Epigenetic Basis of Twin Discordance in Diseases: Future Benefits

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ABSTRACT

Monozygotic twins share the same genotype since they are derived from the same zygote. However, monozygotic twin siblings frequently present many phenotypic differences, such as their susceptibilities to diseases. These isogenic individuals are not entirely identical. They exhibit phenotypic incompatibility for many features, from birth weight to complex diseases. Recently, several studies have been published showing that phenotypic differences, especially in monozygotic twins, are being induced from prenatal period to life-long epigenetic differences. Epigenetic studies on twins have a great potential to contribute to our understanding of complex diseases, such as cancer, autoimmune disorders, psychiatric disorders and neurological diseases. Since monozygotic twins are genetic clones (genetically identical), they are considered as perfect models for studying the role of environmental factors as determinants of complex diseases and phenotypes. In this review, a number of intrauterine effects and genetic mechanisms that may affect phenotypic, genotypic, and epigenetic differences between monozygotic twins were described and effects of epigenetic mechanisms on complex diseases were mentioned. Further work on epigenetic changes in diseases using incompatible monozygotic twin models, would lead to new developments in medical therapies.

Keywords: Monozygotic twins, Epigenetic mechanisms

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Introduction

Several theories about the origin of monozygotic (MZ) twins have been proposed, such as the theory of the co-dominant axes, the cell repulsion theory, the depressed calcium levels theory, the blastomere herniation theory and the zona pellucida assisted binary fissions theory. However, it is still not clear why MZ twinning occurs. Delayed fertilization or implantation can be one of the mechanisms that may lead to MZ twinning. Traditionally, it has been thought that dizygotic twins result from fertilization of two distinct ova by two separate spermatozoa, while monozygotic twins are the product of a single ovum and sperm that subsequently divides to form two embryos (1). According to this hypothesis, the polarization of the embryoblast occurs in the post zygotic period. In this model, the number of fetuses, chorion, and amnion are determined by the time of polarization. The events that trigger polarization are post zygotic gene mutations, and abnormalities in cell surface proteins as well as in formation of zona pellucida. One can speculate that MZ twinning may be a result of both environmental and genetic factors. A combination of mechanical stresses on the preimplantation embryo and genetic factors that modify embryonic integrity or growth and tissue differentiation may act on MZ twinning (2). Post-zygotic splitting becomes more unlikely as the time passes, and that kind of splitting has never been observed in vitro. In 2013, Herranz offered an alternative theory based on two principals: 1. Monozygotic twinning occurs at the first cleavage division of the zygote and 2. Subsequent chorionicity and amnionicity is determined by the degree of fusion of amniotic membranes within the zona pellucida (3).

Monozygotic twins are often described as being identical both phenotypically and genetically. Nevertheless, these isogenic individuals are not completely identical, and show phenotypic discordance for many traits from birth weight to a range of complex diseases. Most twin researches rely on the assumption that dizygotic twins share approximately 50% of the same genes, whereas monozygotic twins share 100%. However, among these isogenic twins, small phenotypic differences can be found such as different hair styles, minor body shape changes, and different personality traits. These differences are due to epigenetic changes in twin pairs. Figure 1 summarizes the possible post-zygotic genetic effects that may account for MZ twin discordance. In this review, we will focus on certain epigenetic changes, specifically DNA methylation, identified in certain diseases.

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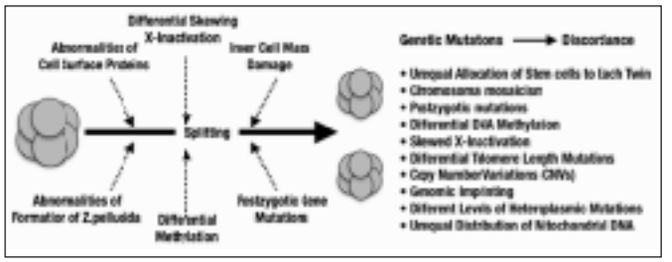


Figure 1: Possible triggers for splitting of the zygote (left) and (epi)genetic mechanisms that may cause MZ twin discordance (right).

