Identical Twins, Identical Fates? An Introduction to Epigenetics

by
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Part I – Coming Home

Elise was excited as she boarded the bus. She had just finished her first year at college and was looking forward to her first night back home since winter break, which would include mom's spaghetti and meatballs and catching up with her family. She couldn't wait to curl up on the couch with her cat Ziggy snuggled next to her, and she was hopeful that her sister, Shannon, would be willing to join her there, since they had talked very little since Elise's last visit home.

As she settled in for the four-hour bus ride home, Elise pulled out her iPhone, put on some music, and started looking through old photographs. She came across a few of her and her sister taken at Christmas—the last time they had seen each other. Looking at Shannon was like looking in the mirror. After all, they were identical twins. Elise recalled all of the pranks that she and Shannon used to pull in school when they were kids. In 5th grade, they once made it all the way to lunchtime before their teachers realized that they had swapped classes and were impersonating one another!

Shannon and Elise used to have so much fun together, but things had changed. Elise was worried about her sister and the serious health troubles she had been having over the past year and a half. And she couldn't help but wonder to herself, "Are the same troubles heading my way?"

Questions

1. What exactly are twins, and how do they arise? Your response should distinguish between the two different types of twins.
2. Are identical twins completely identical? Why or why not?
3. What can studying twins tell us about the *genetic influence* on a particular trait?

Part II – The Diagnosis

Elise stared out the window of the bus at the rush-hour traffic that had befallen travelers on the other side of the highway. She recalled that night back in November when her mother called her at school to share the fateful news about her sister. "Shannon has been diagnosed with schizophrenia," was what she had said. The words had dropped into the pit of Elise's stomach.

She had known that something was going wrong with her sister. The summer before she left for college, Elise noticed changes in Shannon's behavior. Despite being an avid swimmer and lifeguard, Shannon quit her highly coveted swim camp instructor position just two weeks into the summer. She seemed withdrawn and unmotivated, and had also unexpectedly decided not to attend college in the fall, despite Elise's and her parent's efforts to convince her otherwise. But Elise did not get to see the worst of Shannon's behavior, when she began having hallucinations and couldn't seem to carry on a coherent conversation with her parents.

Elise had done some research about schizophrenia after hearing of her sister's diagnosis. She did not like what she found out. Apparently, schizophrenia has a tendency to run in families. In fact, studies indicate that a sibling of a schizophrenic has a 10-fold higher risk of developing schizophrenia over the general population. Elise began to worry about her own mental health. She decided she would do some further investigation into the disease once she got home for summer break.

Questions

You are encouraged to consult reliable sources (such as your textbook and other online and print resources) to answer some of these questions. The review articles listed below address Question #3.

1. What causes genetic variation? For example, what causes some people to have curly hair and others to not? What causes some people to have a genetic disease such as cystic fibrosis and others to not?
2. What does it mean when a trait or a disease "runs in families"?
3. What could be some possible *genetic* and *non-genetic* causes of Shannon's schizophrenia?

Review Articles

Gejman, P.V., Sanders, A.R., and Kendler, K.S. (2011). Genetics of schizophrenia: new findings and challenges. *The Annual Review of Genomics and Human Genetics* 12: 121–44.

Roth, T.L., Lubin, F.D., Sodhi, M., and Kleinman, J.E. (2009). Epigenetic mechanisms in schizophrenia. *Biochim Biophys Acta* 1790(9): 869–877.

Rutten, B.P. and Mill, J. (2009). Epigenetic mediation of environmental influences in major psychotic disorders. *Schizophrenia Bulletin* 35(6): 1045–1056.

Part III – Just How "Identical" Are We?

Elise had been home from college for a week, and she was still preoccupied with Shannon's diagnosis and her own potential risk for mental illness. Elise expressed her anxiety and concerns to her mother one night after dinner. "Elise," her mother said, "your concerns are perfectly valid, and you have every reason to want to get more information. Why don't we make you an appointment to consult with a psychiatrist?" Elise decided to make the appointment the next day.

* * * * *

Elise left Dr. O'Brien's office feeling that some of the weight had been lifted from her shoulders. On the car ride home, she thought about the things that Dr. O'Brien had said to her during their consultation.

