(Epi) genetics and twins

van Dongen, J.

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The continuing value of twin studies in the omics era

Abstract

The classical twin study has been a powerful heuristic in biomedical, psychiatric and behavioral research for decades. Twin registries worldwide have collected biological material and longitudinal phenotypic data on tens of thousands of twins, providing a valuable resource to study complex phenotypes and their underlying biology. In this review, we consider the continuing value of twin studies in the current era of molecular genetic studies. We conclude that classical twin methods combined with novel technologies represent a powerful approach to identify and understand the molecular pathways underlying complex traits.

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Introduction

The **classical twin design** has been used for decades to estimate the importance of genetic and environmental influences on complex trait variation. Its results have contributed to the awareness that variation in almost every conceivable facet of the human condition is influenced by genetic variation (BOX 1). Traits include intrinsic physical, medical, and biochemical characteristics; life outcome variables such as income, divorce and mortality; and behavioral traits, including apparently trivial ones such as TV watching and internet use. In fact, for many human phenotypes, **heritability** estimates derived from twin studies initially encouraged the search for the responsible genetic variation. Through their collaboration in genome-wide association study (GWAS) consortia, large twin registries (TABLE 1; Supplementary information S1 (table)) are nowadays also making an important contribution to identifying the genetic variation underlying complex traits and disorders.

Twins offer unique opportunities to genetic research that extend beyond the analysis of phenotypic heritability (BOX 2). Twin designs can provide insight into the genetic etiology of disease development over time, and aid in the detection of biomarker profiles for medical conditions. For heritable traits, the comparison of **discordant monozygotic (MZ) twins** represents a powerful improvement over the traditional **case–control study** to search for disease-associated biological marks. The power of this design is illustrated in a recent study that compared the DNA methylation patterns of MZ twins discordant for systemic lupus erythematosus (SLE), which identified several genomic regions in which DNA methylation changes were associated with the disease[1]. Novel applications of the classical twin design can provide fundamental insights into the biological mechanisms underlying complex traits. For example, gene expression studies in monozygotic and dizygotic (DZ) twins have highlighted that variation in genome-wide expression between individuals is due to both genetic and environmental influences, and that the importance of these influences may vary across genes and tissues[2, 3].

This review addresses the continuing value of twin studies. We describe various twin study designs with examples of traditional applications, and we describe how twin approaches are now used for tracing disease-causing mutations and for studying a variety of other newly emerging phenotypes (*e.g.,* the **epigenome, transcriptome, metabolome, proteome** and **microbiome**). We address the use of discordant MZ twins to identify biological mechanisms associated with complex traits, for the inference of causality, and for the genome-wide analysis of genotype-by-environment (G×E) interaction at **variability gene**s. We also discuss various questions that can be addressed by contrasting data from MZ and DZ twins to establish the heritability of biological marks and to unravel the shared etiology of associated traits. A range of twin studies is presented, focusing on the initial level of the DNA sequence, down to its expression and intermediate phenotypes such as metabolites, and ultimately the clinical endpoints of interest**.**

Box 1: The history of the classical twin study

The scientific study of twins goes back to 1875, when Francis Galton published his seminal paper *The history of twins, as a criterion of the relative powers of nature and nurture*[104]. However, Galton was unaware of the distinction between monozygotic (MZ) and dizygotic (DZ) twins. The first papers to contrast the similarity of MZ and DZ twins were published by Poll (1914)[105] and Siemens (1924)[106], whose interest was pigmented nevi (common moles), a phenotype still being studied intensively today because of its importance as a risk factor for melanoma[107]. Not much later, the first twin registries were founded, and power calculations showing that very large sample sizes were needed to obtain reliable estimates of heritability stimulated the foundation of new large registries in the 1980s[108, 109]. Consolidation of these registries, new methods for **zygosity assessment**, and improved survey methods coincided with a growing awareness that genetic influences affected a wide range of traits of biomedical and social significance, and an increase in funding to mount large studies. Worldwide, many countries have now set up twin registries[110-112], which have established collections of longitudinal data in twins across age categories from birth[113] to death[34]. Within the last twenty years, very large twin studies have been carried out through mailed, telephone, and internet surveys. Methods linking twin registry data to national databases containing information on cancer and mortality[114], or outcomes of population screens[115] have provided population-based estimates of heritability on samples as large as 44,000 twin pairs.

The continuing importance of twin study designs

Quantitative analysis of genetic and environmental influences

The classical twin design has traditionally been used to study the heritability of disease-related phenotypes and clinical endpoints (TABLE 2). This design has also been widely applied to estimate the extent to which different traits are influenced by the same or different genetic and environmental factors[4].

Multivariate twin models of symptoms of anxiety and depression, for example, provided evidence that comorbidity of these disorders is due to genetic influences that affect the vulnerability to both disorders, but that different environments determine whether a vulnerable person develops major depression or generalized anxiety disorder[5, 6]. Longitudinal data can be analyzed in a similar way: genetic variation in IQ from age 1 to 16 is largely attributable to the same genetic influences[7], and the increase in heritability[8] is due to amplification of genetic effects with age. The classical twin design can be extended to model polygenic G×E interactions, by testing whether the heritability of a trait varies across different levels of environmental exposures[9]. The heritability of body mass index (BMI) is moderated by physical activity: the higher the level of physical activity, the smaller the genetic influence on BMI[10].

Box 2: The classical twin design

In the classical twin design, the extent to which phenotypic variation in a trait (V_P) is due to genetic (V_G) and environmental (V_E) influences is estimated: $V_P = V_G + V_E$. Genetic variance can be further decomposed into additive genetic variance (V_A) and variance due to non-additive genetic effects (dominance variance, V_D): $V_G=V_A+V_D$. Most twin studies, unless they are very large, consider the narrow-sense heritability (h^2), which refers to the proportion of variation that is due to additive genetic variance: $h^2=V_A/V_P$. Environmental influences (V_E) comprise those that are shared by family members ("the common environment", V_C) and influences that are unique to each individual ("the unique environment", V_U): $V_E=V_C+V_U$.

These unobserved variance components can be estimated from the observed resemblance (*i.e*. the phenotypic covariance) in MZ and DZ twin pairs. Monozygotic (MZ) twins are derived from a single fertilized egg cell and share (nearly) 100% of their segregating genes, while dizygotic (DZ) twins are derived from two distinct zygotes and share on average 50% of their segregating genes. Twins of both types share 100% of the common environment and 0% of the unique environment. Therefore, the phenotypic covariance of MZ twins is expected to equal $V_A + V_D + V_C$ and the phenotypic covariance of DZ twins is expected to equal $0.5V_A + 0.25V_D + V_C$. These expectations are the input (structural equations) for genetic structural equation modeling (GSEM), a technique by which **maximum likelihood** estimates of variance components are obtained from twin data. GSEM obtains the expected MZ and DZ covariances given the equations above, and compares the outcome to the covariances observed in the data. The maximum likelihood estimates of V_A, V_D, V_C, and V_E are those estimates that predict covariances that are most consistent with the observed data. With MZ and DZ data, V_C and V_D cannot be estimated simultaneously. V_D is estimated if there is stronger evidence for non-additive effects (if the MZ correlation is more than twice as large as the DZ correlation) and V_C is estimated if there is stronger evidence for common environmental effects (if the MZ correlation is less than twice as large as the DZ correlation). In extended-twin family designs, the information from additional types of family relations together with the information from twins allows for estimating V_A, V_D, V_C and V_E simultaneously.

