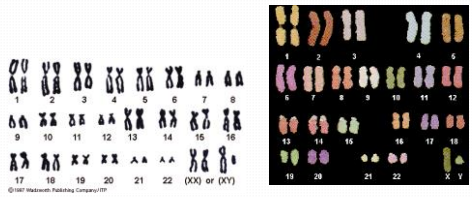
Lecture 2

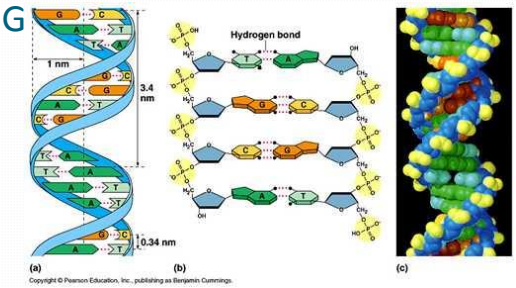
Related to Neurobiology of Reward

WHAT ARE GENES?



GENES ARE LINED UP IN LINEAR ORDER ALONG OUR 23 CHROMOSOMES

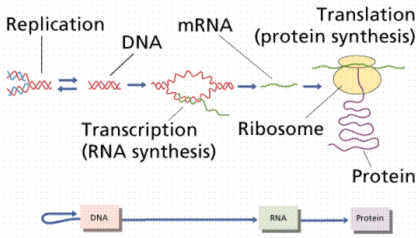
G



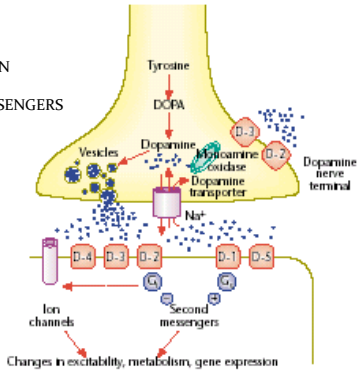
4 LETTERS IN THE DNA ALPHABET: **A G C T** & 3 LETTERS FORM A DNA WORD

DNA LANGUAGE

- A single strand of DNA is made of letters:
  ATGCTCGAATAAATGTGAATTTGA
- The letters make words:
  ATG CTC GAA TAA ATG TGA ATT TGA
- The words make sentences:
  <ATG CTC GAA TAA> <ATG TGA ATT TGA>
- These "sentences" are called genes. Genes tell the cell to make other molecules called proteins.
- Protein are required for the structure, function, and regulation of the body's cells, tissues, and organs.

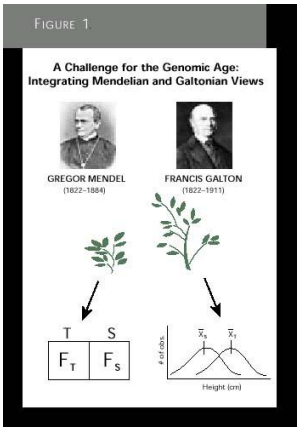


SYNTHESIS
RELASE
INACTIVATION
RECEPTORS
SECOND MESSENGERS



Quantitative genetics

- It is effectively an extension of simple Mendelian inheritance in that the combined effects of one or more genes and the environments in which they are expressed give rise to continuous distributions of phenotypic values.

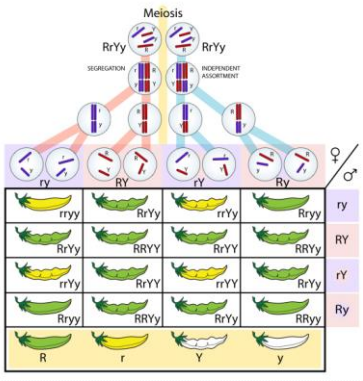


Non-mendelian traits

How will we balance the Mendelian notion of discrete heritable characteristics with the Galtonian idea of continuous trait variation?

Mendel

- Mendel noticed that certain characterisitics of pea plants could be readily divided into distinct categories (for example, short versus tall plants as shown in the diagram).
- His categorical approach allowed analysis by counting the members of each category, and, as illustrated, calculating their frequencies. Comparisons of categorical trait frequencies in carefully conducted breeding experiments led him to formulate the notion of the gene as the fundamental particulate unit of inheritance.