Epigenetic changes

Epigenetics can be defined as all meiotically and mitotically inherited modifications in gene expression that are not encoded in DNA sequence. Epigenetic modifications of chromatin and DNA have been recognized as important permissive and suppressive factors in controlling the expressed genome via gene transcription. There are four major epigenetic mechanisms within the cell which are; posttranslational modifications of histone proteins, methylation of DNA, RNA-associated silencing and genomic imprinting (4,5). Histone modifications such as methylation and acetylation are more flexible (short-term) modifications whereas in differentiated adult cells, DNA methylation is more stable long-term modification (6). Histone methylation is frequently associated with gene silencing. In contrast, core histone acetylation is generally associated with relaxed chromatin structure and permit or facilitate access to gene regulatory proteins (Figure 2). DNA methylation is one of the most extensively studied epigenetic marks and will be the focus of this review. It is involved in transcriptional gene silencing, plays important roles during mammalian development and its perturbation is often associated with human diseases (5). Covalent addition of a methyl group to a 5'-position in the cytosine pyrimidine ring in the DNA sequence is termed DNA methylation (5). A family of enzymes called DNA methyl transferases catalyze DNA methylation by using S- adenylyl - methionine as the methyl group donor (6,7). In humans, DNA methylation preferentially occurs on cytosines in the context of CpG dinucleotides (Figure 3) (9). Regarding the CpG content, two groups of sequences can be defined: CpG poor regions and CpG rich regions which is named as “CpG islands”. CpG islands are parts of the genome with a high density of CpG dinucleotides and are present in the promoter regions of almost 50% of all genes. The CpG poor regions in inactive genes are usually methylated to suppress their expression. For most cell types in the body, these epigenetic changes become fixed once cells are differentiated or exit the cell cycle. However, in normal developmental or disease situations, some cells undergo major epigenetic “reprogramming”.

Epigenetic changes influenced by intrauterine environment are classified as trans-generational epigenetic effects because a maternal environmental factor can have epigenetic effects or even epigenetically independent toxic effects on the developing fetus (offspring or the F1 generation) and, if the fetus is female, on developing germ cells that are going to contribute to the grandchildren (the F2 generation) (10,11). (Figure 4)

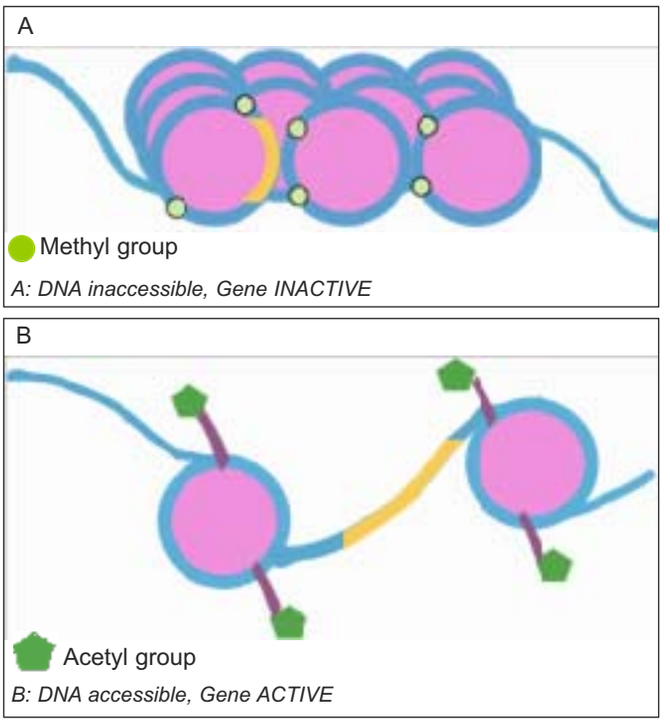


Figure 2: Histone methylation is frequently associated with gene silencing (A), whereas core histone acetylation is generally associated with relaxed chromatin structure (B).

Figure 3: Methylated DNA Sequence

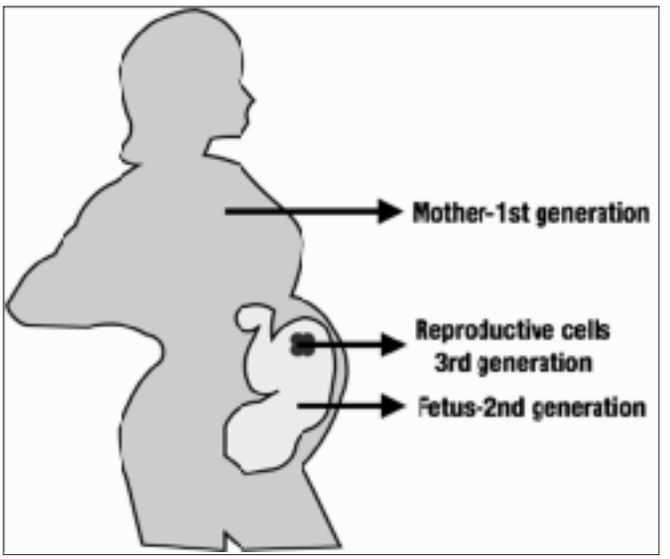


Figure 4: Mitotically heritable epigenetic variation can arise in somatic tissue within a lifetime and can alter the phenotype.

