non-classical study designs, data based on twin registers can integrate with other resources to boost research in virtually every field of human research. Probably the best example is provided by the participation of twin biobanks in many of the large association studies (GWAS and EWAS) that have been published in the last decade.

An additional feature empowering twin registers relies on their orientation to collaborative work. The community of twin registers has a long history of successful alliances. The very nature of their origin as research resources and their scientific environment imply, on the one hand, the existence of matching data across different registers and, on the other hand, the need for very large samples in order to find answers to some of the research questions investigators are interested in. In these circumstances, collaboration is not only practicable, but also a must. Multiple consortia and collaboration initiatives have seen the light as an answer to those needs. The GenomeEuTwin (Peltonen, 2003), EuroDiscoTwin (Willemsen et al., 2015), or the CODATwins (COllaborative project of Development of Anthropometrical measures in Twins) (Silventoinen et al., 2015) consortia are just a few examples of associative efforts, joining together data from a large number of twin cohorts in order to advance in the analysis of the genetic and environmental underpinnings of human complex phenotypes. Other initiatives, such as the International Network of Twin Registries (INTR; Buchwald et al., 2014) have emerged from the International Society for Twin Studies, aiming to foster collaboration and serve as a platform for networking and establishing research relationships between twin registers and the global scientific community.

These collaborative efforts have a parallel outcome on infrastructures related to the registers, such as biobanks. In the same way that registers multiply their scientific impact when joining efforts, the effective use of biobank resources depends on their accessibility. Building a centralized database for the research community allows storing of raw and processed data, reference data for case-control studies and imputation, and linking to clinical phenotypes, so that data can be effectively used not only by single research groups, but also in collaborative multicenter and consortium projects. For instance, the advent of the GWAS method took advantage of such multicenter collaborations in order to lead to the successful identification of thousands of variants that are robustly associated with complex disease phenotypes. The big databases permit research on genetic, methylation, expression level, available protein, lipid, metabolite level information, and on disease/phenotype level. In Europe, for instance, a range of biobanks that joined in the Biobanking and BioMolecular Resources Research Infrastructure and national hubs (e.g., www.bbmri.nl) generated -omics data by the same platforms and shared these combined with existing phenotype data.

Nowadays, the advancement of scientific knowledge requires such collaborations to gain explanatory power and optimize the invested resources. Twin registers, and associated biobanks, have an enormous potential that multiply when joining efforts, and new or growing registers are always welcome to this endeavor. In this article, we have outlined what we feel are the main principles and recommendations for the establishment and management of a twin register, from its inception to its actual development. As pointed out before, our intention has not been to enumerate a detailed checklist of actions, or a complete step-by-step technical guide on this process, but rather to highlight the main aspects that, from our perspective, need to be taken into account for being able to make the difference between an isolated initiative and a successful long-lasting scientific resource.

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Disclosure of Interests

The authors declare no conflict of interest.

References

Ayorech, Z., Selzam, S., Smith-Woolley, E., Knopik, V. S., Neiderhiser, J. M., DeFries, J. C., & Plomin, R. (2016). Publication trends over 55 years of behavioral genetic research. *Behavior Genetics, 46*, 603–607.

Bechger, T. M., Boomsma, D. I., & Koning, H. (2002). A limited dependent variable model for heritability estimation with non-random ascertained samples. *Behavior Genetics, 32*, 145–151.

Bell, J. T., & Spector, T. D. (2011). A twin approach to unraveling epigenetics. *Trends in Genetics, 27*, 116–125.

Bell, J. T., & Spector, T. D. (2012). DNA methylation studies using twins: What are they telling us? *Genome Biology, 13*, 172.

Berg, K. (1988). Variability gene effect on cholesterol at the Kidd blood group locus. *Clinical Genetics, 33*, 102–107.

Boomsma, D., Busjahn, A., & Peltonen, L. (2002). Classical twin studies and beyond. *Nature Reviews Genetics, 3*, 872–882.

Boomsma, D., Helmer, Q., Nieuwboer, H. A., Hottenga, J. J., de Moor, M. H., van den Berg, S. M., … de Geus, E. J. (2018). An extended twin-pedigree study of neuroticism in the Netherlands Twin Register. *Behavior Genetics, 48*, 1–11.