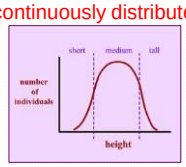


Galton

- Galton, a contemporary of Mendel's, applied emerging Darwinian concepts of evolution to what we would now call complex traits, such as "human talent."
- He pointed out that many heritable characteristics varied continuously (as illustrated by the distributions of pea plant height, although Galton did not study peas), and articulated the idea that such traits reflected the interplay between "nature and nurture."

Quantitative Traits

- But many behavioral traits are not like these clear-cut, have-it-or-don't-have-it phenotypes. People vary from being quite shy to very outgoing. But is shyness a discrete trait or merely a descriptive adjective for one end of a continuous distribution?
- In this chapter, we will discuss the genetics of quantitative, continuously distributed phenotypes.

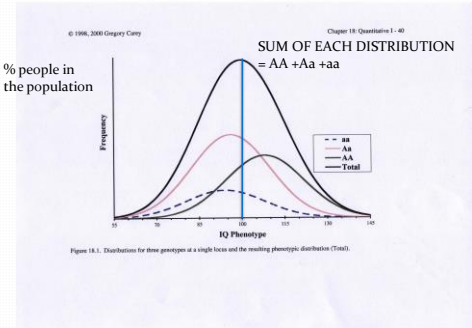


IQ

- Let us begin the development of a quantitative model by considering a single gene with two alleles, *a* and *A* .
- Define the *genotypic value* (aka *genetic value*) for a genotype as *the average phenotypic value for all individuals with that genotype*.

Genotypic value

- For example, suppose that the phenotype was IQ, and we measured IQ on a very large number of individuals.
- Suppose that we also genotyped these individuals for the locus "A".
- The genotypic value for genotype *aa* would be the **average** IQ **of all individuals who had genotype *aa*.**
- Hence, the means for genotypes *aa*, *Aa*, and *AA* would be different from one another, but there would still be variation around each genotype.



Variation around the three genotypes

- The first point to notice about the figure is the variation in IQ around each of the three genotypes, *aa* , *Aa* , and *AA* .
- Not everyone with genotype *aa*, for instance, has the same IQ.
- The reasons for this variation within each genotype are unknown.
- It would include environmental variation as well as the effects of loci other than the one genotyped or even *noise* in measuring IQ.

Means of genotypes differ

- A second important feature about the figure is that the means of the distributions for the three genotypes differ.
- The mean IQ (i.e.,the genotypic value) for *aa* is 94, that for *Aa* is 96, and the mean for *AA* is 108.
- This implies that the locus has some influence on individual differences in IQ.

Dominance

- A third feature of note in the figure is that the genotypic value of heterozygote is not equal to the average of the genotypic values of the two homozygotes.
- The average value of genotypes *aa* and *AA* is (94 +108)/2 =101, but the actual genotypic value of *Aa* is 96.
- This indicates a certain degree of *dominant gene action* for allele *a*.
- Allele *a* is not completely dominant; otherwise the genotype value for *Aa* would equal that of *aa*. Hence, the degree of dominance is incomplete.

Allele frequencies differ

- A fourth feature of importance is that the curves for the three genotypes do not achieve the same height.
- This is due to the fact that the three genotypes have different frequencies in the population.
- In the calculations used to generate the figure, it was assumed that the allele frequency for *a* was 0.4 and the frequency for *A* was 0.6, giving the genotypic frequencies as 0.16 (*aa*), 0.48 (*Aa*),and 0.36 (*AA*).
- Consequently,the curve for *Aa* has the highest peak,the one for *AA* has the second highest peak, and that for *aa* has the smallest peak- representing the number of individuals in the population carrying these alleles.

- A final feature of note is that the phenotypic distribution of IQ in the general population (the solid line in the figure) looks very much like a normal distribution.
- The phenotypic distribution is simply the sum of the distributions for the three genotypes. For example, the height of the curve labeled "Total " when IQ equals 100 is the distance from the horizontal axis at 100 to the curve for genotype *aa* plus the distance from the horizontal axis at 100 to the curve for genotype *Aa* plus the distance from the horizontal axis at 100 to the curve for genotype *AA* .
- Often social scientists mistakenly conclude that the phenotypic distribution must be trimodal because it is the sum of three different distributions.