Diet and DNA methylation

Many nutrients are promoted before and during pregnancy to women for prevention of deficiencies and for improvement of gestational outcomes in the offspring. Animal studies sug-

gest that these nutrients may have long-lasting health effects. Maternal excess folate or methyl donor intake during pregnancy in animal models causes weight gain or metabolic syndrome in offspring (12,13). These effects may be more pronounced when offsprings are fed with a high fat diet (9). For example, high dietary levels of methyl donors (betaine and choline) or methyl metabolism cofactors (folate and vitamin B12) (9 - 60 times the recommended level) fed to viable yellow agouti (Avy) mice during pregnancy and lactation increased cytosine methylation of the Avy gene (11) and increased the proportion of offspring with brown coat color and lean phenotype compared with yellow coat color and obese phenotype through epigenetic modification of gene expression (13,14). Intake of vitamins A and D during pregnancy can also affect gene expression in offspring (15,16). In animal studies, excess folic acid may exacerbate weight gain, fat accumulation, and inflammation caused by consumption of a high fat diet (17). Mothers exposed to high folic acid (HFA), have increased weight gain and reveal hyperphagia and hyperdypsia after the end of HFA exposure. HFA exposed female offspring have increased weight gain and disturbed feeding behavior, and also have impaired glycemic control.

Adipose tissue appears to be an important endocrine organ which produces hormones such as leptin and estrogen as well as cytokines that play an important role for cell signaling. Cytokines secreted by adipose tissue are called adipokines. Leptin is a type of hormone called an adipokine that is released exclusively from fat cells. Leptin interacts with brain to signal the body to eat less and burn more calories. Adiponectin is another type of adipokine. Unlike leptin, the leaner the body is, the more adiponectin the fat cells release. Its production is abnormally low in obese individuals, particularly in those with visceral obesity (18). HFA induces changes in hormones and blood adiponectin levels. When compared with matched controls, female offspring of HFA exposed mothers had increased insulin and decreased adiponectin levels, and male offspring of HFA exposed mothers had increased levels of leptin, when compared with corresponding controls (16). In humans, high erythrocyte folate status during pregnancy was associated with increased fat mass of children at six years of age (19). Folic acid appears to influence energy and lipid metabolism by modulating DNA methylation and gene expression patterns. However, the effects of excess folic acid intake on metabolic syndrome risk and adiposity in adulthood remains poorly understood.

The discordant MZ twin model and epigenetics

From the late 19th century onwards, twin studies played an important role in genetics. MZ and DZ twins are "experiments of nature" that provide an excellent opportunity to analyses environmental and genetic influences on phenotypes of humans. In classical twin studies, comparison of the resemblance (concordance rates or correlations) for a disorder or a quantitative trait between MZ and DZ twins facilitates research into the etiology of population variation. Twin studies contributed enormously to current genetic insights. For example, multiple sclerosis (MS) was thought to be a non-genetic disorder until a large study was done in Canadian twins (20).

The logical basis of classical twin studies is the assumption that MZ twins are genetically identical, whereas DZ twins share 50% of their segregating genes on average, and are as genetically different as are ordinary siblings. Similarity in MZ twin pairs can be established by means of concordance rates. When twins have the same disease, they are said to be concordant for that disorder. Conversely, when only one member of the twins is affected and the other is not, they are said to be discordant for the disease. The cause of this discordance is not known, but recent evidence suggests that differences in DNA methylation may be involved. One possibility is that DNA methylation patterns can differ between twins, even though their DNA is identical. Interestingly, a significant proportion of identical twins have an unaffected twin (Table 1), and MZ twin

Table 1: Concordance rates in monozygotic and dizygotic (7,23,26)

Disease	Concordance Rate (%)	
	Monozygotic Twins	Dizygotic Twins
Non-Traumatic Epilepsy	70	6
Multiple sclerosis	18	2
Type 1 Diabetes	40	5
Schizophrenia	46	15
Bipolar Disease	62	8
Autism	58-60	
Osteoarthritis	32	16
Psoriasis	72	15
Cleft lip with or without cleft palate	30	2
Systemic Lupus Erythematosus	22	0
Rheumatoid arthritis	12	3
Various Cancer Types *(breast, leukemia, gastrointestinal tract, lung, bladder, pancreas etc.)*	0-16	

Data from referances 7., 23. and 26.

discordance can be particularly important to investigate the environmental component of diseases. However, for most of the diseases, identical MZ twins show a concordance rate that varies between 5% and 75%, that is a frequency at least 2 to 5 times higher than in fraternal twins. Examples include, neurological (21) and mental disorders (22), autoimmune diseases (20), childhood leukemias (24), cardiovascular diseases (25), cancer (26), and even age-related macular degenerations (27).

