Influence of Epigenetic Differences on the Etiology of Bipolar Disorder and Schizophrenia

Zhiyu Yan
University College London, London, WC1H 0AJ, U.K.

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Abstract: Schizophrenia and bipolar disorder are two mental disorders that have attracted worldwide attention. However, The role of DNA methylation in epigenetics in schizophrenia and bipolar disorder is unclear. This paper investigates the role of epigenetics in the pathogenesis of schizophrenia and bipolar disorder. Genome-wide analyses of monozygotic twins have identified specific genetic loci at which DNA methylation may be responsible for schizophrenia and bipolar disorder. Certain conclusions were made by genome-wide DNA methylation analysis of DNA samples from identical twins with inconsistent major psychiatric disorders. In twins with schizophrenia, the largest differential methylation region associated with mental illness was significant hypomethylation of the ST6GALNAC1 promoter, which overlaps with previously reported rare schizophrenia genomes. The average difference in DNA methylation at this locus is 6%, but there is considerable variation between families, with some twins even showing a 20% difference in methylation. These results suggest that DNA methylation differences play a role in phenotypic differences in identical twins and may influence the etiology of schizophrenia and bipolar disorder to some extent.

1 INTRODUCTION

Schizophrenia and bipolar disorder are two related mental disorders that are common across the globe (Patel et al. 1996). Schizophrenia is a highly inherited neuropsychiatric disease, which is mainly manifested in the presence of psychotic symptoms, but also characterized by dysfunctional emotional response and cognitive changes. Although people have succeeded in identifying the gene variants associated with schizophrenia, they are still uncertain about the pathogenic genes of the pathogenesis of the disease and how their functions are regulated (Hannon et al. 2016). Bipolar disorder is an extremely debilitating mental illness. It is characterized by paroxysmal mood swings. Patients often experience both manic and depressive moods, and often have cognitive impairment. People with this disease have serious destructive attacks, frequent recurrence and serious psychosocial disorders. The disease usually begins in adolescence and even in the late childhood of some patients, much earlier than previously thought (Miklowitz et al. 2008). Since the two diseases may have the same etiology, the symptoms of schizophrenia and bipolar disorder overlap and can be classified as major psychosis (Cardno et al. 2002). Bipolar disorder and schizophrenia have strong aggregation in the family. Quantitative genetic analysis showed that both had strong genetic components. However, although the heritability of schizophrenia and bipolar disorder is estimated to be 70%, the disease consistency of monozygotic twins with the same DNA sequence is not 100% (Cardno et al. 2000). This means that non-genetic and environmental factors are also important in the etiology of the diseases.

Epigenetics is a rapidly developing field that includes regulatory mechanisms of gene expression that do not involve genotype change. Epigenetic mechanism mainly realizes heritable changes in gene expression during mitosis through DNA methylation and chromatin structure changes, but does not change genomic DNA sequence. And the study of epigenetics is increasingly relevant to neuroscience. Epigenetic mechanisms involve brain development and neuronal differentiation. Epigenetic regulation involves multiple levels of gene expression, with direct modifications from DNA and histone tails that regulate transcription levels to interactions with messenger RNA that regulate translation levels (Roy et al. 2015). It is generally believed that epigenetic dysfunction of human brain can be related to a series

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of mental diseases, including psychosis. In recent years, researchers have studied the brains of psychiatric patients and healthy controls, and found that there are significant epigenetic changes in the genome related to schizophrenia and bipolar disorder (Mill et al. 2008). But how the epigenome plays a role in schizophrenia and bipolar disorder is not well understood. The role of DNA methylation in epigenetics in schizophrenia and bipolar disorder is unclear.

Monozygotic twins carrying the same disease mutation can be clinically quite different, and investigating inconsistent monozygotic twins pairs is a useful method for discovering disease-related epigenetic mechanisms, as It can detect the epigenome independently of potential variation of genome sequence (Bell et al. 20011. A study found substantial differences in DNA methylation variation between monozygotic twins, suggesting that epigenetic variation can lead to phenotypic inconsistencies between humans with the same gene (Kaminsky et al. 2009).