"It was good of you to come in to see me, Elise. You are absolutely right to have concerns for yourself when your identical twin has been diagnosed with schizophrenia. Research shows that schizophrenia is almost 50% heritable, and since you share nearly identical DNA with your sister, that puts you at a higher risk for developing this disease as well."

"Fifty percent may sound like a scary number, but remember that schizophrenia is a very complex disease, and 50% of what causes schizophrenia is due to things *other* than your DNA."

"Well, like what? What else could be contributing to Shannon's schizophrenia that wouldn't necessarily affect me?" Elise asked.

Dr. O'Brien replied, "There are many, many environmental influences that seem to play a role in the development of this disease, such as increased stresses and anxiety, or difficult relationships with other people. Interestingly, there is some groundbreaking research that is going on that suggests that the *environment* itself might even play a role at influencing one's DNA at the *molecular level*. This concept is called *epigenetics*. An example of epigenetics in nature is the calico cat. Each calico cat has a unique orange and black fur color pattern because of alterations, called epigenetic changes, which occur within the cells that produce coat color during the cat's development. Research in the field of epigenetics suggests that individuals with schizophrenia appear to have some of these epigenetic changes to their DNA that are due to environmental influences, and that these alterations could be contributing to their development of mental illness."

"But wouldn't I also have these 'epigenetic alterations' in my DNA?" Elise asked.

"Not necessarily, because you and Shannon have not experienced completely identical environments throughout your lives. For example, you and Shannon have had different teachers and jobs throughout high school. And I also understand that you spent many childhood summers with a friend and her family out in the Grand Canyon, while your sister was off at swim camps. If you are interested, I can give you some literature to read about this subject."

Elise was definitely interested. She took the articles and headed home.

Questions

1. Briefly describe what you know about the structure of DNA and how DNA is packaged in a cell.
2. At the *molecular level,* speculate on some ways that the environment might have an influence on DNA and its packaging.

Part IV – What Really is "Epigenetics"?

Despite being three weeks into her summer break, Elise felt like she was back in school. The more she read about the topic of epigenetics, the more fascinated she became, and she found herself spending most of her days on the Internet doing research. Elise had learned about genetics in her general biology class and thought she had a pretty good idea of how the Laws of Mendel worked, but this whole field of epigenetics seemed to take the idea of inheritance to another level. She was particularly fascinated by an article that Dr. O'Brien had given her regarding epigenetic differences between identical twins. The article suggested that during one's lifetime epigenetic changes occur to one's DNA that can affect gene expression, and therefore whether or not one will express a certain trait. These epigenetic changes are influenced by one's environment and behaviors, so despite having identical DNA, identical twins will not always have the same epigenetic changes, and therefore, will not always express the same traits.

In this article, researchers examined a particular type of epigenetic modification called DNA *methylation,* whereby a cytosine base becomes methylated through the action of an enzyme called a DNA *methyltransferase*. The reaction is shown below in Figure 1.

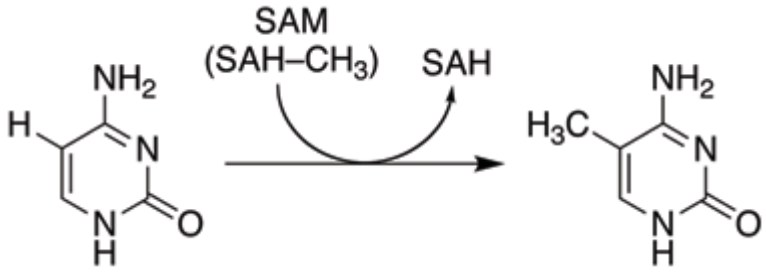

Figure 1. Methylation of cytosine to form 5-methylcytosine. SAM (S-adenosyl methionine) serves as the source of the methyl group, giving SAH (S-adenosyl-L-homocysteine) as a by-product.