There are some differences between Mendelian diseases and diseases that shows complex inheritance. Sickle cell anemia is an example for a Mendelian inherited disorder. If one MZ twin has sickle cell disease, the other twin always has the disease as well. On the other hand, diabetes mellitus is an example for a multifactorial disorder and when one MZ twin has type 1 diabetes mellitus, in only approximately 40% of such twin pairs the other twin has the disease as well. Greater concordance in MZ versus DZ twins is an evidence of a role of a genetic component for the disease, whereas disease concordance less than 100% in MZ twins is an evidence that non-genetic factors play a role in the disease (7). Such factors could include environmental influences, such as exposure to infection or diet, as well as other effects, such as somatic mutation, effects of aging, or epigenetic changes in gene expression in one twin in comparison to the other.

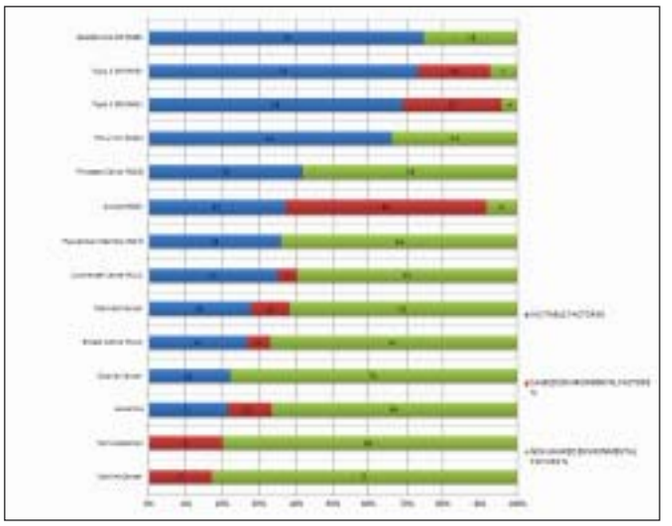
Environmental influences, stochasticity and mirror imaging

"Environmental differences" are one type of non-genetic factors that results in epigenetic differences in MZ twins. MZ twins are exposed to different nutritional conditions related to the formation and vascularization of placenta. Because the intrauterine environment of MZ twins can differ, it is possible that intrauterine differences contribute to differences in epigenetic states. Studies investigating placental epigenetic dysregulation have identified altered DNA methylation of genes involved in placental development, including trophoblast differentiation, angiogenesis, and endocrine signaling (28). Placental epigenetic dysregulation, in particular altered DNA methylation, has been proposed as a mechanism underlying the aberrant gene expression in the pathophysiology of IUGR. Intrauterine growth restriction (IUGR) is a common issue in twin pregnancies, affecting approximately 12 to 47% of all twin pairs (29). Severe discordant growth is a major contributor to perinatal morbidity and mortality in MC twin pregnancies. Growth discordance is 2.5 - fold higher in MC versus dichorionic twin pregnancies. Discordant birth weight in MZ twins is a result of unequal territory, which may be due to unequal division of the inner cell mass at twinning with subsequent discrepant placental territoriality (30). The other causes of IUGR include genetic predisposition, in utero crowding, uneven allocation of blastomeres, uneven blood supply, and placental dysfunction (for example, placental abruption, infarcts, stem vessel thrombosis, velamentous insertion of the cord, and single umbilical artery). Some of these events, such as unequal division of blastomeres or uneven vascularization of the placenta, can be considered as non-shared early exposures, which can be classified as environmental or stochastic, depending on the adopted definition.

After birth, any variety in environmental exposure, such as diet, smoking, toxin exposure and infection, may contribute towards twin discordance. Moreover, early phenotypic differences arising in twins can potentially cause different effects of shared exposures, leading to dissimilarity between the twins. Certain cases of twin discordance might potentially be stochastic in origin, however it is difficult to separate these from environmental effects and gene-environment interactions, since the causal mechanisms are not clearly understood. Stochastic events, such as unequal division of the inner mass cells during twinning or unequal allocation of the developmental markers or precursor cells to different somatic lineages, have been reported as potential sources of discordance in MZ twins (30,31). Examples include discordance for eye or hair color and fingerprint profiles, cases of mirror twinning (affecting up to 25% of MZ twins), and major malformations (32).