Because the role of DNA methylation in schizophrenia and bipolar disorder is unclear, it is not known exactly which DNA sites are involved. The new study proposes genome-wide analysis to identify the genetic loci most affected by the two diseases.

This paper investigates the role of epigenetics in the pathogenesis of schizophrenia and bipolar disorder. Genome-wide analyses of monozygotic twins have identified specific genetic loci at which DNA methylation may be responsible for schizophrenia and bipolar disorder. Genome-wide analysis of DNA methylation variations in identical twins caused by schizophrenia and bipolar disorder, with genetically damaged DNA extracted from a unique twin. Many DNA methylation differences associated with disease were found, many of which were located near genes previously associated with psychosis. The results agree with the hypothesis that epigenetic changes can influence the causes of schizophrenia and bipolar disorder (Emma et al. 2011).

Table 1: Group of monozygotic twin pairs utilised in the study, values shown are average plus standard deviation.

	Schizophrenia-discordant twin pairs	Bipolar Disorder-discordant twin pairs	Psychosis-discordant twin pairs
Sex (males:females)	8:3	2:9	10:12
Ethnicity	10 Caucasian, 1 unknown	10 Caucasian, 1 Afro-Caribbean	20 Caucasian, 1 unknown, 1 Afro-Caribbean
Time discordant (years)	10.4 ± 10.6	14.6 ± 10.7	12.6 ± 10.6
Age of onset (years)	20.0 ± 4.6	21.7 ± 12.3	20.9 ± 9.3

2 METHODOLOGY

Since monozygotic twins share the same genetic sequence, studying epigenetic changes in diseases in inconsistent identical twins is a powerful approach because it allows independent epigenetic assessment of any potential genome sequence variation.

Inconsistent DNA methylation in schizophrenia or bipolar disorder was measured in 22 pairs of twins (44 individuals) using the Illumina Infinium HumanMethylation27 BeadChip. Standard protocols is used to extract genomic DNA from whole blood of 22 pairs of inconsistent monozygotic twins recorded in the Maudsley Bipolar disorder and Schizophrenia Twin Study. In the experiment, the twins were clinically diagnosed by at least two psychiatrists and two psychologists to ensure mental inconsistencies. On average, the twins had been ill for 12.6 (+ 10.6) years when they were taken blood (Table 1). The EZ 96-DNA methylation Kit (Zymo Research, CA, USA) is used to replicate 500 nm of genomic DNA per person with sodium bisulfite. In order to study genome-wide DNA methylation, Illumina Infinium human methylation 27 beadchip was mainly used. The chip investigated 27578 CpG sites related to about 14000 genes. Also, Illumina GenomeStudio software play a important role in extracting the signal strength of each probe and perform an quality control inspection when all data sets are available. The probes with p value of 0.05 (n = 733) detected in all samples were removed, and the probes with poor quality were strictly controlled.

This experiment mainly uses microarray data analysis. In the experimental analysis procedures, all calculations and statistical analyses are performed in the R statistical analysis environment. The ratio of the normalized signals of methylated probes to the sum of the normalized signals of methylated and unmethylated probes calculates the relative

methylation levels of each detected CpG site. This gives an mean β value for each CpG loci, which is from 0 to 1, where 0 means unmethylated and 1 means fully methylated.

In genome-wide correlation analysis, variable probes are recorded by calculating the standard deviation of the entire data set, and then those probes whose standard deviation is less than the estimated standard deviation are filtered out. Two independent ranking tests were analyzed. The first is the standard paired T-test, which looks at the meaning of differences in DNA methylation between the members of each twin pair. The another test was used to measure the size of the methylation difference, with a calculated Δβ-value describing the average difference in methylation between the members of each twin pair (Δβ refers to the unaffected minus the affected twin). The results of the two tests were sorted by P value and size. Next, the two ranking lists were added together to produce the final CpG site ranking list. The table shows the CpG sites with the greatest differences in DNA methylation and the most consistent in all twins. For statistical analysis of the magnitude of change observed at each locus for affected and unaffected twins, the custom weighted T-test was used.

