Epigenetics as an explanation for phenotypic variation in monozygotic twins

MARINA POMERANTSEVA

INTRODUCTION

Researchers often use twins as natural samples to test hypotheses regarding the contribution of genetic factors to different phenotypes, especially diseases. The classical method is comparing traits in identical, or monozygotic (MZ) twins to those of dizygotic (DZ) twins. This method has had a significant impact on our current understanding of etiologic factors in many diseases which do not follow simple Mendelian law (i.e. complex diseases), including schizophrenia, bipolar disease, and major depression.

Twin Studies

Monozygotic twins originate from a single fertilized egg which separates and becomes two separate embryos. Dizygotic twins originate from two separate eggs fertilized by different sperm. Monozygotic twins are often described as physically and genetically identical. Most twin studies rely on the assumption that monozygotic twins share 100% of the same genes, whereas dizygotic twins share approximately 50% of the same genes. Accordingly, it is reasonable to assume that all monozygotic twin pairs should share the same heritable diseases, and half of dizygotic twin pairs should share such diseases. Much of the time, however, this is not the case.

The chief purpose of twin studies is to detect the genes responsible for a given disease. Over the past two decades, there have been a series of discoveries of genes that contribute to human diseases. Most of such disease genes, however, are those that cause simple Mendelian diseases like sickle cell anemia, hemophilia, cystic fibrosis and Duchene muscular dystrophy. On the contrary, the genes underlying complex diseases that exhibit a heritable component but do not follow Mendelian laws have for the most part remained unidentified. The origins of the heritable component in diabetes, schizophrenia, asthma, inflammatory bowel disease, and the overwhelming majority of cancers, for example, have not been found.

In twin studies of complex diseases, one of the difficulties is the rate of discordance (phenotypic dissimilarity) of monozygotic twins. Discordance of monozygotic twins reaches 30% for idiopathic epilepsy, 30-50% for diabetes, 50% for schizophrenia, 70% for multiple sclerosis and rheumatoid arthritis and 80% for breast cancer (Boomsung et al., 2002).

Environment as an Explanation of Discorance

This significant discordance in disease between MZ twins cannot be explained purely based on chromosomal DNA sequence (Chak Ravarti, and Little 2003). The difference in disease concordance in MZ twins is an example of a more general phenomenon, that of phenotypic differences between genetically identical organisms. These differences have usually been attributed to

Marina is a current student at Touro.

the effects of environment, as a cause for differences that remain after genetics are accounted for (Plomin and Daniels, 1987). Differences due to environmental factors, however, are difficult to measure. An example of a significant environmental effect on disease risk is the effect of smoking on lung cancer (Alberg and Samet, 2003), but direct evidence of other measured environmental effects on phenotype is rare. There is more and more evidence that the assumption that non-genetic differences have to be due to the environment is not so accurate after all.

There were a series of studies conducted on over 100 MZ pairs of twins which test the importance of environment as a cause for twin dissimilarity (Bouchard et al, 1990; Marcon et al., 2002). The data of MZ pairs raised together (MZT) was compared to the data of twins raised apart (MZA). The degree of dissimilarity between the MZT and the MZA was assumed to be the result of different environments (Bouchard et al, 1981). A series of tests were administered to each pair of MZA and MZT twins, and the correlations of their scores on each scale were calculated. Surprisingly, the correlations within MZT and MZA twin pairs on personality measurements were almost identical. Both intra-class correlations are less than 1, and the difference between them is not significant.

Other studies have yielded similar results. Svensson, et al (2003) studied the etiology of migraine headaches, gathering data from 80 pairs of twins. The authors found that susceptibility to migraine was mostly inherited and that twins separated earlier had even greater similarity in migraine headaches. Migraine reports were almost similar in MZA compared to the MZT group; rMZA=.58 and rMZT =.46.

The results of the studies cited above show that intra-class correlations factors rMZT and rMZA are always far less than 1. Therefore, there is always a substantial non-genetic component of heritability. At the same time, rMZT and rMZA are not significantly different. Thus, different environments cannot explain the fact that the rMZT is less than 1. Therefore, the remaining variation in phenotype is not due to environmental factors. In many other recent studies, researchers also came to the conclusion that the pure heritage-environment approach is not enough to explain the twins data described above (Wong, 2005; Haque, 2008).