Boomsma, D., Willemsen, G., Vink, J. M., Bartels, M., Groot, P., Hottenga, J. J., … van der Kleij, F. (2008). Design and implementation of a twin-family database for behavior genetics and genomics studies. *Twin Research and Human Genetics, 11*, 342–348.

Buchwald, D., Kaprio, J., Hopper, J. L., Sung, J., Goldberg, J., Fortier, I., … Harris, J. R. (2014). International network of twin registries (INTR): Building a platform for international collaboration. *Twin Research and Human Genetics, 17*, 574–577.

Castillo-Fernandez, J. E., Spector, T. D., & Bell, J. T. (2014). Epigenetics of discordant monozygotic twins: Implications for disease. *Genome Medicine, 6*, 60.

Chen, C., Grennan, K., Badner, J., Zhang, D., Gershon, E., Jin, L., & Liu, C. (2011). Removing batch effects in analysis of expression microarray data: An evaluation of six batch adjustment methods. *PLoS One, 6*, e17238.

Dick, D. M., Barr, P. B., Cho, S. B., Cooke, M. E., Kuo, S. I., Lewis, T. J., … Su, J. (2018). Post-GWAS in psychiatric genetics: A developmental perspective on the 'other' next steps. *Genes, Brain and Behavior*, *17*, e12447.

Duarte, I. F., Diaz, S. O., & Gil, A. M. (2014). NMR metabolomics of human blood and urine in disease research. *Journal of Pharmaceutical and Biomedical Analysis, 93*, 17–26.

Ehli, E. A., Abdellaoui, A., Fedko, I. O., Grieser, C., Nohzadeh-Malakshah, S., Willemsen, G., … Hottenga, J. J. (2017). A method to customize population-specific arrays for genome-wide association testing. *European Journal of Human Genetics, 25*, 267–270.

Finnicum, C. T., Dolan, C. V., Willemsen, G., Weber, Z. M., Petersen, J. L., Beck, J. J., … Ehli, E. A. (2017). Relative telomere repeat mass in buccal and leukocyte-derived DNA. *PLoS One, 12*, e0170765.

Fisher, R. A. (1919). The genesis of twins. *Genetics, 4*, 489–499.

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., … Esteller, M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 10604–10609.

Galton, F. (1875). The history of twins, as a criterion of the relative powers of nature and nurture. *Fraser's Magazine, 12*, 566–576.

Garcia-Closas, M., Egan, K. M., Abruzzo, J., Newcomb, P. A., Titus-Ernstoff, L., Franklin, T., … Rothman, N. (2001). Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiology, Biomarkers & Prevention, 10*, 687–696.

Goldberg, J., Miles, T. P., Furner, S., Meyer, J. M., Hinds, A., Ramakrishnan, V., … Levy, P. S. (1997). Identification of a cohort of male and female twins aged 65 years or more in the United States. *American Journal of Epidemiology, 145*, 175–183.

Goodrich, J. K., Davenport, E. R., Beaumont, M., Jackson, M. A., Knight, R., Ober, C., … Ley, R. E. (2016). Genetic determinants of the gut microbiome in UK twins. *Cell Host & Microbe, 19*, 731–743.

Grigorenko, E. L., & Ravich-Shcherbo, I. V. (1997). Russian psychogenetics: Sketches for the portrait. In P. M. R. E. L. Grigorenko & R. J. Sternberg (Ed.), *Psychology in Russia: Past, present, and future* (pp. 84–121). New York, NY: Nova Science Publishers.

Groen-Blokhuis, M. M., Middeldorp, C. M., van Beijsterveldt, C. E., & Boomsma, D. I. (2011). Evidence for a causal association of low birth weight and attention problems. *Journal of the American Academy of Child & Adolescent Psychiatry, 50*, 1247–1254.

Hogervorst, J. G., Godschalk, R. W., van den Brandt, P. A., Weijenberg, M. P., Verhage, B. A., Jonkers, L., … Schouten, L. J. (2014). DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiology, Biomarkers & Prevention, 23*, 2703–2712.

Holland, N. T., Smith, M. T., Eskenazi, B., & Bastaki, M. (2003). Biological sample collection and processing for molecular epidemiological studies. *Mutation Research, 543*, 217–234.