3 RESULTS

DNA methylation at a single CpG site illustrated important difference in monozygotic twins. Analytical methods was used to determine the biggest differences in DNA methylation at specific CpG sites. Many sites in the whole genome is identified which showed differences in disease-related DNA methylation (Figures 1 & 2).

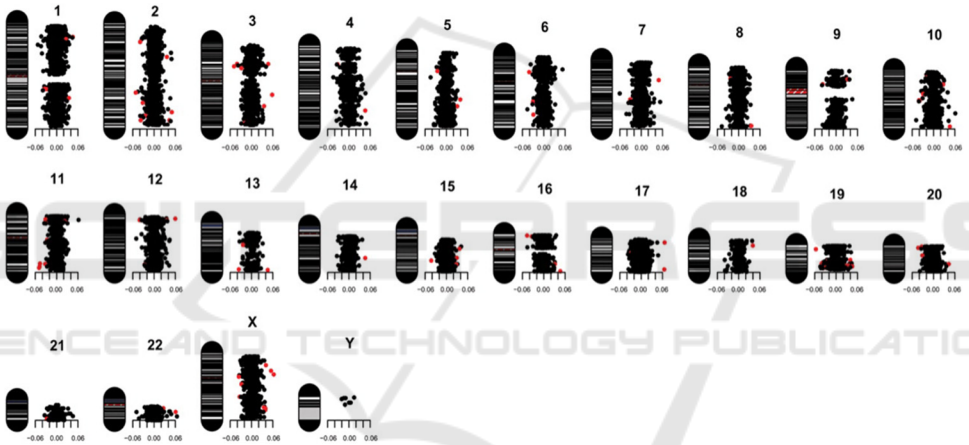

Figure 1: Characteristic map of each CpG locus in all 44 individuals of psychotic discordant twins.

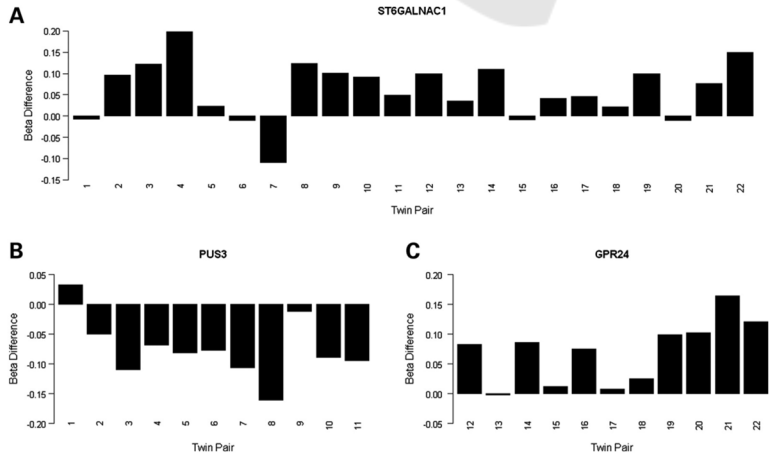

Figure 2: DNA methylation differences (Δβ-value) for the top-ranked probes from (A) the combined psychosis-discordant analysis group: ST6GALNAC1 (cg13015534), (B) Schizophrenia-discordant analysis group: PUS3 (cg02659232) and (C) the bipolar disorder-discordant analysis group: GPR24 (cg21342728).

Table 2 shows the genomes closest to the differentially methylated CpG sites associated with the eight top diseases in the three experimental groups (Schizophrenia, bipolar disorder and combined psychosis). By analyzing the location of 100 CpG sites with the highest methylation difference associated with psychosis, the representation of CpG sites in CpG island was significantly insufficient.

Table 2: The first eight of the three test groups had differential methylation CpG sites.