The researchers examined genome-wide methylation patterns in several twin pairs of various ages. Representative data from their analysis is shown below in Figures 2 and 3:

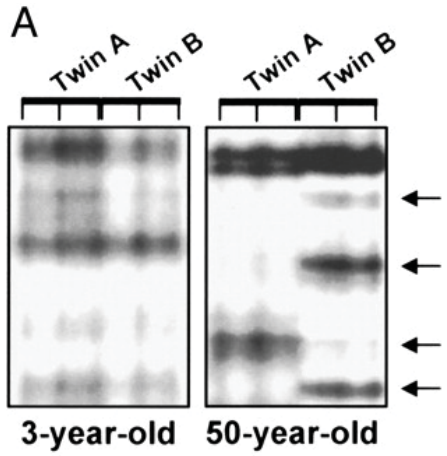

Figure 2. Differential DNA methylation between two sets of monozygotic twins, one set at age 3 (left), one set at age 50 (right) using AIMS (amplification of inter-methylated sites). Different bands, corresponding to sibling-specific changes of DNA methylation, are indicated with arrows. (Panel A of Figure 2 in Fraga et al., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *PNAS* 102:10604–10609. Copyright 2005 National Academy of Sciences, USA.)

Elise read this article several times, and began to feel a little bit better about her "genetic future." According to this article, it seemed that as time went on she and Shannon would become more and more epigenetically dissimilar, even though they did carry the same genes. Perhaps she would not share the same fate as her sister after all.

Questions

You are encouraged to consult reliable sources (such as your textbook and other online and print resources) to answer some of these questions. The article by Singh et al. (2003) referenced below is a useful resource for Question #2.

1. Examine the data shown in Figures 2 and 3. Carefully compare the DNA methylation profiles from the 3-year-old twins versus the 50-year-old twins and summarize your observations. Which set of twins (3-year-old or 50-year-old) have the most similar DNA methylation profiles? Provide a brief explanation of your observations.
2. What types of environmental factors can influence DNA methylation?
3. Aside from DNA methylation, what other types of epigenetic modifications can occur within the genome to influence gene expression?
4. Do you think Elise needs to be worried about her own mental health? Why or why not? If you were a health-care professional, what would you advise Elise to do?

Reference

Singh, S.M., Murphy, B., and O'Reilly, R.L. (2003). Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. *Clinical Genetics* 64(6): 451–460.