Similar experiments were done with animal strains that were inbred for many generations which consequently have almost identical chromosomal DNA sequence. MZ and DZ twins can be generated by artificial fertilization and artificial twinning procedure (Keith and Machin, 1997). In these procedures, the environment can be strictly controlled, which is not possible with humans.

In a series of experiments designed to find the relative contributions of genes, environment and other factors to laboratory animal phenotype, Gartner (1990) was able to demonstrate that the majority of random non-genetic variability in mice was not due to the environment. Genetic causes of differences were reduced by using inbred animals, but reduction of genetic variation did not substantially reduce the amount of observed variation in phenotypes such as body weight or kidney size. Strict standardization of the environment within a laboratory did not have a major effect; only 20-30% of the variability could be attributed to environmental factors, with the remaining 70-80% of non-genetic variation due to some other factors.

The results of the experiments showed that despite the complete genetic similarity of all the mice and identical pre- and post-natal environments, the MZ twin pairs exhibited a greater degree of phenotypic similarity among co-twins than did the DZ twin pairs. A possible conclusion from these facts is that there exists some mechanism of heritability that is neither genetic nor environmental. This mechanism would be generated before the twinning stage and would influence the

zygote. Gartner and Baunack (1981) referred to this non-genetic influence as the "third component", after genes and environment.

Multiple species of animals have recently been cloned. Experiments using cloned animals also allow separating the effects of chromosomal DNA sequence from other factors that can influence phenotype. Although the offspring in these cloning experiments have the same genome as the donor animals, they exhibit a variety of phenotypic abnormalities that obviously cannot be attributed to genetic causes (Edwards et al, 2003). The differences found include diseases as well as other, more subtle, differences. The most famous of these cloning experiments was performed on sheep, but along with seemingly healthy lambs, many clone siblings died prenatally as a result of overgrowth, pulmonary hypertension and body wall defects (Rhind et al, 2003). Clones in other species also show considerable variation in lifespan and disease phenotypes in comparison to normal members of that species (Carter et al, 2002; Wellsatal, 2004).

This demonstrates that significant phenotypic variation, including fatal disease, can emerge from animals that have an identical, cloned genetic background. These early examples of cloned animals were subjected to intense scrutiny in highly supervised and controlled environments, yet they still exhibited disease in an inconsistent fashion. If environmental factors were the source of this phenotypic variation, then one would expect the same emergence of disease among non-cloned members of these species. Thus, in the case of cloned animals, it is also impossible to explain phenotypic differences by genetics and environment only.

Epigenetics and DNA methylation

Many researchers consider epigenetics as a not-accounted-for mechanism that explains heritability of complex non-Mendelian diseases. By definition, epigenetics refers to modification in gene expression that is controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure (Heinkoff and Matzker, 1997).

DNA methylation is a subject of extensive research because this process plays a unique role in organism development. DNA methylation involves the addition of a methyl group to the 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring This modification can be inherited through cell division. DNA methylation is typically removed during zygote formation and, in some cases, reestablished through cell division during development. DNA methylation is a crucial part of cellular differentiation. DNA methylation stably alters the gene expression pattern in cells in such a way that cells can "remember where they have been." In other words, cells programmed to be pancreatic islets during embryonic development remain pancreatic islets throughout the life of the organism without continuous signals telling them that they need to remain islets. In addition, DNA methylation suppresses the expression of viral genes and other deleterious elements that have been incorporated into the genome of the host over time. DNA methylation also forms the basis of chromatin structure, which enables cells to form the myriad characteristics necessary for multi-cellular life from a single, immutable sequence of DNA.

The addition of a methyl group to DNA usually takes place in the region of CpG islands. CpG islands are clusters of cytosine and guanine repeats, commonly found near a gene-coding DNA region. Methylation of CpG islands adjacent to a gene often leads to its inactivation (Cedar, 1988). As a result, the expression of a sequence of DNA can be modified by becoming "silenced" or "switched off".