The concept of mirror-image MZ twins is based on inverse laterality. This may suggest that the event that originated the twins began after the cells of the embryonic plate started to lateralize but before formation of the primitive streak (1). The primary difficulty with this subject seems to be the variation scale of asymmetry, varying from situs inversus totalis to right and left-handedness. Although individual cases of situs inversus have been reported, there does not seem to be an excess of situs inversus neither among MZ twins, nor of MZ twins among cases with situs inversus (31). Discordant handedness (right and left handedness) has been commonly reported in MZ twins suggesting some degree of cerebral hemisphere dominance asymmetry as recently confirmed by functional magnetic resonance brain imaging (30,33). Many subtler kinds of mirror-image asymmetry, such as asymmetries in nevi, hair whorl direction, tooth patterns, unilateral eye and ear defects, and even tumor locations and undescended testicles have been reported (34). Most complex phenotypes arise as a result of the interplay between genetics and environment. In epidemiology, it is of interest to determine what proportion of the phenotypic variance each of these factors can explain. Greater phenotype concordance in MZ twins would point to a higher contribution of genetics to the disease (35-38). The heritable and environmental factors contributing to certain diseases can be seen on table 2.

Table 2: Heritable and environmental factors that contribute to formation of certain diseases. Percentages in parenthesis near the disease names are concordance rates.



The rates were obtained from published studies of gestational diabetes, type 1 diabetes, and type 2 diabetes (33), psoriasis (35), stomach, colorectum, pancreas, lung, breast, cervical, uterine, ovary, prostate, bladder and leukemia cancers (35); autism (36); myocardial infarction (37).

Twin models to study diseases:

Twin studies can basically be subdivided in classical and case-control studies. Classical twin studies make use of the identical genetic background of MZ twin pairs and DZ twins that share almost 50% of their genetic background. The degree of phenotypic similarity is evaluated by means of twin concordance rates or correlation coefficients. Concordance can be expressed as either pairwise or probandwise rates. The pairwise concordance rate illustrates the proportion of affected pairs concordant for the disease, while the probandwise rate provides an estimation of the risk that one twin will develop the disease if her/his twin has been already diagnosed. Concordance rates of diseases with a substantial genetic component are expected to be significantly different in MZ compared to DZ twins (40). Different from classical twin studies, the co-twin control method is the best suited method to study the impact of specific genes or environmental risk factors on the development of disease (41). Twin pairs who are discordant for a given phenotype are considered as matched pairs and the healthy co-twin serves as a control for the affected twin.

There are at least 17 and 22 twin registries within and outside Europe, respectively. Twins and their family members are often enthusiastic participants of these registries (42). Many research projects about genetic epidemiology and medical, behavioral and psychiatric genetics are carried on for clarifying the etiology of these diseases. Here we focused on recent genome-wide efforts across five major types of disease.

Psychiatric disorders:

Schizophrenia (SZ) and bipolar disorder (BD) are two related psychiatric conditions that together contribute significantly to the global burden of disease.

Schizophrenia is a severe chronic mental disorder affecting about 0.5% of the world populations (43). While it is believed that the etiology of SZ is multifactorial, genetic epidemiological studies have indicated that schizophrenia is highly heritable (44). Genome-wide association studies (GWAS) have identified a number of common single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) associated with SZ (45,46). Although some CNVs were replicated and have not been found in mentally healthy cohort, they are not unique to SZ (47). A recent large GWAS of schizophrenia reported 108 independent genetic variants that met the level of genome-wide significance (45). However, these common variants altogether explain only a small portion of genetic risk. Recently, whole-genome sequencing (WGS) analysis of 8 families with monozygotic (MZ) twin pairs discordant for schizophrenia were analyzed to assess potential association of de novo mutations (DNMs) or inherited variants with susceptibility to schizophrenia. Eight non-synonymous DNMs were identified and shared by twins, which were either located in previously reported schizophrenia risk genes (p. V24689I mutation in TTN, p. S2506T mutation in GCN1L1, IVS3+1G>T in DOCK1). By searching the inherited rare damaging or loss-of-function (LOF) variants and common susceptible alleles from three classes of schizophrenia candidate genes, genetic alterations in several schizophrenia risk genes, including GAD1, PLXNA2, RELN and FEZ1, were identified. Certain inherited copy number variations (CNVs; including a large deletion at 16p13.11) were also implicated for SZ. as well as missense DNMs and inherited risk variants. This might suggest that DNMs, inherited rare damaging variants and common risk alleles may lead schizophrenia susceptibility altogether. These results show that schizophrenia is caused by a combination of multiple genetic factors, with each DNM/variant showing a relatively small effect size (48).