In multivariate twin models, extending the set of equations for the expected covariances allows the modelling of the cross-twin–cross-trait covariance, i.e. the covariance of trait 1 in one twin with trait 2 in the co-twin. To estimate to which degree the clustering of different traits or comorbidity of disorders is explained by genetic and environmental influences, the same principles apply as for the expected covariances of twins (*e.g.*, MZ twins are expected to share 100% of genetic influences that overlap between traits while DZ twins are expected to share 50%, resulting in a larger cross-twin–cross-trait covariance for MZ twins if the association between traits has a genetic basis).

Extending twin models with data from other relatives (their parents, siblings, spouses or offspring) enhances statistical power[11] and allows for testing of a much wider range of hypotheses about the causes of human variation, including the role of cultural transmission, social interactions among relatives[12], **genetic non-additivity** and various mechanisms of **assortative mating**[13, 14]. The offspring-of-twins design is a powerful tool for studying intergenerational associations between environmental variables and outcomes in children[15]. Also, comparing the phenotypic similarity of children of female MZ twins (who are socially cousins but genetically half-siblings) to the similarity of children of male MZ twins gives insight into the differential importance of paternal and **maternal effects**; if paternal and maternal effects are equally important, children of male twins and female twins are expected to be equally similar. For birth weight, larger correlations have been observed in children of female twins compared to children of male twins, highlighting the importance of maternal effects for this trait[16].

Classical twin methods continue to be a valuable addition to genetic association studies, for example to establish the proportion of the heritability that can be explained by newly identified SNPs from GWAS[17]. The current discussion about "missing heritability" largely stems from the (often great) disparity between estimates of total heritability from twin studies and the proportion of variance accounted for by SNPs from GWAS[18-20], for which many explanations have been proposed[21] including implications that heritability estimates from twin studies may be too high. In our later section on testing classical assumptions, we discuss the relevance of recent molecular findings in twins in the light of the current discussion on "missing heritability".

The value of discordant twins

Data from MZ and DZ twins allow for the examination of causal relations in the comorbidity of traits. In this case, information from discordant twins is used in a design referred to as the **co-twin control method**. This method was first used to study the association between smoking and lung cancer[22], and has since been applied to investigate a wide variety of medical hypotheses, for example to provide evidence against the efficacy of vitamin C in preventing the common cold[23]. The value of the co-twin control design for distinguishing between associations that reflect causality and associations due to confounding effects of genes or environmental factors (i.e. if two traits are affected by the same genetic or environmental influences, rather than one trait causing the other) is further exemplified by several recent studies on complex traits, as described below.

Experimental studies in which depressive patients are exposed to various types of exercise regimes suggest that regular exercise causes a reduction in anxious and depressive symptoms. To examine whether this causal relationship is present at the population level, twins discordant for

exercise behavior were studied[24]. MZ twins who exercised more than their co-twin did not have fewer symptoms of anxiety and depression. The relationship between exercise behavior and depression was explained by shared genetic influences, rather than by a cause–effect relationship. In another twin study, a reciprocal causal relationship between depression and migraine was revealed[25]. In MZ pairs discordant for depression, only the depressed twin had an increased risk of migraine, and in MZ pairs discordant for migraine, only the twin with migraine had an increased risk of depression. Furthermore, a co-twin control study of anthropometric traits and cancer found a positive correlation between height and risk of breast and ovarian cancer and indicated correlations between BMI and several types of cancer in some population subgroups[26].

The comparison of discordant MZ twins offers an alternative to the traditional case–control study. Here, the primary interest is not the inference of causality, but to identify factors associated with a trait of interest that differ between cases and controls who are perfectly matched for age, sex, and genetic background, and partly matched for early environmental influences.

Molecular phenotypes and the causes of quantitative trait variation

Technological advances allow an assessment of the extent to which twins resemble each other at the level of molecular processes that contribute to their phenotypic similarity[27]. Thereby, the comparison of discordant MZ twins can lead us into novel pathways associated with disease. A unique advantage of the MZ twin design is the ability to study biological discordance against an equivalent genetic background. Divergence of epigenetic profiles in MZ twins depends on the locus and has been documented for both younger and older age groups[28-30]. In fact, differences in DNA methylation and gene expression are already evident in newborn MZ twins[31, 32]. Clearly, environmental and stochastic factors start *in utero* and operate throughout life.

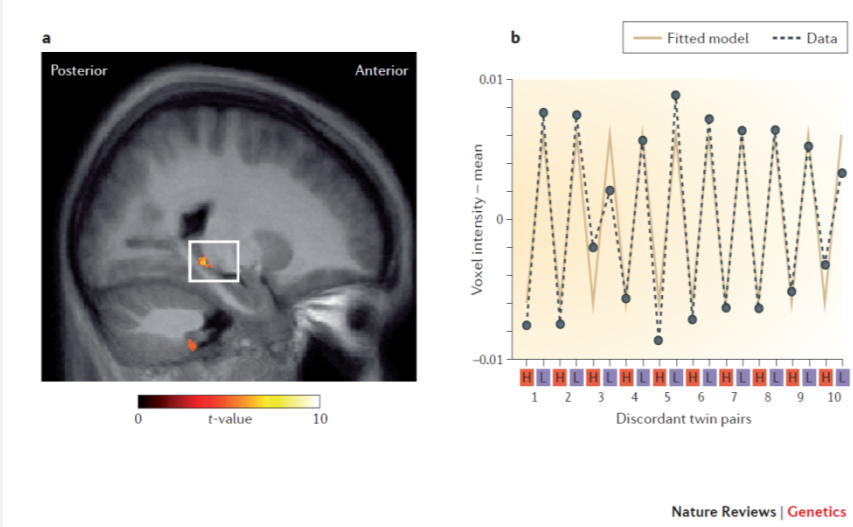
In addition to traditional organismal quantitative traits (such as height and BMI) molecular characteristics — such as gene expression levels, the methylation state of CpG sites in the DNA and the concentration of metabolites in blood and urine — may also be regarded as quantitative traits. Variation in molecular traits measured in groups of MZ and DZ twins can be analyzed using the classical twin method like any other phenotype. Multivariate twin analyses address questions that are not easily resolved in any other study design, such as: to what extent is the epigenetic regulation and expression of genes across genomic regions influenced by shared genetic factors and to what extent is each region influenced by unique factors? And: to what degree do common genetic and environmental mechanisms underlie biological variation across different cells and tissues[33]?

The availability of genome-wide DNA marker data allows for novel approaches to study G×E interactions in which MZ twins can play a vital role. By studying variation in a phenotypic trait of interest in MZ twins, it is possible to see not only whether some genotypes confer higher *levels* of risk for that

trait, but also whether some contribute to its *variability*; high variability in the expression of a trait from a common genetic background could explain phenotypic differences between MZ co-twins. Of interest, genetic and environmental factors may influence disease through different pathways (BOX 3). Twin studies can be used to identify aspects of disease that are most related to the underlying genetic liability of individuals, and thereby help to establish clinical criteria and phenotypic definitions that will facilitate the success of GWAS. Other approaches such as the offspring-of-twins design may provide insight into **trans-generational inheritance** of epigenetic regulation and the importance of maternal effects and **imprinting** on epigenetic marks, though such studies have not yet been published.