The epigenetic approach may help explain the results of the studies discussed above. The fact that the differences found in MZT are similar differences in MZA, for a large number of traits,

suggests that in such twins stochastic events may be a more important cause of phenotypic differences than specific environmental effects. If the emphasis is shifted from environment to stochasticity, it may become clear why MZ twins reared apart are not more different from each other than MZ twins reared together. It is possible that MZ twins are different in some traits not because they are exposed to different environments but because those traits are determined by meta-stable epigenetic regulation on which environmental factors have only a modest impact. From this point of view, MZ twin discordance for complex, chronic, non-Mendelian disorders such as schizophrenia, multiple sclerosis or asthma could arise as a result of a chain of epigenetic events in one of the twins. During embryogenesis, childhood and adolescence, there is ample opportunity for tissue differentiation and stochastic factors to accumulate in only one of the two identical twins (Petronis, 2004; Petronis et al, 2003). In addition, some external environmental aspects, such as nutrition, medication, addictions, and other factors can influence certain phenotypes by changing epigenetic profiles (Jaenish and Bird, 2003).

The mechanism of methylation, "silencing" of genes, is not yet entirely clear. There are several approaches to explain the phenomenon. First, the methylation of the DNA itself may physically impede the binding of transcriptional protein to the gene by occupying the place near the gene, which is otherwise suitable for proteins to stay close to the gene. Second, the electric field of methyl may diminish electric forces nearby due to polarization and consequently diminish electrical interactions between a gene and its proteins. Many researchers, however, give more complex explanations for the molecular mechanism of "silencing". For such mechanisms, methylation of the cytosine residue in the DNA molecule, and acetylation and other modifications of histones have been described (Machin, 1996).

Epigenetic studies may help in the identification of the molecular effects of the environmental factors. There are many environmental events that result in epigenetic changes (Hursting et al, 2003; Ross, 2003; Waterland and Tirtle, 2003). The advantage of the epigenetic studies is that identification of molecular epigenetic effects of environmental factors might be easier and more efficient than direct epidemiological studies.

To evaluate the influence of a given environmental factor on the epigenetic status of one of a pair of MZ twins, researchers seek genes or regions of DNA that are methylated differently in cells of two twins, if one of them experienced an environmental influence. In most cases, researchers try to evaluate epigenetic status by applying one of two methods. In the first method, special enzymes are applied to the sample, representing the DNA. These enzymes are chemical compounds which react differently to methylated and non-methylated regions of DNA. In this case, the intensity of the chemical reaction between an enzyme and a region of DNA investigated is proportional to the number of methylated sites. To separate a genomic region of interest, special chemicals are applied to the genome which multiply the yield of the DNA region investigated to the total effect of the chemical reaction. The second method of investigating the epigenetic status of a given sample involves studying the emission of the sample which is characteristic to methylated parts of the DNA. The intensity of this emission under some standard conditions is proportional to the number of methylated sites (Petronis, 2002).

Kaminski, et al (2009) investigated DNA methylation profiles in 114 monozygotic and 80 dizygotic twins. The purpose of the research was to identify not only phenotypic, but also epigenetic differences in monozygotic and dizygotic twins. As epigenetic factors can contribute to phenotypic outcomes, a DNA methylation analysis of several different tissues was conducted. Epigenetic signals of 6,000 unique genomic regions in MZ twins were registered. The results of

the experiments showed that DZ co-twins exhibit higher epigenetic differences in cells than MZ co-twins. In general, this epigenetic difference may result from DNA sequence difference between co-twins. In the case discussed, however, the authors conducted special experiments to show that difference in DNA sequence cannot be greater than .05%, which is why the authors consider the observed difference to be a real epigenetic difference.

The results of the study suggest that in addition to identical DNA, epigenetic similarity may also contribute to phenotypic similarities in MZ co-twins. DZ co-twins are more different from each other than MZ co-twins not because they possess some DNA sequence differences, but because they originated from epigenomically different zygotes. This leads to the conclusion that epigenetic factors themselves may be inherited. This means that molecular mechanisms of heritability may not be limited to DNA sequence differences.

Epigenetic twin studies are providing insight into molecular causes of differential susceptibility to a disease in genetically identical organisms. If this approach is successful, the results may be generalized to singletons.

The results of many years of research in the field of twin studies give reason to think that some phenotypic changes may be inherited not only through direct genetics, but also through epigenetic changes. It is easier to change the epigenetic code through the environment than through DNA sequence, and it may be passed on to the next generation. From this point of view it is possible that some unhealthy habits, such as drug abuse or smoking, pose a danger to future generations.

CONCLUSION

The field of epigenetics as a new direction in twin studies is of great interest to me personally. As a monozygotic twin, my sister and I have always been wondering what the cause of the differences between us is. We also notice that the differences increase drastically as we get older. Epigenetics has provided an explanation to satisfy our curiosity.

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