Holzinger, K. J. (1929). The relative effect of nature and nurture influences on twin differences. *Journal of Educational Psychology, 20*, 241–248.

Iacono, W. G., Heath, A. C., Hewitt, J. K., Neale, M. C., Banich, M. T., Luciana, M. M., … Bjork, J. M. (2017). The utility

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of twins in developmental cognitive neuroscience research: How twins strengthen the ABCD research design. *Developmental Cognitive Neuroscience*. Advance online publication. doi:10.1016/j.dcn.2017.09.001

Johnson, W., Turkheimer, E., Gottesman, I. I., & Bouchard, T. J. Jr. (2010). Beyond heritability: Twin studies in behavioral research. *Current Directions in Psychological Science, 18*, 217–220.

Johnson, W. E., Li, C., & Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics, 8*, 118–127.

Kaprio, J., & Silventoinen, K. (2011). Advanced methods in twin studies. *Methods in Molecular Biology, 713*, 143–152.

Keller, M. C., Medland, S. E., & Duncan, L. E. (2010). Are extended twin family designs worth the trouble? A comparison of the bias, precision, and accuracy of parameters estimated in four twin family models. *Behavior Genetics, 40*, 377–393.

Keller, M. C., Medland, S. E., Duncan, L. E., Hatemi, P. K., Neale, M. C., Maes, H. H., & Eaves, L. J. (2009). Modeling extended twin family data I: Description of the Cascade model. *Twin Research and Human Genetics, 12*, 8–18.

Knopick, V., Neiderhiser, J., DeFries, J., & Plomin, R. (2017). *Behavior genetics*. New York, NY: Worth Publishers.

Lazar, C., Meganck, S., Taminau, J., Steenhoff, D., Coletta, A., Molter, C., … Nowe, A. (2013). Batch effect removal methods for microarray gene expression data integration: A survey. *Briefings in Bioinformatics, 14*, 469–490.

Leek, J. T., Scharpf, R. B., Bravo, H. C., Simcha, D., Langmead, B., Johnson, W. E., … Irizarry, R. A. (2010). Tackling the widespread and critical impact of batch effects in high-throughput data. *Nature Reviews Genetics, 11*, 733–739.

Lejeune, J., Turpin, R., & Gautier, M. (1959). Mongolism; A chromosomal disease (trisomy). *Bulletin de l'Academie Nationale de Medecine, 143*, 256–265.

Levit, S. G. (1935). Twin investigations in the U.S.S.R. *Character and Personality*, *3*, 188–193.

Li, X., Basu, S., Miller, M. B., Iacono, W. G., & McGue, M. (2011). A rapid generalized least squares model for a genome-wide quantitative trait association analysis in families. *Human Heredity, 71*, 67–82.

Lippert, C., Listgarten, J., Liu, Y., Kadie, C. M., Davidson, R. I., & Heckerman, D. (2011). FaST linear mixed models for genome-wide association studies. *Nature Methods, 8*, 833–835.

Lykken, D. T. (1982). Presidential address, 1981. Research with twins: The concept of emergenesis. *Psychophysiology, 19*, 361–373.

Maes, H. H., Neale, M. C., & Eaves, L. J. (1997). Genetic and environmental factors in relative body weight and human adiposity. *Behavior Genetics, 27*, 325–351.

Malykh, S., Egorova, M., & Meshkova, T. (1998). *Osnovi povedencheskoj genetiki*. Moscow, Russia: Epidaurus.

Martin, N. (1975). The inheritance of scholastic abilities in a sample of twins. II. Genetical analysis of examinations results. *Annals of Human Genetics, 39*, 219–229.

Martin, N. G., & Martin, P. G. (1975). The inheritance of scholastic abilities in a sample of twins. I. Ascertainment of the sample and diagnosis of zygosity. *Annals of Human Genetics, 39*, 213–218.

Martin, N., Boomsma, D., & Machin, G. (1997). A twin-pronged attack on complex traits. *Nature Genetics, 17*, 387–392.

Martin, N. G., Eaves, L. J., Kearsey, M. J., & Davies, P. (1978). The power of the classical twin study. *Heredity (Edinb), 40*, 97–116.

Mayo, O. (2009). Early research on human genetics using the twin method: Who really invented the method? *Twin Research and Human Genetics, 12*, 237–245.