An important strength of twin registries lies in the extensive longitudinal collection of data on a variety of phenotypes. Twin studies have indicated that approximately 20-30% of the overall variation in adult lifespan is accounted for by genetic factors[34]. Longitudinal twin studies can be used to identify biomarkers associated with aging: a co-twin control analysis demonstrated that telomere length at advanced age is predictive of survival[35]. MZ twins with the shortest telomeres at baseline had a three-fold greater risk of death during a follow-up period of 7 years than their co-twins with the longest telomere measurements (relative risk (RR) = 2.8). The discordant MZ twin design and the classical twin design have received much interest in recent years for studying molecular biology. The following sections will provide an overview of findings from such studies.

Box 3: The value of twins in neuroimaging genetics



Imaging genetics is a form of association analysis in which the phenotype is a measure of brain structure or function (*e.g.*, physiological response of the brain during information processing)[116, 117]. Brain imaging studies in twins have

contributed substantially to the knowledge that individual differences in brain structure[118] and function[119] are highly heritable. A group of ten male MZ twin pairs and their non-twin brothers had their brains scanned in a functional magnetic resonance imaging (fMRI) study while they had to memorize a short span of digits (digit-memory task)[120]. Before they were asked to recall the digits they memorized, a distraction task was presented in which objects (*e.g.* fruit, vegetables and tools) had to be categorized. When they were distracted by the object categorization task, many men used brain areas associated with language for recalling the digits they had memorized. These men took longer to provide the answer than did those who resorted to a visual-spatial memory system to encode the numbers. MZ twins used the same strategy more often than their non-twin brothers, indicating that there are qualitative differences in how individuals think, and that these differences have a substantial genetic component.

Another design in imaging genetics compares disease-discordant and disease-concordant MZ twins to assess whether genetic and environmental risk factors for psychiatric disorders act on the same brain regions. Comparisons of discordant MZ twins can highlight brain regions that are susceptible to environmental risk factors. Contrasting MZ twins who both score high on the disease phenotype to those who both score low can be used to identify brain characteristics that are related to genetic risk for disease. An imaging study of bipolar disorder that made use of this design found that white matter pathology in the frontal lobe may be central to the genetic risk of developing bipolar disorder, whereas widespread grey matter abnormalities may be more related to environmental effects and reflect effects of the illness itself[121]. A study of MZ twins discordant or concordant for anxious depression found that environmental risk is highlighted in the left temporal lobe (see the figure)[122]. Most notable were the lower grey matter volumes in the left posterior hippocampus, which contains the main afferent and efferent connections of the hippocampus to the rest of the temporal lobe, in high-risk twins from discordant pairs. The Figure illustrates the striking differences in discordant MZ twins, both at the group and individual pair level. The boxed region in **panel A** shows the left parahippocampal area where a significant volume reduction was found in the high risk twin compared to the low risk co-twin from MZ twin pairs discordant for anxious depression. The reduction was not evident in MZ pairs concordant for high risk of depression, when compared to MZ twin pairs concordant for low risk of depression. The within-pair comparison of discordant MZ pairs most likely reveals differences related to environmental exposures, while the between-pair comparison of concordant-high and concordant-low pairs is more likely to reveal differences in genetic vulnerability. Therefore, changes in the left-parahippocampal area may be specific to an environmentally driven etiology of anxiety and depression. Colours represent the effect size (t-value from paired t-test) of the comparison of grey matter volume between discordant

twins. **Panel B** shows the relative responses (individual voxel intensity minus mean voxel intensity in all twins) of ten discordant twin pairs at the most significant voxel in the left parahippocampal area (H= twin with high risk of anxious depression, L=low risk co-twin). Although a significant overall volume reduction was found in the group of discordant pairs, this Figure illustrates that there is large variation in volume difference across individual discordant pairs. Figure is reproduced, with permission, from [122] © (2007) Elsevier.

Tracing the origin of new mutations

Identifying sequence differences between twins

Although MZ twins originate from one zygote, there is some evidence that their somatic cells are not always identical at the DNA sequence level[36]. A study of healthy MZ twins and singletons suggested that **copy number variations** (CNVs) may accumulate with aging in a dynamic fashion[37]. By comparing CNVs in longitudinally collected blood samples of MZ pairs, both increases and decreases in CNV content were found after ten years (between co-twins and within individual twins). This may reflect fluctuations in the proportions of peripheral blood cells carrying aberrant DNA. By comparing copy numbers in buccal cells of twins and their parents, Ehli *et al* found evidence for a pre-twinning *de novo* duplication in a healthy twin pair (present in both twins but not in their parents) and a post-twinning *de novo* deletion in one twin from a pair of twins concordant for attention problems[38]. A comparison of CNVs in the blood of MZ pairs discordant for **congenital diaphragmatic hernia** and **esophageal atresia** found no evidence for structural genomic differences between twins[39]. All these studies made use of microarrays, which cover a limited portion of the total content of structural variation in the genome[40]. The application of whole-genome sequencing techniques may unravel many more sequence differences between MZ twins, including single nucleotide substitutions.

In 2010, Baranzini *et al* published the first study that applied whole-genome-sequencing technology in discordant MZ twins [41]. The study entailed a combination of techniques — including whole-genome sequencing, RNA sequencing and genome-wide SNP microarrays — to measure multiple molecular marks in $CD4^+$ cells from female twins discordant for multiple sclerosis (MS). Only a small fraction of SNPs and structural variants differed within twin pairs, but no differences were replicated across methods. This study should be interpreted as exploratory, however, as only three discordant pairs were studied. Larger studies are needed to establish whether molecular differences may explain discordance for MS and other diseases in MZ twin pairs.

Table 1 | Twin registries worldwide (for a full list see Supplement)

Country	Twin Registry Name	Registry Characteristics	Age	Website	N twins/ subjects (approx.)[A]	N twins/subjects with DNA available (approx.)[A]	Biospecimens (available for at least subset of the sample)
Africa							
Guinea-Bissau	Bandim Health Project Twin Registry	Population-based with ongoing longitudinal data collection	0-30	www.bandim.org	2,500 (twins and singleton controls)	200 twin pairs	Whole blood, plasma
Asia and Australia							
Australia	Australian Twin Registry	Population-based with ongoing longitudinal data collection	0-90	www.twins.org.au	66,000	12,000 (twins and other family members)	Serum, plasma, buccal
China	Chinese National Twin Registry (CNTR)	Population-based with ongoing longitudinal data collection	All	cntr.bjmu.edu.cn	35,000 twin pairs	3,200	Serum, DNA
Korea	South Korean Twin Registry (SKTR)	Volunteer preschoolers, cohort of school children, volunteer young adults	0-30	www.ktrc.org	10,000 twin pairs	800 twin pairs	Hair, saliva
Japan	Keio Twin Registry	Adult and adolescent twins from the general population in the Tokyo area	14-30	totcop.keio.ac.jp; kts.keio.ac.jp; kotrec.keio.ac.jp	4,000 twin pairs (plus other family members)	600 twin pairs	Buccal, blood
Sri Lanka	Sri Lankan Twin Registry	Voluntary twin registry component and a population-based database with ongoing data collection	6-94	www.ird.lk/Twin%20Registry.php	35,000	Plans to collect DNA from 4,000	Buccal