Analysis group	Rank	Gene name	Chromosome	Paired t-test P-value	Mean Δβ (minimum–maximum)	Weighted Δβ P-value	Weighted q-value
Psychosis	1	ST6GALNAC1	17q25.1	4.03E − 04	0.06 (−0.11–0.20)	1.19E − 07	7.97E − 04
	2	ACADL	2q34	2.49E − 04	0.05 (−0.05–0.17)	1.59E − 06	3.19E − 03
	3	TBC1D10A	22q12.2	8.56E − 04	0.06 (−0.05–0.23)	9.40E − 08	7.97E − 04
	4	PUS3	11q24.2	7.66E − 04	−0.05 (−0.16–0.06)	1.71E − 06	3.19E − 03
	5	FXR2	17p13.1	1.74E − 03	0.06 (−0.09–0.26)	9.03E − 08	7.97E − 04
	6	TSP50	3p21.31	4.92E − 04	0.04 (−0.05–0.12)	2.26E − 05	1.12E − 02
	7	PCOLN3	16q24.3	1.27E − 03	0.04 (−0.06–0.21)	1.79E − 05	1.03E − 02
	8	SOX1	13q34	1.04E − 03	0.04 (−0.04–0.13)	2.64E − 05	1.18E − 02
Schizophrenia	1	PUS3	11q24.2	7.66E − 04	−0.07 (−0.16–0.03)	5.16E − 05	0.10
	2	SYNGR2	17q25.3	8.29E − 04	0.07 (0.01–0.13)	9.82E − 05	0.14
	3	KDELR1	19q13.3	1.25E − 03	−0.06 (−0.14–0.01)	3.07E − 04	0.18
	4	PDK3	Xp22.11	7.54E − 04	0.06 (0.00–0.14)	3.67E − 04	0.18
	5	PPARGC1A	4p15.1	1.85E − 03	0.06 (−0.02–0.12)	2.89E − 04	0.18
	6	ACADL	2q34	3.74E − 03	0.07 (0.00–0.17)	7.81E − 05	0.12
	7	FLJ90650	5q23.1	4.19E − 04	0.05 (−0.01–0.09)	6.98E − 04	0.19
	8	TUBB6	18p11.21	3.54E − 03	0.06 (−0.01–0.18)	1.78E − 04	0.16
Bipolar disorder	1	GPR24	22q13.2	1.30E − 03	0.07 (0.00–0.16)	7.59E − 05	0.17
	2	TLE6	19p13.3	1.97E − 03	−0.09 (−0.21–0.01)	1.54E − 05	0.12
	3	STAB1	3p21.1	1.63E − 03	−0.07 (−0.18–0.02)	8.11E − 05	0.17
	4	PPYR1	10q11.2	5.13E − 04	−0.06 (−0.12–0.01)	3.44E − 04	0.25
	5	CTNNA2	2p12	3.59E − 03	0.09 (0.00–0.21)	1.56E − 05	0.12
	6	ST6GALNAC1	17q25.1	3.06E − 03	0.06 (−0.01–0.15)	2.82E − 04	0.23
	7	C1orf35	1q42.13	5.30E − 03	0.06 (−0.01–0.18)	1.88E − 04	0.23
	8	IQCH	15q23	3.94E − 03	0.05 (−0.06–0.10)	7.81E − 04	0.30

In all 22 pairs of uncoordinated monozygotic twins, the methylation difference in the promoter site of the gene sequence ST6GALNAC1 was the highest, and the methylation degree of the affected individuals was lower than that of the unaffected monozygotic twins. The highest differentially methylated CpG locus in discordant schizophrenic twins is located upstream of the gene encoding PUS3, which is highly methylated in affected twins. The higher ranked CpG locus in inconsistent comorbid psychiatric pairs is located upwards of the gene site GPR24, which is methylated in diseased twins.

Although the influence of the top loci in each diagnostic group was in the same direction, the significance of methylation difference between the diseased and non-diseased twins at some loci was different, suggesting familial heterogeneity even at the top loci. The first psychotic differential methylation site in the ST6GALNAC1 promoter showed binomial Δβ values as high as 0.20 (Table 2). Psychosis has significant clinical heterogeneity, so it is generally accepted that there are some rare etiologies that are highly influential in some cases. Individual twins may also vary greatly in methylation at sites associated with particular diseases (Merikangas et al. 2009). Therefore, DNA methylation differences in individual twins to find the greatest specific changes involved In families. Many new and known psychiatric candidate genes show significant differences in DNA methylation between patients of one or more pairs of twins, and some sites can be calculated with Δβ values greater than 0.60 between diseased and non-diseased twins.