In BD, the genetic contribution to the disease is high and estimated to be approximately 85%, however the concordance rate of BD in mono-zygotic twins is only 40-70% (49), indicating that environmental factors are probably involved as well. Recently, the top differentially methylated site across all 22 psychosis-discordant MZ twin pairs was shown to be located in the promoter region of a gene that codes alpha-N-acetylgalacto saminide alpha-2,6-sialyltransferase 1 (ST6GALNAC1), which was hypomethylated in affected individuals compared with their corresponding unaffected twins. For BD, epigenetic changes that affect psychosis, such as DNA methylation, were also previously implicated for the genes GPR24 and CTNNA2 (50). These studies show that epigenetic factors are contributors of certain psychiatric disorders.

Cardiovascular diseases

The risk of developing a cardiovascular disease (CVD) de-

pends on both lifestyle and genetical background. Previous twin studies have indicated that heritable factors may account for as much as 30% to 60% of the variation in risk (39). In adults, altered DNA methylation at genes including those encoding insulin (INS) and GNAS antisense RNA 1 (GNASAS) is associated with the risk of myocardial infarction, although there was no investigation of the effect of altered DNA methylation on gene expression (51). It was also shown recently that childhood obesity is associated with hypermethylation of the proopiomelanocortin (POMC) gene, which is involved in energy homeostasis and in which genetic polymorphisms are associated with early-onset obesity and adrenal insufficiency (52). Peri/postnatal Epigenetic Twins Studies highlight the influence of non-shared intrauterine environment, genetic variations, and shared environment on epigenomic profile. Analysis of DNA methylation in MZ and DZ pairs revealed that the largest contributor of variation was the combined effects of non-shared intrauterine environment and stochastic factors, which highlights the importance of the intrauterine environment on shaping the neonatal epigenome (53). To our knowledge, only one EWAS of a CVD-related phenotype has been performed in twins. Using DNA methylation profiles of purified CD14+ monocytes from 15 type 1 diabetes-discordant pairs of MZ twin children, the presence of 132 genomic locations has been shown to differ in DNA methylation in all twins with type 1 diabetes (54). These CpG regions were rich in genes that are involved in immune function, including HLA-related genes (HLA-DQB1) and regulatory factor X-associated protein (RFXAP). Both HLA-DQB1 and RFXA previously shown to be associated with type 1 diabetes and the pro-inflammatory cytokine tumor necrosis factor. However, the small sample size, and the use of whole blood material may influence the overall DNA methylation status. More studies with MZ/DZ twins would lead to better understanding of the epigenetic contribution to cardiovascular diseases.

Autoimmune diseases

Autoimmune diseases can be referred as immune system problems that lead one's own immune cells to attack their own cells. Systemic lupus erythematosus (SLE), rheumatoid arthritis, and dermatomyositis are three autoimmune diseases with overlapping clinical signs and symptoms. DNA methylation status of these diseases were investigated by Javierre et al. with a genome-wide DNA methylation study (55). SLE is a chronic multiorgan disease characterized by acute and chronic systemic inflammation. Autoantibodies against nuclear and cytoplasmic antigens have been detected in patients with SLE, which is thought to play a role in pathogenesis of the disease. In the study by Javierre et al., DNA methylation of WBCs was assayed with a platform that included 1,505 CpG sites from 807 genes related with SLE, rheumatoid arthritis, and dermatomyositis (55). The cohort included five discordant MZ twin pairs for each disease and 30 unrelated normal controls. The authors found no differences in DNA methylation of the five MZ twin pairs for rheumatoid arthritis and dermatomyositis, but forty-nine genes had significant differences in DNA methylation between SLE and healthy MZ twins. Among these genes are IFNGR2, MMP14, LCN2, CSF3R, PECAM1, CD9, and AIM2(55). There are significant differences in genes encoding for immune function, such as defense response, cell activation, immune response, cell proliferation, and cytokine production, all of which are potentially relevant in autoimmune inflammatory diseases.

Type-1 diabetes mellitus (T1D) is an autoimmune disease characterized by the destruction of insulin-producing beta islet cells through a T-cell mediated mechanism in the pancreas. In a study by Rakyan et al. (54), DNA methylation profiles of 27,458 different CpG sites within 14,475 promoters were investigated in type-1 diabetes mellitus (T1D) in 15 discordant MZ twins. In this study, fifty-eight T1D-associated hypermethylated methylation variable positions (T1D-MVPs) and seventy-four hypomethylated T1D-MVPs were identified in the T1D-affected twins. Several of these T1D-MVPs associated genes or gene products are known to be associated with T1D or immune responses. These include; the HLA class II gene, HLA-DQB1, which carries the highest single genetic risk for T1D along with HLA- DRB1; RFXAP, an HLA class II regulating element; NFKB1A, an important regulator of apoptosis and inflammatory immune responses; TNF, a key inflammatory cytokine associated with T1D in animal models; and GAD2 which encodes GAD65, a major T1D auto-antigen involved in disease etiology. Further investigation of the epigenetics status of genes by means of their role in T1D would help more to enlighten the role of epigenetic mechanisms in this disease.