Table 1 | continued							
Europe							
Denmark	The Danish Twin Registry (DTR)	Population-based with ongoing longitudinal data collection	0-107	www.sdu.dk/dtr	170,000	20,000	Serum, plasma, buffy coat, saliva, buccal, urine
Finland	Finnish Twin Cohort study	Population-based with ongoing longitudinal data collection	11-100+	www.twinstudy.helsinki.fi	45,000 (plus family members)	14,600 (twins and family members)	Whole blood, serum, plasma, saliva, urine, fat and muscle by biopsy
Netherlands	Netherlands Twin Register (NTR)	Population-based with ongoing longitudinal data collection	0-100	http://www.tweelingenregister.org/en/	87,500 (plus family members)	18,000	DNA, RNA, cell lines, serum, plasma, buccal, urine, stool
Norway	Norwegian Twin Registry (NTR)	Population-based with ongoing longitudinal data collection	18+	www.fhi.no/twins	40,000	4,800	Whole blood, buccal swabs, plasma
Sweden	Swedish Twin Register (STR)	Population-based with ongoing longitudinal data collection	5-100+	http://ki.se/ki/jsp/polopoly.jsp;jsessionid=acR0ziTHzWEcIO_cNC?l=en&d=9610	194,000	44,600	Whole blood, serum, saliva
UK	TwinsUK registry	Population-based with ongoing longitudinal data collection	18-90	www.twinsuk.ac.uk	12,000	7,000	Whole blood, serum, plasma, buffy coat, saliva, buccal, urine, skin, fat, muscle

Table 1 | continued							
North America							
USA	Mid-Atlantic Twin Registry (MATR)	Population-based, ascertained at birth	0-94	www.matr.vcu.edu	56	1,5	Whole blood, serum, plasma, buffy coat, saliva, buccal
USA	NAS-NRC	Male twins born between 1917-27, both of whom served in the military, mostly during World War II	85-95	iom.edu/Activities/Veterans/TwinsStudy.aspx	31,848	700+	Blood and other materials collected for various investigations
USA	Minnesota Twin Family Study	Ongoing population - based longitudinal study	11-47	mctfr.psych.umn.edu	5,000 (plus family)	10,000 (twins and family members)	Blood-derived or saliva-derived DNA
USA	Wisconsin Twin Panel (WTP)	Population based, longitudinal data, extensive phenotypic characterization, follow up of selected samples	0-23	www.waisman.wisc.edu/twinresearch	19,638 twins (plus parents and sibs)	3,489 (twins, parents, sibs)	Saliva, buccal
South America							
Cuba	Cuban Twin Registry	Population-based with ongoing longitudinal data collection	All	-	55.400 twin pairs	250 twin pairs	Blood-derived DNA

[A] Numbers refer to individual twins (rather than twin pairs) unless indicated otherwise. This table shows a selection of some of the large twin registries worldwide. For a more comprehensive table see Supplementary information S1 (table).

Table 2 | Heritability estimates from twin studies			
Trait	Heritability	Number of twin pairs (or study type for multiple data sets)*	Refs
Anthropometric			
Height	M: 0.87-0.93[A]; F: 0.68-0.90[A]	30111	126
Body Mass Index (BMI)	M: 0.65-0.84[A]; F: 0.64-0.79[A]	37000	127
Birth weight	0.42	2009[B]	128
Metabolic and cardiovascular			
Diabetes, Type 1	0.88	22650	129
Diabetes, Type 2	0.64	13888	130
Coronary heart disease	M: 0.57; F: 0.38	10483	131
Systolic blood pressure	0.42	1617[C]	132
Diastolic blood pressure	0.40	1617[C]	132
Markers for cardiovascular disease in blood		12000 twins	133
High density lipoprotein(HDL) level	0.66		
Low density lipoprotein (LDL) level	0.53		
Triglyceride level	0.54		
Glucose level	0.53		
C-reactive protein (CRP) level	0.43		
Diseases and characteristics of the brain and CNS, psychiatric disorders			
Alzheimer's Disease	0.48	662	134
Parkinson's Disease	0.34	46436 twins	135
Migraine	0.34-0.57[A]	29717	136
Multiple Sclerosis	0.25-0.76[A]	Review	137
Attention-Deficit Hyperactivity disorder	0.76	Review	138
Autism Spectrum Disorders	0.71	11535 twins	139
Schizophrenia	0.81	Meta-analysis	140
Major Depression	0.37	Meta-analysis	141
EEG measures of brain activity		Meta-analysis	119
Alpha power	0.79		
P300 amplitude	0.60		
MRI measures of brain structure		Review	118
Total Brain volume	0.66-0.97		

Table 2 | continued			
Frontal lobe volumes	0.90-0.95		
Hippocampal volumes	0.40-0.69		
Skeletal features and disorders			
Bone mineral density	0.60-0.80	Review	[142]
Osteoarthritis	0.40-0.70	Review	[143]
Rheumatoid arthritis	0.60	13502	[144]
Asthma and pulmonary function			
Asthma	0.60[D]	21135	[145]
Forced Expiratory Volume in one second (FEV(1))	0.61	4314 twins	[146]
Forced Vital Capacity (FVC)	0.55	4314 twins	[146]
Peak Expiratory Flow (PEF)	0.43	4314 twins	[146]
Cancer			
Prostate cancer	0.42	21000	[114]
Breast cancer (in females)	0.27	23788	[114]
Colorectal cancer	0.35	44788	[114]
Aging			
Mortality	0.25	Review	[34]
Telomere length	0.56	175	[35]
Lifestyle and life events			
Exercise participation	0.48-0.71[A]	37051	[89]
Dietary patterns	0.41-0.48	3262[C]	[90]
Smoking initiation	M: 0.37; F: 0.55	Meta-analysis	[147]
Smoking persistence	M: 0.59; F: 0.46	Meta-analysis	[147]
Alcohol abuse/dependence	0.50-0.70	Review	[148]
Stressful life events	0.28	Meta-analysis	[92]

Abbreviations: CNS, central nervous system; EEG, Electroencephalography; **F**,females; **M**,males;* Not that numbers refer to twin pairs unless stated otherwise and most heritability estimates refer to the narrow-sense heritability (h^2, Box 2)

[A] Range of heritabilities from different countries or study samples

[B] Female twin pairs with child (offspring-of-twin design)

[C] Only females

[D] The original paper reports estimates for various age categories from 3-71 years, separately for males and females.

Timing the occurrence of de novo mutations

A unique advantage of studying disease-causing mutations in MZ twins is that the developmental timing of *de novo* mutations[42] may be tracked if DNA from multiple cell lines is available for both twins. Vadlamudi *et al* were able to determine the timing of a mutation in the sodium channel α1 subunit gene (*SCN1A)* that causes **Dravet's syndrome**, by sequencing DNA from several embryonic tissue lineages from a pair of discordant MZ twins[43]. As the mutation was present in all analysed cell lines of the affected twin but not in those of the unaffected co-twin, it was concluded that the mutation had probably occurred at the two-cell stage in the pre-morula embryo. For any disease caused by *de novo* mutations, information about the timing of mutagenesis is of major importance for genetic counselling. Mutations that occurred in parental gametes are associated with a negligible risk of recurrence in additional offspring. By contrast, parental germ-line **mosaicism** for the mutation is associated with a high recurrence risk because many existing parental gametes will carry the mutation.