Some CpG loci ranked higher in schizophrenic discordant and bipolar disorder discordant twin analyses, but DNA methylation changes were reversed between diseases, suggesting that epigenetic mechanisms can be disease identified. In monozygotic twins with dissonant schizophrenia and

bipolar disorder, the CpG loci in the ZNF659 promoter was the highest of 100 sites and was hypomethylated in bipolar disorder twins and hypermethylated in schizophrenia twins (Figure 3). Among the 100 highest-ranked schizophrenia and bipolar disorder loci, several other studies have shown commonly large diversities in the alternative diagnostic group in the opposite direction.

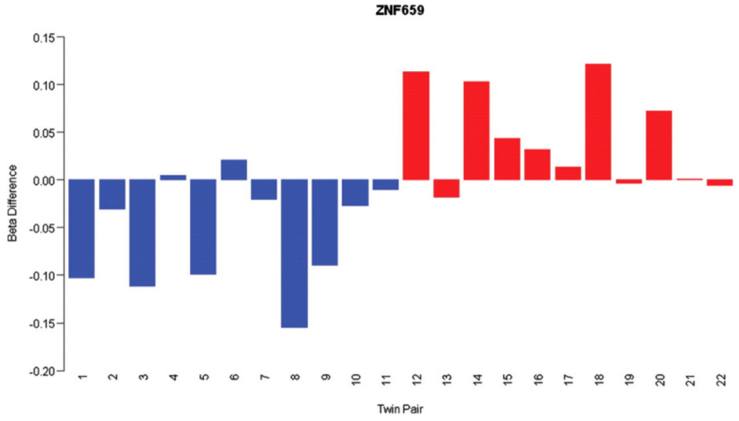

Figure 3: DNA methylation differences (Δβ-value) for a CpG loci located at ZNF659 (cg18267381), where Schizophrenia group is blue bars, and Bipolar disorder group is red bars.

Investigating that the validation and reproduction of disease-associated unmethylated at the ST6GALNAC1 gene loci. The Sequenom EpiTYPER data, which accurately repeated arrays of specific CpG sites on the Illumina platform, showed hypommethylation in diseased psychiatric twins (mean ¼ 35% methylation in psychotic twins and ¼ 41% methylation in unaffected twins). Notably, validation data for schizophrenic twins showed greater differences in DNA methylation (an average of 15% hypomethylation) between the diseased and undiseased twins than was spotted at this locus. The overall level of DNA methylation in the brain (85%) was higher than that in the blood (40%). Although no overall significant difference was found between psychiatric patients and the control group at the dominant site of DNA methylation array (CpG4 sequenom analysis), thirty (13.3%) psychiatric patients tested illustrated clear (27%) methylated and large difference at this CpG and many similar CpG loci (Fig. 4).