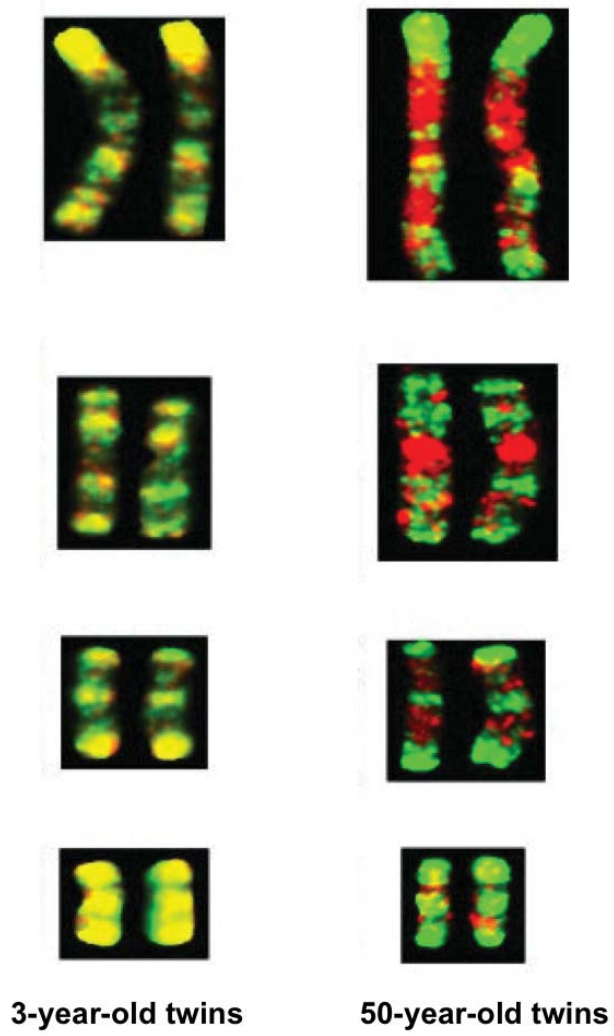

Figure 3. Mapping of chromosomal regions with differential DNA methylation in monozygotic twins using comparative genomic hybridization for methylated DNA. Four representative chromosomes pairs are shown. Methylated DNA from one twin was labeled with a red fluorescent dye, while methylated DNA from the other twin in the pair was labeled with a green dye. Both sets of twin DNA were hybridized to normal metaphase chromosomes. The yellow color represents equal amounts of red and green dye hybridizing to the chromosomes, indicative of similar levels of DNA methylation at those particular chromosomal locations. (Figure 3 of Fraga et al., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *PNAS* 102:10604–10609. Copyright 2005 National Academy of Sciences, USA.)

Part V – What Does the Research Say?

Dr. O'Brien took off her reading glasses and rubbed her temples. It had been a long day of office visits, rounds at the hospital, and reviewing the latest literature on schizophrenia at her desk. Her recent office visit with Elise, whose identical twin sister had been diagnosed with schizophrenia, had prompted her to revisit some studies that had been conducted showing a link between epigenetic modifications and schizophrenia.

In particular, certain studies demonstrated that the DNA methylation patterns of select genes were abnormal in the brains of patients with schizophrenia as opposed to non-psychotic control subjects. The abnormal methylation patterns led to the abnormal expression of these genes. One such gene encodes REELIN, a glycoprotein secreted by GABAergic interneurons, which activates signaling pathways important for many neurological processes responsible for brain development and adult brain functioning. Figure 4 shows a schematic diagram of the REELIN signaling pathway.

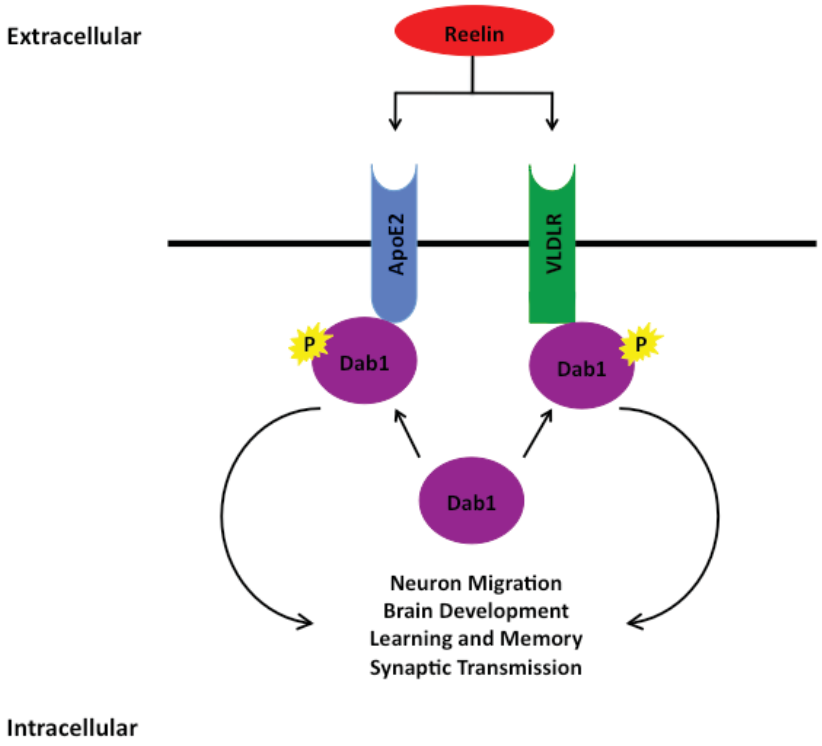

Figure 4. Schematic diagram of REELIN signaling. Reelin is a secreted glycoprotein capable of binding to several receptors, including apolipoprotein E receptor 2 (ApoE2) and very low density lipoprotein receptor (VLDLR). Binding of reelin to these receptors leads to phosphorylation of the intracellular adaptor protein disabled-1 (Dab-1), which is then capable of activating many downstream signaling pathways important in neurological function.