Phenotypic impact of epigenetic variation

DNA methylation and disease

Besides *de novo* mutations in the DNA, epigenetic variation may be another important source of phenotypic variation and discordance in MZ twins. The following example illustrates this point. In 1997, a pair of MZ girls was born; one of them was healthy but the other had a severe spinal malformation in which the spinal cord was duplicated. This defect resembled a condition in mice with a mutation in the *Axin* gene, but no mutation was found in this gene in the twins. Oates *et al,* however, found increased methylation of CpG sites at the *AXIN* promoter in the affected twin as compared to the unaffected, which may have suppressed gene expression and caused the malformation[44].

Although epigenetic variation has not yet been investigated in large twin studies, several small studies illustrate the promise of the discordant twin design for epigenetics, including studies of Alzheimer's Disease[45], autism[46], Bipolar Disorder[47, 48], birth weight [49], cancer[50], and systemic lupus erythematosus (SLE)[1]. In MZ twins discordant for autoimmune disorders (SLE, rheumatoid arthritis, and dermatomyositis), Javierre *et al* identified a global decrease in DNA methylation (hypomethylation) in SLE-affected twins, and regional DNA methylation changes at 49 genes that were enriched for immune function. Many of the genes that were hypomethylated in SLE-affected twins also showed increased expression compared to the healthy co-twin[1]. Integrated studies of DNA methylation and gene expression in discordant twins[51] are particularly valuable to identify loci at which epigenetic regulation may be associated with disease. Importantly, the dynamic nature of epigenetic variation makes results of epigenetic studies more difficult to interpret compared to genetic studies. Alternatively to being the cause of disease discordance, epigenetic differences may also reflect the effects of disease or the effect of an

event occurring in one twin that triggered both the disease and the epigenetic changes independently. Some twin registries have collected longitudinal biological samples, which allow for identifying epigenetic differences between twins that were already present prior to onset of discordance for some diseases. Functional studies will ultimately be required to verify the effect of epigenetic variation.

The classical twin design provides information about the importance of genetic influences on epigenetic variation: comparison of the level of DNA methylation at the imprinted *IGF2–H19* locus in MZ and DZ twins showed that variation in DNA methylation at this locus is mainly determined by heritable factors before middle age[52]. High heritability of epigenetic variation has also been observed for some other loci[53, 54], although the average heritability across all loci seems to be low[55].

Differential miRNA expression and disease.

The role of **non-coding RNA**s such as **microRNA** (miRNA)[56-58] is relatively unexplored. Sarachana *et al* measured miRNA expression in **lymphoblastoid cell lines** in a sample of MZ twins and sibling pairs discordant for autism and observed differential regulation of a number of miRNA transcripts[59]. For two differentially expressed brain-specific miRNAs, the putative target genes — *ID3* and *PLK2*, which have been implicated in circadian rhythm signaling and modulation of synapses — were validated by experiments involving knockdown or over-expression of these miRNAs. By combining miRNA data and mRNA expression data, dysregulation of miRNA expression was found to contribute to alterations in target gene expression, which in turn may contribute to disease pathology of autism. Te *et al* measured miRNA expression in MZ twins discordant for Lupus Nephritis and observed differential expression of several miRNAs[60]. Among the gene targets of the most important miRNAs were primarily genes with a role in **interferon (IFN) signaling**. Together, these studies indicate that the discordant MZ twin design will be a valuable approach to explore the role of miRNA expression in complex disease.

Gene expression variation: causes and disease links

There is wide variation in the heritability of transcript expression across the genome[2, 61]. To identify **expression quantitative trait loci** (eQTLs), variation in expression across tissues of healthy female twin pairs was investigated in a "matched co-twin analysis" [62]. In the initial stage, SNP associations were tested in one twin of each pair. Although this method of eQTL identification does not require twins the co-twins in this study served to replicate and validate the identified eQTLs, thus providing extra confidence in the findings.

A frequent use of twin studies is to identify gene expression alterations (on a shared genetic background) that are associated with various disease states; such genes may provide mechanistic insight into disease pathogenesis. A study of gene expression in subcutaneous fat of obesity-discordant MZ twins

detected differential expression of a range of genes[63]. Differentially expressed genes included those involved in inflammatory pathways (up-regulated in obese twins) and in mitochondrial branched-chain amino acid (BCAA) catabolism (down-regulated in obese twins). Interestingly, the largest increase in expression in obese twins was reported for the gene encoding the inflammatory cytokine osteopontin (*SPP1*), which has previously been associated with obesity and insulin resistance in mice. Other diseases for which gene expression changes have been identified in discordant MZ twins include rheumatoid arthritis[64], bipolar disorder[65], schizophrenia[66], and type 1 diabetes[67, 68]. A comparison of the skeletal muscle transcriptomes in MZ twins discordant for postmenopausal estrogen-based hormone replacement therapy (HRT) highlights the insights that may be obtained from MZ twins discordant for drug treatment, regarding the long-term effects of drug therapies[69]. Several pathways were differentially regulated in twins who received hormonal treatment, and expression differences correlated significantly with differences in muscle performance between the twins. Large twin studies estimating the heritability of expression of individual transcripts have not yet been published.

Metabolomics

Metabolites may serve as biomarkers of health and disease[70] and can be quantified in body fluids and tissue samples by approaches such as **mass spectrometry (MS)** and **^{1}H NMR spectroscopy.** Nicholson *et al* published the first metabolomics study based on ^{1}H NMR spectroscopic analysis of urine and blood plasma from MZ and DZ twin pairs[71], showing that familial factors (genetic influences and family environment) explain on average 42% of the variation in individual metabolite peak heights in plasma and 30% of the variation in urine. In two GWASs of metabolite profiles, data from twins allowed the proportion of variance in metabolite levels explained by significant SNPs to be compared with the proportion explained by the total genetic or familial variance[72, 73]. Heritability estimates of metabolic measures based on data from 221 MZ and 340 DZ twin pairs ranged between 23%–55% for amino acids and other small-molecule metabolites[72]. Estimates were higher for lipids (48%–62%) and lipoproteins (50%–76%). Although for most direct metabolite measures the total variance explained by significantly associated SNPs was 10% at most, higher estimates of explained variance were observed for certain metabolites ratios. The highest explained variance (25%) was observed for the ratio of linoleic acid to other polyunsaturated fatty acids (LA/PUFA). The twin based heritability for this ratio was 62%, implying that 40% of the total heritability can be ascribed to SNPs, which is high compared most other (clinical) phenotypes.

While traditional enzymatic methods usually provide composite measures of metabolites, ^{1}H NMR gives more detailed insight into the behavior of individual metabolites in pathways. In a direct comparison, similar estimates of heritability were found for most composite lipid measures based on either

enzymatic methods or ^{1}H NMR[72]. This supports the notion that high resolution metabolomics techniques are reliable.