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by an autoimmune attack on myelin and neurological degeneration with formation of plaques. Methylation at greater than 1.5 million CpG dinucleotides was assessed in one study of MS discordant MZ twins (56). The twins differed at 176 sites. However, these differences were not consistent between twin pairs. In this study, more than 18,000 genes were expressed but the transcript abundance was not significantly different between twins. Robust gene-expression differences were not observed between MS-affected and unaffected twins in CD4+ lymphocytes either. This study did not find any convincing evidence for epigenetic or transcriptome differences that could explain MS discordance in MZ twins.

Alleles at or near the class II loci HLA-DRB1 and HLA-DQB1 contribute significantly to the genetic risk of MS. The MHC class II transactivator (MHC2TA) is the major controller of expression of class II genes, and methylation of the promoter of this gene has already been shown to alter its function. Another study analyzed DNA from MS discordant MZ twins to detect methylation of the CpG islands in the MHC2TA promoter (57). This study did not find the expected effect of methylation of the MHC2TA promoter pIV, which would contribute to MS twin discordance. Therefore, further

studies are needed to clear if epigenetic changes play role on MS or not.

Psoriasis is a common autoimmune skin disease characterized by T cell-mediated hyperproliferation of keratinocytes in the epidermis. In one study, the methylation status of 27.578 individual CpGs predominantly distributed in the promoters of 14.475 coding genes throughout the genome and gene expression profiling targeting 25.000 annotated genes was performed on CD4+ and CD8+ cells (58). The study revealed no significant difference in DNA methylation or gene expression between unaffected and affected twins. Nonetheless, combined analysis of DNA methylation and gene expression identified genes where differences in DNA methylation between unaffected and affected twins were correlated with differences in gene expression, such as IL13, *ALOX5AP, PTHLH, TNFSF11*, which are known to be involved in immune responses and associated with psoriasis. Furthermore, among these genes, significant enrichment of the ones that play role in biological pathways in CD4+ cells were identified, whereas no significant enriched pathways found in CD8+ cells. A significant portion of the genes were found to be involved in immune responses, positive regulation of response to stimulus, and immune system processes. These results suggest that, in addition to global epigenetic targeting as a mechanism for twin disease discordance, there may be an impact of epigenetics on specific cell types, as well. (58).

Cancer

Despite global research efforts, cancer remains one of the leading causes of death in economically developed countries. Large numbers of genes have been found to be hyper- or hypomethylated in different types of cancer tissues at various stages of tumorigenesis. The first epigenetic alteration identified in cancer was the global reduction of methylation levels in cancer patients compared with healthy subjects in a primary human tumor tissue (59).

Even though only a minority of CGI promoters are methylated in normal somatic cells, there are a number of oncogenes identified that show a loss of methylation at their promoters and associated activation of gene expression in tumor tissues. These include key genes in tumorigenesis such as RRAS (60), MAGE1(61), XAGE1A (62) and MASPIN (63). Overall, the global loss of methylation is a key abnormality in cancer cells and is associated with repressive chromatin marks and silencing of genes within these regions.

Hypermethylation can affect at least 5% of all promoter CGIs that are normally unmethylated in somatic cells. The hypermethylation at individual genes is generally associated with stable gene silencing and can be specific to tumor type (64). Many tumor suppressor genes have been identified in the last two decades that are hypermethylated and generally repressed in multiple types of cancer including RB1 (65), CDKN2A (66), MLH1 (67), VHL (68) and BRCA1 (69).

Two recent studies of cancer-discordant MZ twins have provided interesting findings (70,71). High-resolution profiling of DNA methylation in whole blood samples from breast cancer discordant MZ twins led to the identification of DOK7 hypermethylation as a blood-based epigenetic biomarker that can be traced years before tumor diagnosis (70). DOK7 encodes a docking protein that acts as a substrate and activator of receptor tyrosine kinase. The authors identified 403 positions that were differentially methylated in breast cancer in whole blood from 15 MZ twin pairs discordant for the disease. Among these positions they found a hypermethylated site located in an alternative promoter of the *DOK7* gene with a methylation difference of only 2%. Further analysis revealed that the methylation signal spreads upstream of the promoter. Interestingly, the methylation of *DOK7* was also found approximately 4.7 years before tumor diagnosis, on average, implying that the epigenetic change occurs early in the development of the disease (70).