It seemed clear to Dr. O'Brien that epigenetics played an important role in the clinical course of schizophrenia. But what epigenetic modifications were the most critical? And, more importantly, could these epigenetic changes somehow be reversed pharmalogically as a form of therapy for patients with this disease?

Dr. O'Brien put on her reading glasses again. She decided to review the REELIN papers one more time before finally heading home.

Figures 5–7 represent some of the data Dr. O'Brien reviewed that evening at her desk:

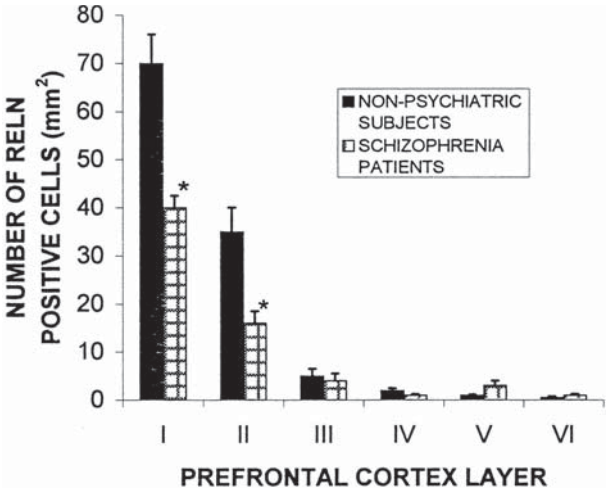

Figure 5. Mean number of REELIN (RELN) positive neurons detected by immunohistochemistry in prefrontal cortex layers I-VI in non-psychiatric control subjects and patients with schizophrenia. (Figure 3 of Impagnatiello et al., 1998. A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *PNAS* 95:15718–15723. Copyright 1998 National Academy of Sciences., USA.)

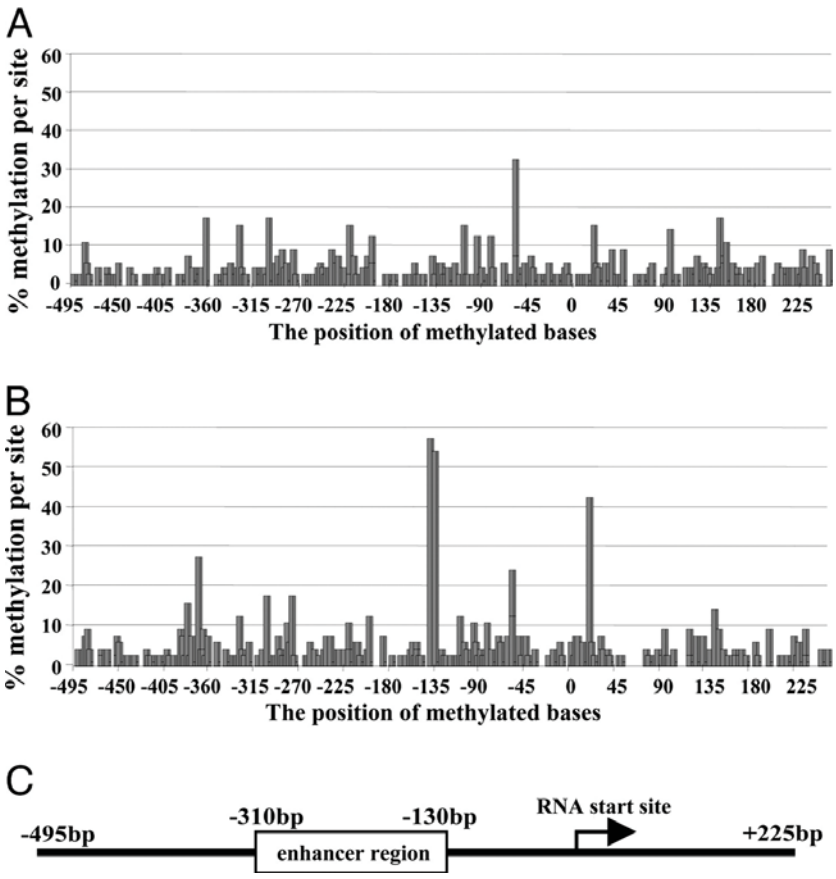

Figure 6. Levels of methylation of the REELIN promoter in sections of the prefrontal cortex taken post-mortem from non-psychotic control brains (panel A) or schizophrenic brains (panel B). Levels of 5-methylcytosine are mapped against specific positions along the REELIN promoter, as shown in panel C. (Figure 1 of Grayson, et al., 2005. Reelin promoter hypermethylation in schizophrenia. *PNAS* 102: 9341–9346. Copyright 2005 National Academy of Sciences, USA.)