Similarly to differentially expressed genes, differential levels of other molecules can be linked to disease pathogenesis. After detecting differences in serum and fat tissue lipid profiles in MZ twins discordant for obesity[74], Pietiläinen *et al* performed a simulation of **lipid bilayer dynamics** using **lipidomic** and gene expression data from the twins, providing novel functional insights into the biological pathways that underlie adipocyte expansion[75]. This study illustrates how findings from discordant twin studies may encourage and guide further functional or bioinformatic approaches to obtain in-depth mechanistic insights into the pathological mechanisms underlying complex traits and disease.

To date, there have been few proteomic studies in twins. A twin study of serum protein levels, as measured by antibody arrays, found that a relatively small proportion of the variation was attributable to familial factors; however, experimental variation in this study was relatively large[76].

Tissue-specificity of molecular variation

In concordance with the majority of molecular and genetic epidemiological studies, most twin studies have been based on peripheral blood. But how well does a molecular profile in blood cells reflect epigenetic and gene expression changes associated with different phenotypes and diseases in relevant tissues? Epigenetic changes arising at later stages of development and throughout life are more likely to be limited to specific tissues or even cells. DNA methylation profiles of MZ twins discordant for major psychosis suggest that epigenetic changes related to psychosis may be reflected in peripheral blood[77]. In this study, the most significant methylation change in psychosis-affected twins, *i.e.* hypomethylation at the promoter of the *ST6GALNAC1* gene, was also evident in postmortem brain tissues of some psychosis patients. However, large studies are warranted to establish how well molecular profiles in blood reflect those occurring in tissues relevant to disease, since molecular characteristics, particularly epigenetic and gene expression profiles are known to be largely tissue-specific. Although many of the relevant disease tissues are difficult if not impossible to obtain from large groups of living subjects, several twin registries are collecting biological samples from a variety of sources other than blood, including saliva, buccal cells, hair, skin, fat, muscle, urine and stool (Table 1; Supplementary information S1 (table)).

An issue of particular relevance to MZ twins, and possibly also DZ twins[78], is **chimerism**. Twins can exchange fetal blood through vascular connections between their circulatory systems. As a result, MZ twins can be hematopoietic chimaeras, and a variable fraction of cells derived from the hematopoietic stem cells (*e.g.* peripheral blood cells) in each twin may actually originate from the co-twin. This process can have implications for the detection of genetic or epigenetic events related to discordance originating *in utero*, since

some cells in unaffected twins may carry the genetic or epimutation of the co-twin. A study of twins discordant for transient neonatal diabetes mellitus type 1 (TNDM1) found that buccal cells only displayed hypomethylation of the *TNDM1* locus in the affected twins, while the same epigenetic change was evident in blood samples from both twins[79]. The issue may likewise influence the results of DNA sequence analysis of blood samples from MZ twins[80], although a study in healthy twins suggested that MZ twin concordance for SNPs and copy number in blood versus buccal cells is highly similar[81].

Host genetic influences on the microbiome

Studies of the human gut microbiome have revealed considerable variation in the composition of microbial communities between individuals. It remains to be established to what degree this variation is controlled by host genetics[82], but greater similarity has been observed in family members compared to unrelated individuals. A few studies have explored the role of host genetics by comparing various measures of the microbiome in small groups of MZ and DZ twins, but findings have so far been inconclusive, with some studies suggesting that the **microbiota** is slightly more similar in MZ twins compared to DZ twins[83, 84] and others observing comparable levels of similarity of the fecal microbiome of MZ and DZ twins[85]. An important factor in the comparison of similarity of individuals is the level that is compared: the overlap between relatives may be small at the organismal level, but might be larger at relevant functional levels (*e.g.* the degree to which microbial genes and metabolic pathways are shared).

A few studies in twins searched for microbial signatures associated with disease. A comparison of the fecal microbial communities in (concordant) obese and lean MZ twins showed that obesity is associated with various changes, including reduced bacterial diversity and differences in the representation of specific bacterial genes and metabolic pathways[85]. In MZ twins discordant for inflammatory bowel diseases, certain gastrointestinal bacterial populations differed in abundance among individuals with different clinical phenotypes of Crohn's disease, which is relevant to our understanding of the pathogenesis behind inflammatory bowel diseases[86]. MZ twins discordant for ulcerative colitis differed in the composition of the microbiota and in the expression of human RNA transcripts related to oxidative and immune responses in the mucosal epithelium[87]. In affected twins, fewer RNA transcripts correlated with bacterial genera than in unaffected twins, suggesting that ulcerative colitis may be associated with a loss of interaction between the mucosal transcriptional profile and the colonic microbiota.

The interplay of genes and environment

Genetic and environmental influences in many cases do not act independently. Gene–environment correlation (*r*GE) refers to the situation that exposure to certain environments is under genetic control[88]. For instance, twin but also adoption studies have found that lifestyle factors (*e.g.* exercise participation[89]

and diet[90]), life events (*e.g.*, divorce[91]) and life circumstances (*e.g.* family environment and social support[92]) are moderately heritable. Thus, influences that are usually considered as measures of 'environment' might often be better described as external factors that are partly under genetic control[93]. By contrast, G×E interaction refers to the scenario where different genotypes have different reactions to the same environmental exposure[94, 95]. By comparing differences in serum lipid levels in MZ twins across pairs with different genotypes, it was found that the Kidd (*JK*) blood group locus is associated with variability in total cholesterol level[96]. A similar approach was used to test whether interaction of the serotonin transporter gene (*SLC6A4*) length polymorphism with environmental stress is associated with MZ discordance for depression; no evidence was found for this hypothesis[97].

Testing classical assumptions

MZ twins share all their segregating genes while DZ twins share on average 50%

The assumptions of the classical twin method and the interpretation of results have always been a subject of debate (for a detailed discussion of the difficulties related to the concept of heritability, see[98]). A first assumption is that MZ twins are genetically identical, for which it has now been proven that there are exceptions to the rule. Still, the difficulty of various whole-genome sequencing efforts to find any replicable differences between MZ twins[39, 41] suggests that DNA sequence differences between MZ twins are not large, although an exact estimation of somatic sequence variation (given the nontrivial error rate in sequencing itself) has not been reported.

The availability of genome-wide marker data also allows us to address the assumption that DZ twins share on average 50% of their segregating genetic material, by estimating the true amount of genetic material that DZ twins inherited from the same parent (*i.e.* identity-by-descent (**IBD) sharing**). From genome-wide microsatellite marker data, Visscher *et al* demonstrated that the proportion of IBD sharing in most (95%) DZ twins and siblings lies within the range of 42-58%, with an average very close to 50%[99]. Using the empirical IBD measure instead of assumptions about genetic sharing, the heritability of height was estimated at 0.86, *i.e.* highly consistent with results from traditional twin studies, providing perhaps the most pertinent evidence to support the estimates of narrow-sense heritability from twin studies.