McArdle, J. J., & Prescott, C. A. (2005). Mixed-effects variance components models for biometric family analyses. *Behavior Genetics, 35*, 631–652.

McCarthy, S., Das, S., Kretzschmar, W., Delaneau, O., Wood, A. R., Teumer, A., … Haplotype Reference Consortium. (2016). A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics, 48*(10), 1279–1283. doi:10.1038/ng.3643

McGue, M. (2010). The end of behavioral genetics? 2008. *Behavior Genetics, 40*, 284–296.

McRae, A. F., Powell, J. E., Henders, A. K., Bowdler, L., Hemani, G., Shah, S., … Montgomery, G. W. (2014). Contribution of genetic variation to transgenerational inheritance of DNA methylation. *Genome Biology, 15*, R73.

Merriman, C. (1924). *The intellectual resemblance of twins*. Princeton, NJ: Psychological Review Co.

Meulenbelt, I., Droog, S., Trommelen, G. J., Boomsma, D. I., & Slagboom, P. E. (1995). High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *American Journal of Human Genetics, 57*, 1252–1254.

Min, J. L., Lakenberg, N., Bakker-Verweij, M., Suchiman, E., Boomsma, D. I., Slagboom, P. E., & Meulenbelt, I. (2006). High microsatellite and SNP genotyping success rates established in a large number of genomic DNA samples extracted from mouth swabs and genotypes. *Twin Research and Human Genetics, 9*, 501–506.

Minica, C. C., Dolan, C. V., Kampert, M. M., Boomsma, D. I., & Vink, J. M. (2015). Sandwich corrected standard errors in family-based genome-wide association studies. *European Journal of Human Genetics, 23*, 388–394.

Neale, M. C., & Eaves, L. J. (1993). Estimating and controlling for the effects of volunteer bias with pairs of relatives. *Behavior Genetics, 23*, 271–277.

Neale, M. C., Eaves, L. J., & Kendler, K. S. (1994). The power of the classical twin study to resolve variation in threshold traits. *Behavior Genetics, 24*, 239–258.

Nygaard, V., Rodland, E. A., & Hovig, E. (2016). Methods that remove batch effects while retaining group differences may lead to exaggerated confidence in downstream analyses. *Biostatistics, 17*, 29–39.

Peltonen, L. (2003). GenomEUtwin: A strategy to identify genetic influences on health and disease. *Twin Research, 6*, 354–360.

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Petronis, A. (2006). Epigenetics and twins: Three variations on the theme. *Trends in Genetics, 22*, 347–350.

Petronis, A. (2010). Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature, 465*, 721–727.

Polderman, T. J., Benyamin, B., de Leeuw, C. A., Sullivan, P. F., van Bochoven, A., Visscher, P. M., & Posthuma, D. (2015). Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics, 47*, 702–709.

Poll, H. (1914). Über Zwillingsforschung als Hilfsmittel menschlicher Erbkunde. *Zeitschrift für Ethnologie, 46*, 87–105.

Posthuma, D., & Boomsma, D. I. (2000). A note on the statistical power in extended twin designs. *Behavior Genetics, 30*, 147–158.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., … Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics, 81*, 559–575. doi:10.1086/519795.

Rahmioglu, N., & Ahmadi, K. R. (2010). Classical twin design in modern pharmacogenomics studies. *Pharmacogenomics, 11*, 215–226.

Rende, R. D., Plomin, R., & Vandenberg, S. G. (1990). Who discovered the twin method? *Behavior Genetics, 20*, 277–285.

Schadt, E. E., Lamb, J., Yang, X., Zhu, J., Edwards, S., Guhathakurta, D., … Lusis, A. J. (2005). An integrative genomics approach to infer causal associations between gene expression and disease. *Nature Genetics, 37*, 710–717.

Siemens, H. W. (1924). *Zwillingspathologie: Ihre Bedeutung; ihre Methodik, ihre bisherigen. Ergebnisse* [Twin pathology: Its meaning; its method; results so far]. Berlin: Springer Verlag.