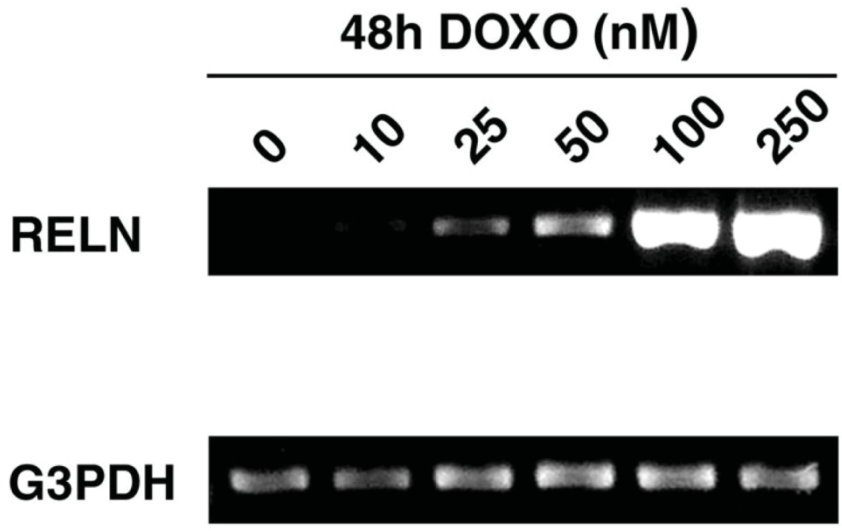

Figure 7. Measurements of REELIN (RELN) expression by RT-PCR analysis. NT-2 neuronal precursor cells were grown for 48 hours in the presence of varying concentrations (0-250nM) of the DNA methyltransferase inhibitor Doxorubicin (DOXO). G3PDH expression serves as a control. (Portion of Figure 1A from Marija Kundakovic, Ying Chen, Erminio Costa, and Dennis R. Grayson, DNA Methyltransferase Inhibitors Coordinately Induce Expression of the Human Reelin and Glutamic Acid Decarboxylase 67 Genes, *Molecular Pharmacology* March 2007 71: 644–653. Used with permission.)

Questions

You are encouraged to consult reliable sources (such as your textbook and other online and print resources) to answer some of these questions. The NCBI's Online Mendelian Inheritance in Man (OMIM) is a useful resource for examining the role of reelin in the brain.

1. What is the role of reelin in the brain? According to Figure 5, how does the expression of reelin in the prefrontal cortex of schizophrenic patients differ from reelin expression in non-psychotic subjects?
2. Study the promoter methylation data shown in Figure 6. How does the overall level of methylation of the reelin promoter in schizophrenic brains compare to the methylation of the reelin promoter in non-psychotic control brains? What would be the most probable effect of this methylation pattern on the expression of reelin in patients with schizophrenia?
3. What is the enzyme responsible for methylating DNA? How does a drug like doxorubicin affect DNA methylation?
4. In reference to Figure 7, what is the effect of doxorubicin treatment on the expression of reelin in NT-2 cells? How does increasing amounts of doxorubicin affect reelin expression in these cells?
5. Based on these data, might a drug like doxorubicin be a potential treatment for schizophrenia? Why or why not? What additional experiments should be performed before a drug like doxorubicin goes into clinical trials?

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Roth, T.L., Lubin, F.D., Sodhi, M., and Kleinman, J.E. (2009). Epigenetic mechanisms in schizophrenia. *Biochim Biophys Acta* 1790(9): 869–877.

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