MZ twins share environmental influence to the same degree as DZ twins

Now that the classical twin design is being used to study epigenetic variation, it is becoming evident that novel attention has to be paid to the assumption that MZ twins share environmental influences to the same degree as DZ twins. Since MZ twins are derived from a single zygote, they may start out with more similar epigenomes than DZ twins, who originate from two zygotes with unique epigenetic profiles. DZ twins may thus start with more epigenetic differences

than MZ twins due to a cause that is not necessarily related to genetic differences. Although this hypothesis remains to be tested, an important observation in this light has been provided by a comparison of a small groups of MZ twins that were either monochorionicor dichorionic. The DNA methylation profiles of buccal epithelial cells were more similar in dichorionic MZ twins than in monochorionic MZ twins[55], and this may be related to the timing of splitting of the zygote. Thus, differences in epigenetic resemblance of monochorionic and dichorionic twins may be due to epigenetic divergence of embryonic cells that takes place after the blastomeric stage. Although this issue requires further study in larger samples, it illustrates that prenatal developmental processes related to twinning may influence the epigenetic resemblance of twins. Importantly, if MZ twins are epigenetically more similar than DZ twins due to non-genetic causes, the heritability of phenotypes that are epigenetically regulated may be overestimated.

Table 3 | **MZ and DZ twin concordance for complex disease**

	Probandwise concordance[A] (%)		
	MZ twins	DZ twins	refs
Type 1 diabetes	42.9	7.4	[129]
Type 2 diabetes	34	16	[130]
Multiple sclerosis	25.3	5.4	[149]
Crohn's disease	38	2	[150]
Ulcerative colitis	15	8	[150]
Alzheimer's disease	32.2	8.7	[134]
Parkinson's disease	15.5	11.1	[151]
Schizophrenia	40.8	5.3	[152]
Major depression	31.1[B]/47.6[C]	25.1[B]/42.6[C]	[153]
Attention-deficit hyperactivity disorder (ADHD)	82.4	37.9	[154]
Autism spectrum disorders	93.7	46.7	[155]
Colorectal cancer	11	5	[114]
Breast cancer	13[C]	9[C]	[114]
Prostate cancer	18	3	[114]

[A] Defined as 2C/(2C+D), where C is the number of concordant affected twin pairs and D is the number of discordant twin pairs. [B] Concordance in male twin pairs. [C] Concordance in female twin pairs.

Twin concordance and disease liability

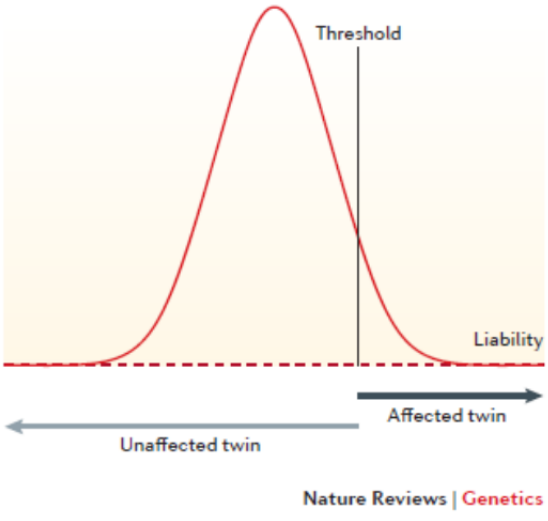
Relationship between heritability and discordance rates in MZ twins

A high concordance of MZ twins on its own does not imply a high heritability, as illustrated by concordance for measles. Before immunization was introduced,

concordance was close to 100% in both MZ and DZ twins[100]. This indicates that, despite the high concordance in MZ twins, genetic differences between individuals actually contribute little to differences in the vulnerability to this infectious disease. Likewise, a high rate of disease discordance in MZ twins does not rule out the importance of genetic influences. Although MZ twins are usually remarkably similar in appearance, MZ twins discordant for disease are often observed (TABLE 3). It is generally assumed that liability to disease is continuous, and disease becomes evident once a threshold is passed. The probability of observing discordant MZ twins thus depends on the heritability of the underlying liability and on the level of the threshold[101]. Especially for rare disorders (for which the threshold is high), many affected MZ twins are discordant even if the heritability is high (*e.g.*, schizophrenia, ADHD, autism, MS or type 1 diabetes,). From the dimensional view of disease liability it also follows that despite striking differences in clinical appearance, discordant MZ twins can be quite similar in terms of underlying disease liability (FIGURE 1).

Trait concordance in MZ twins, penetrance and disease-risk prediction.
The presence of disease-discordant twins indicates that genomes cannot completely predict the disease outcome of individuals, even if most variation in disease outcome *between* individuals is caused by genetic differences. For example, for schizophrenia, despite the high heritability of 80% the probandwise concordance between MZ co-twins is only 40-50%. The fact that MZ twin concordance for common disorders is not always high has important implications for genomic risk prediction and the ethical concerns that have been raised in this light. Even if we knew all the genetic variants that contribute to differences in disease risk between individuals, we would still not be able to predict with certainty the disease risk of all individuals based on their DNA sequence.

Figure 1| Liability threshold model and disease discordance in MZ twins. The liability threshold model assumes that multifactorial diseases result from an underlying continuous character (liability) that is normally distributed in the population[123]. If the combined effects of genetic and environmental influences push an individual's liability across a certain threshold level, the individual is affected. In the population, the proportion of individuals with a liability above the threshold is reflected in the disease prevalence. In discordant MZ twin pairs, only one twin has a liability above the threshold, although the liability of the unaffected twin may also be high. The red arrow displays the potential range of liabilities of affected twins from discordant MZ twin pairs, and the blue arrow displays the potential range of liabilities of unaffected twins. A comparison of MZ twins discordant for congenital diaphragmatic hernia and oesophageal atresia found no differences in genomic structural variation between co-twins[39]. However, structural events in relevant genomic regions that may have contributed to the genetic predisposition of both twins were detected in several pairs; these events were rarely observed in individuals from a healthy control population. A metabolomic study of MZ twins discordant for schizophrenia found that, relative to healthy individuals in concordant pairs, the unaffected twins from discordant female pairs showed similar (though smaller) metabolic changes than the affected co-twins[124]. These examples illustrate that the liability of unaffected twins from discordant pairs may also be elevated. However, this feature does not argue against the value of studying discordant MZ twin pairs to search for the molecular events that caused the affected twin to pass the threshold, or events that protected the unaffected twin. Of interest, a study of neurofibromatosis type 1 (NF1) in MZ twins with the same causal mutation in the *NF1* gene but highly variable disease phenotypes revealed considerable variation between twins in DNA methylation at the *NF1* gene[125].



Conclusions

Insights that can be obtained from twin studies extend far beyond the classical estimates of heritability. Traditional comparisons of the phenotypic resemblance of twins have been extended to studies of molecular variation across biological samples, providing functional insights into the underlying biology of heritable traits. The study of discordant MZ twins is a powerful method to identify DNA sequence variants, epigenetic variation, and metabolites associated with disease.

One might feel that there are few aspects of the human condition that have not been investigated in twins; however, new aspects emerge all the time. We have emphasized the value of twin studies to refine phenotypic and clinical definitions and to evaluate biomarkers for disease, but the use of twins can go even further. In recent years, political scientists, sociologists and even economists have become engaged in twin studies. A study of MZ twins who were infected with HIV through blood-transfusion at birth but who had strikingly different clinical outcomes used the identical genetic background of twins as a model to study the evolutionary processes and population dynamics that shape viral diversity[102].