Silventoinen, K., Jelenkovic, A., Sund, R., Honda, C., Aaltonen, S., Yokoyama, Y., … Kaprio, J. (2015). The CODATwins Project: The cohort description of collaborative project of development of anthropometrical measures in twins to study macro-environmental variation in genetic and environmental effects on anthropometric traits. *Twin Research and Human Genetics, 18*, 348–360.

Slagboom, P. E., Droog, S., & Boomsma, D. I. (1994). Genetic determination of telomere size in humans: A twin study of three age groups. *American Journal of Human Genetics, 55*, 876–882.

Snieder, H., van Doornen, L. J., & Boomsma, D. I. (1997). The age dependency of gene expression for plasma lipids, lipoproteins, and apolipoproteins. *American Journal of Human Genetics, 60*, 638–650.

Suhre, K., Meisinger, C., Doring, A., Altmaier, E., Belcredi, P., Gieger, C., … Illig, T. (2010). Metabolic footprint of diabetes: A multiplatform metabolomics study in an epidemiological setting. *PLoS One, 5*, e13953.

Sukhanov, S. (1900). O psihozah u bliznetsov [On psychosis in twins]. *Klinicheskij jurnal*, (4), 341–352.

Sumathipala, A., Yelland, L., Green, D., Shepherd, T., Jayaweera, K., Ferreira, P., & Craig, J. M. (2018). Twins as participants in randomized controlled trials: A review of published literature. *Twin Research and Human Genetics, 21*, 51–56.

Sun, Z., Chai, H. S., Wu, Y., White, W. M., Donkena, K. V., Klein, C. J., … Kocher, J. P. (2011). Batch effect correction for genome-wide methylation data with Illumina Infinium platform. *BMC Medical Genomics, 4*, 84.

Thorndike, E. L. (1905). *Measurements of twins*. New York, NY: The Science Press.

Tworoger, S. S., & Hankinson, S. E. (2006). Collection, processing, and storage of biological samples in epidemiologic studies: Sex hormones, carotenoids, inflammatory markers, and proteomics as examples. *Cancer Epidemiology, Biomarkers & Prevention, 15*, 1578–1581.

van Beijsterveldt, C. E., Overbeek, L. I., Rozendaal, L., McMaster, M. T., Glasner, T. J., Bartels, M., … Boomsma, D. I. (2016). Chorionicity and heritability estimates from twin studies: The prenatal environment of twins and their resemblance across a large number of traits. *Behavior Genetics, 46*, 304–314.

van Dongen, J., BIOSconsortium, Bonder, M. J., Dekkers, K. F., Nivard, M. G., van Iterson, M., … Boomsma, D. I. (2018). DNA methylation signatures of educational attainment. *npj Science of Learning, 3*, 1–14.

van Dongen, J., Nivard, M. G., Willemsen, G., Hottenga, J. J., Helmer, Q., Dolan, C. V., … Boomsma, D. I. (2016). Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nature Communications, 7*, 11115.

van Dongen, J., Slagboom, P. E., Draisma, H. H., Martin, N. G., & Boomsma, D. I. (2012). The continuing value of twin studies in the omics era. *Nature Reviews Genetics, 13*, 640–653.

Vaught, J. B., & Henderson, M. K. (2011). Biological sample collection, processing, storage and information management. *IARC Scientific Publications*, (163), 23–42, https://www.ncbi.nlm.nih.gov/pubmed/22997855.

Willemsen, G., Ward, K. J., Bell, C. G., Christensen, K., Bowden, J., Dalgard, C., … Spector, T. (2015). The concordance and heritability of type 2 diabetes in 34,166 twin pairs from international twin registers: The Discordant Twin (DISCOTWIN) Consortium. *Twin Research and Human Genetics, 18*, 762–771.

Wright, F. A., Sullivan, P. F., Brooks, A. I., Zou, F., Sun, W., Xia, K., … Boomsma, D. I. (2014). Heritability and genomics of gene expression in peripheral blood. *Nature Genetics, 46*, 430–437.

Yudin, T. I. (1907). O shodstve psihoza u bratjev i sester. [On similarity of psychosis in brothers and sisters]. *Sovremennaya psihiatria*, (10), 337–342.

Zwijnenburg, P. J., Meijers-Heijboer, H., & Boomsma, D. I. (2010). Identical but not the same: The value of discordant monozygotic twins in genetic research. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *153B*, 1134–1149.

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