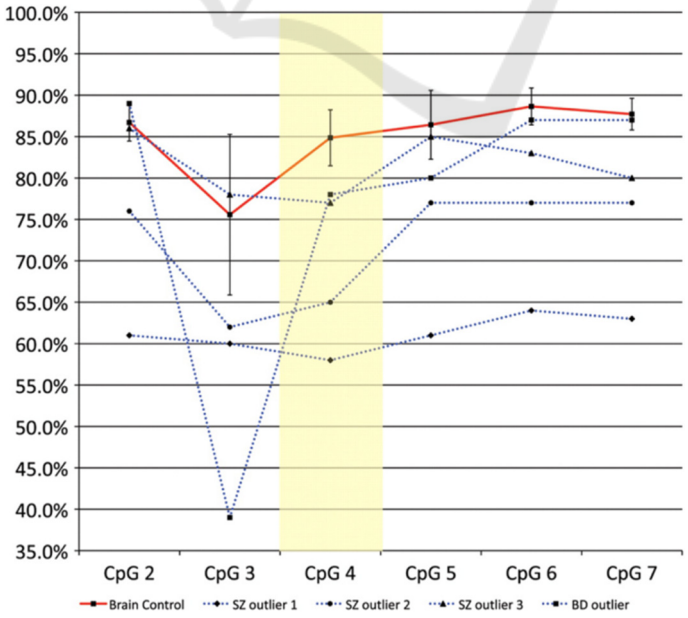

Figure 4: DNA methylation at several CpG sites in the ST6GALNAC1 promoter of psychotic patients was compared with postmortem brain tissue of controls.

This hypomethylated ST6GALNAC1 promoter was not found in any of the control groups in this study, indicating consistent and consistent methylation in the area. These brain data flesh back large heterogeneity in blood data between twins (Figure 1), with some affected siblings showing hypomethylation of up to 20%, but other twins showing smaller differences. These data suggest that major changes associated with disease in this site may affect some patients.

4 DISCUSSION

A genome-wide approach was used to comprehensively analyze disease-related DNA methylation differences in monozygotic twins with discorrelating schizophrenia and bipolar disorder. It is preliminarily found that no significant changes in total DNA methylation between the diseased and undisased twins, but there were important disease-related changes between the twins at identified sites on the genome. Some methylation differences persisted in the comorbidities, while other differences may be specific to schizophrenia or bipolar disorder. Although large differences were found in nearly all pairs of discordant twins in each diagnostic category, other differences related to specificity for schizophrenia or bipolar disorder were found for only one or a few pairs of twins. Although these sites in the experiment had not previously been linked to mental illness, evidence of DNA methylation differences in genes involved in mental illness could still be found. This illustrates the data of this study and provides more demonstrations to support the usage of DNA methylation in the etiology of schizophrenia and bipolar disorder.

The first total difference in methylation combined with mental illness found in the study was a CpG loci (17q25.1) in the ST6GALNAC1 promoter, and it is hypomethylation at this site in the ill twins. They also found that 0.13% of autopsy brain samples from patients with schizophrenia and bipolar disorder showed significant hypomethylation in extended regions containing the designated CpG locus, which could indicate that epigenetic mechanisms in this area can exist in a subset of patients with psychosis. CpG sites associated with mental illness do not exist in the same CpG island, and differential methylation sites are significantly deficient in classical CpG-rich promoters, suggesting that phenotypic related variations in DNA methylation generally occur outside these regions, which is consistent with data from another epigenomic analysis study (Weber et al. 2007).

However, the study did have some limitations. For example, it was limited to 22 monozygotic twins, calculations are based on standard deviation estimates of the entire dataset from Illumina's array data showing that at a strict Bonferroni-corrected assumed value cutoff. The 22 monozygotic twins in the study provided greater than 80% accuracy in obtaining $\Delta\beta=0.06$, although the ability to find small differences at this level of significance was limited.

5 CONCLUSIONS

The role of epigenetics in the etiology of schizophrenia and bipolar disorder has been demonstrated experimentally. Genome-wide analysis of monozygotic twins found that specific sites of DNA methylation also contribute to the cause of schizophrenia and bipolar disorder. In patients with schizophrenia, the largest differential methylation region associated with psychiatric illness was hypomethylation of the ST6GALNAC1 promoter. The average difference in DNA methylation at this site is 6%. However, there is significant heterogeneity between families, since some twin pairs having as much as 20% difference in methylation. These results suggest that DNA methylation differences play a role in phenotypic differences in identical twins and may influence the etiology of schizophrenia and bipolar disorder to some extent.

This study provides insight into the etiology of mental illness, particularly schizophrenia and bipolar disorder. However, it is not particularly clear how DNA methylation at specific sites affects patients with both diseases. Epigenetics with neuroscience is a hot research direction in recent years, and great progress has been made in the genetic etiology and treatment of mental diseases. In the future, human diseases can be better understood at the epigenome level by studying different epigenetic modifications of DNA.

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