In the coming years, longitudinal phenotypic information coupled with biological material collected by worldwide twin registries (TABLE1 ; Supplementary information S1 (table)) will be an important resource for large-scale molecular studies. To make optimal use of genetic data collected within twin registries, methods for family-based association analysis are being explored[103]. With the increasing interest in rare genetic variants, there may be renewed interest in linkage studies, in which DZ twins can play an important role. Linkage analysis in DZ twins, contrary to the analysis of non-twin siblings, is not affected by age differences within pairs and is less likely to suffer from non-paternity. Next-generation sequencing across multiple tissues and cell types will facilitate the detection of genome-wide SNPs, CNVs and epigenetic variation in discordant twins at an unprecedented scale, suggesting that twins will continue to provide valuable insights to human genetics.

Glossary

Classical twin design

The approach used to estimate the importance of genetic and environmental influences on complex trait variation. The estimate of heritability is based on a comparison of resemblance in monozygotic twins (who share nearly all of their genetic material) and dizygotic twins (who share, on average, half of their segregating genetic material).

Heritability

The proportion of variation in a trait that is due to heritable differences between individuals in a population, i.e. the proportion of variation due to additive genetic effects (narrow-sense heritability) or the proportion of variation due all genetic effects (broad-sense heritability).

Discordant monozygotic twins
(Discordant MZ twins). Twins who derive from a single fertilized egg cell but are dissimilar for a certain characteristic or disease. By contrast, concordant MZ twins are phenotypically similar.
Case–control study
The comparison of individuals with a trait or disease of interest (cases) to controls to identify genes or other aspects associated with the trait. Cases and controls can be unrelated or can be relatives (within-family case-control design).
Epigenome
The entire collection of epigenetic marks, including DNA methylation and histone-modifications, that regulate the expression of the genome. In contrast to the genome, the epigenome is specific to each cell.
Transcriptome
The total set of RNA transcripts that are produced in a cell or tissue by transcription of DNA.
Metabolome
The total set of small molecules (*e.g.*, lipids, amino acids, and sugars) that are the reactants, intermediate or end products of cellular metabolism and that are present in a cell, tissue, or complete organism.
Proteome
The entire complement of proteins that are present in a cell, tissue, or complete organism.
Microbiome
The entire set of genomes of micro-organisms (*e.g.* bacteria, fungi and viruses) that are present in a certain environment, for example in the human gut.
Multivariate twin models
Models used for the simultaneous analysis of multiple traits measured in MZ and DZ twins to estimate the importance of genetic and environmental influences shared ("overlapping") between traits in explaining their clustering, comorbidity or covariance.
Variability genes
A gene that contributes to the variation in a phenotype. The genotypes are associated with phenotypic variance rather than with the mean level or frequency of the trait.
Genetic non-additivity
Refers to genetic effects that contribute to the phenotypic variance in a non-additive manner. These include the effects of interacting alleles at a single locus (dominance) and interactions between different loci (epistasis).
Assortative mating
Refers to the situation whereby a trait is correlated in spouses because it influences partner choice (phenotypic assortment), or because it correlates with certain environments that influence partner choice (social homogamy). It is also called non-random mating.

Maternal effects
Effects that are transmitted from mother to offspring, including genetic effects. The phenotype in offspring can be influenced by: the maternal allele, mitochondrial inheritance, the effects of the prenatal environment (*e.g.* nutrient supply *in utero*), or the maternal supply of RNA or proteins to the egg cell.
Co-twin control method
Method to examine the associations between traits using discordant twins. If MZ twins discordant for trait 1 are also discordant for trait 2, the association between these traits is unlikely to be confounded by underlying shared genetic or early environmental influences.
Trans-generational inheritance
The transmission of a trait across generations (genetic or cultural inheritance). Epigenetic variation may also be transmitted across generations.
Imprinting
The mechanism that can occur at some loci to silence the expression of one of the two alleles, depending on the parent-of-origin of the allele.
Copy number variation
CNV. It refers to large DNA segments (over 1 kb) of which the number of copies is variable (*e.g.* between individuals or between cells within an individual), for example insertions, deletions and duplications.
Congenital diaphragmatic hernia
A birth defect that is characterized by malformation of the diaphragm, lung hypoplasia and pulmonary hypertension.
Oesophageal atresia
A congenital malformation of the esophagus in which the esophagus does not form an open passage to the stomach and may be connected to the trachea.
Dravet's syndrome
A childhood-onset epileptic encephalopathy, also called severe myoclonic epilepsy of infancy.
Mosaicism
The situation where the tissue of an individual consists of two or more genetically distinct cell lines due to somatic mutation, but originally derived from one (genetically homogeneous) zygote.
Non-coding RNAs
RNA transcripts that are not translated into protein but probably serve a regulatory function.
microRNA
A type of non-coding RNA with an average length of 22 nucleotides that has been suggested to play an important role in post-transcriptional gene regulation networks.
Lymphoblastoid cell lines
Cell lines derived from lymphoblasts, which are immortalized, cultured and stored to provide a renewable source of DNA and RNA.

Interferon (IFN) signaling
A signaling system for communication between cells that is involved in the immune response to pathogens and tumors
Expression quantitative trait loci
(eQTLs). Genomic regions that are associated with the level of expression of an RNA transcript. eQTLs can be tissue-specific.
Mass spectrometry
Technique to determine the mass-to-charge ratio of ions (charged particles) based on their separation in an electromagnetic field. The measured ratios and their relative intensities provide information about both identity and abundance of the molecules that gave rise to the ions.
^{1}H NMR spectroscopy
Metabolomics technique providing information about structure and quantity of hydrogen-containing molecules. It is based on the absorption and emittance of radiofrequent energy by hydrogen atoms when placed in a strong magnetic field, with wavelengths depending on the atoms' position in the molecule.
Lipid bilayer dynamics
The dynamic properties of lipid bilayer membranes such as thickness, fluidity, and permeability, that influence the physiological properties of a cell.
Lipidomics
The comprehensive study of the entire set of lipids in biological systems, such as cells, tissues and organs, using metabolomics techniques
Microbiota
The collection of all micro-organisms living in a certain environment, for example the human gut.
Identity-by-descent (IBD) sharing
(IBD sharing). Refers to the proportion of alleles in two individuals that are derived identical by descent from a common ancestor
Monochorionic twins
Twins that share the outer membrane (chorion) surrounding the embryos *in utero*. Monochorionic monozygotic twins result when the zygote splits after day 3 after fertilization.
Dichorionic twins
Twins that do not share the chorion surrounding the embryos *in utero*. Dizygotic twins are always dichorionic. Dichorionic monozygotic twins result when the zygote splits early after fertilization.
Chimerism
The situation where an individual carries some of the genetic material originating from another individual (*e.g.,* originating from the co-twin or originating from the mother).
Zygosity assessment
The assessment whether same-sex twins are monozygotic or dizygotic is often based on the comparison of DNA markers, or alternatively on standardized questionnaires.

Maximum likelihood

Maximum likelihood estimation obtains estimates of population parameters from a dataset by computing the probability of obtaining the observed data (likehood) for a range of different parameter values, and evaluating for which values the probability of observing the data is highest.

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