Epigenome-wide DNA methylation profiles in 41 cancers discordant MZ twin-pairs with affected individuals diagnosed with tumors at different single primary sites: the breast, cervix, colon, endometrium, thyroid gland, skin (melanoma), ovary, and pancreas, were analyzed. No significant global differences in whole blood DNA methylation profiles were observed. Epigenome-wide analyses identified DNA methylation signatures in blood associated with pan-cancer??, at or near SASH1, COL11A2, AXL, and LINC00340 (72). Replication of the four top-ranked signals in an independent sample of nine cancer-discordant MZ twin-pairs showed a similar pattern of association at COL11A2, AXL, and LINC00340, and significantly greater methylation discordance at AXL compared to 480 healthy concordant MZ twin-pairs. The effects at cg02444695 (near SASH1), COL11A2, and LINC00340 were the most promising biomarker candidates (72). Three of these SASH1, COL11A2, AXL, and LINC00340 signals were present up to 5 years prior to cancer diagnosis, indicating the potential clinical utility of whole blood DNA methylation analysis in cancer surveillance.

Caudal duplication anomaly

One of the earliest epigenetic studies using disease discordant MZ twins explored *caudal duplication anomaly*, a condition characterized by duplication of organs in the caudal region (73). The authors used a candidate gene approach to examine DNA methylation at the promoter region of a gene implicated in these anomalies, AXIN1. Hypermethylation of the region was detected in the affected twin in peripheral blood mononuclear cells (PBMCs), but not in buccal cells. Because blood is a mesodermal tissue whereas buccal cells are ectodermal, this result suggests that the epimutation occurred after the differentiation of the three germ layers and highlights the tissue-specific role of epigenetic marks.

Future benefits

The study of epigenetics in disease using discordant MZ twins has present and future medical implications and transla-

tional potential. In contrast to genetic mutations, most epigenetic modifications may be reversible and preventable. The resetting of aberrant epigenetic states in affected cells is an expanding therapeutic approach to treat or prevent diseases. Pharmacological targeting of DNA methylation and histone acetylation and methylation is possible and a promising therapeutic approach. At present, at least 40 new epigenetic drugs are reported to be in development for cancer. These drugs can be grouped as inhibitors of DNA methylation, histone deacetylase inhibitors and inhibitors of histone methylation according to their mechanism of action. A number of DNA methylation inhibitors are currently under investigation, including the pyrimidine nucleoside analogs decitabine and azacitidine, and the nonnucleoside inhibitor hydralazine. Azacitidine and decitabine are both U.S. Food and Drug Administration (FDA) approved for the treatment of a number of myelodysplastic syndrome subtypes, including refractory anemia and chronic myelogenous leukemia (74). The most extensively studied histone decatylase inhibitors (HDACi) target class I and/or II, and/or IV HDACs and a separate set of inhibitors target class III HDACs (sirtuins). Vorinostat, panabinostat, belinostat, tenovin and sirtinol are the examples of HDCAi. Varinostat is FDA approved drug for cutaneous T-cell lymphoma (74). Chaetocin was one of the first histone methylation inhibitors (HMTi) developed and Chaetocin killed human tumor cell lines and primary myeloma cells in vitro whereas normal human B cells were insensitive to the compound. Chaetocin also had potent antitumor activity in vivo but at only as an experimental study (74).

Targeted genome editing is a broadly applicable approach for efficiently modifying essentially any sequence of interest in living cells or organisms. This technology relies on the use of engineered nucleases, which are artificial proteins composed of a customizable sequence-specific DNA-binding domain fused to a nuclease that cleaves DNA in a non-sequence-specific manner. Zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPRs) have allowed biological researchers to edit the genomes of various organisms (75,76). The newly-developed transcription activator-like effector nucleases (TALENs) comprise a non-specific DNA-cleaving nuclease fused to a DNA-binding domain that can be easily engineered so that TALENs can target essentially any sequence. The clustered, regularly interspaced, short palindromic repeat (CRISPR) technology, another important new approach for generating RNA-guided nucleases, such as Cas9, with customizable specificities (76). Although has started to be used, in the future, genome editing mediated by these nucleases can more widely be used to rapidly, easily and efficiently modify endogenous genes in a wide variety of biomedically important cell types and in organisms that have traditionally been challenging to manipulate genetically. Finally, combining these technologies with epigenetic mechanisms would be promising for future therapies.

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