Characteristics of QTLs- quantitative trait locus

- How many genes pairs may be involved in multiple genes?

Two or more pairs

- What does each gene of a pair contribute?

A fixed amount toward the phenotype

- What type of distribution is represented by a population with multiple gene inheritance?

Representing a normal distribution or normal curve

What will be the hypothetically potential intelligence (IQ) of the offspring of these parents?

A = 75 IQ, a = 25, B = 10, b = 5; C = 2.5, c = 1

P1 AA Bb cc X aa Bb CC

	½ ABc	½ Abc
½ aBC	AaBBCc	AaBbCc
½ abC	AaBbCc	AabbCc

1 (113.5 IQ), 2 (118.5 IQ), 1 (123.5 IQ),

Important concepts in quantitative behavioral genetics

- Heritability is the proportion of variance that is explained by genes
- Genetic correlation is the proportion of variance that two traits share due to genetic causes. The genetic correlation, then, tells us how much of the genetic influence on two traits is common to both: if it is above zero, this suggests that the two traits are influenced by common genes.
- Gene-environment interaction G X E
- Gene-environment correlation rGE

Quantification

- Although these are described at a conceptual level, it is important to recognize that behavioral geneticists try to quantify each of them —i.e., arrive at an actual number to estimate these quantities and then judge how important this quantity is for a behavioral phenotype .

Rutter, M., Moffitt, T. E., & Caspi, A. (2006). Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry, 47(3-4), 226-261.*

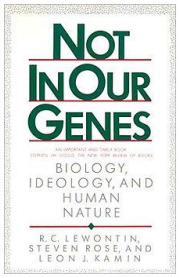
- Historical shifts in attitude about the relative role of genes versus environment in causation of mental disorders
- Beginning of 20th century eugenics movement and the Nazi/Fascist misuse of genetics
- Replaced by Mental Hygiene movement emphasis on family and environment
- Also psychoanalysis
- Behaviorism led by Pavlov and Skinner

- 1950's and 1960's put emphasis on child's upbringing – period of extreme environmentalism
- 1960's to 1980's characterized by major growth in behavioral and psychiatric genetics
- Twin and primarily adoption studies helped convince people of the importance of genes
- 1980's to 1990's – denial of importance of environmental influences concurrently with molecular genetics 'revolution'.

End of determinism

- Era of 1980's (molecular genetics) ends in disillusionment as there are failures to replicate initial gene 'discoveries'.
- Since the 1990's recognition that single genes cannot explain complex traits – rather multifactorial approach; distinction between Mendelian traits and complex traits/disorders.
- All risk factors, genetic and environmental, are probabilistic rather than deterministic.

- Risk alleles extend throughout the normal distribution and not just at the extreme end e.g. cholesterol and heart disease & smoking and lung cancer
- Rise of criticism against behavioral genetics- focused on the dangers of extreme genetic viewpoint
- Reemergence of interest in gene-environment interplay



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Gene expression

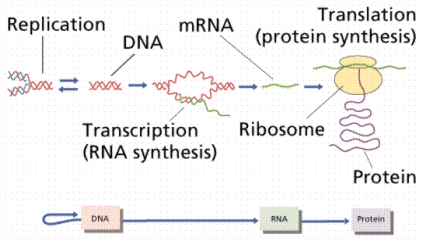
- Gene effects dependent on gene expression which in turn are dependent on a wide range of influences including environment
- The straightforward deterministic view of genetic effects was mistaken for non-Mendelian traits
- Concept of epigenesis – a separate lecture on this

Changing concept of a gene for…. Novelty Seeking

- Genes are NOT for any of these traits
- ACCORDING TO POPULAR PRESS……
- People who crave thrills such as skydiving and whitewater rafting may have a genetic basis for this behavior. Researchers have linked the "novelty seeking," or craving exciting new experiences, personality trait to a gene on chromosome 11. According to an article in *Time* magazine, this was "the first time a normal personality trait [was] firmly linked to a particular gene" (Toufexis, 1996)
- Terminology is a convenient SHORT HAND

Misleading shorthand

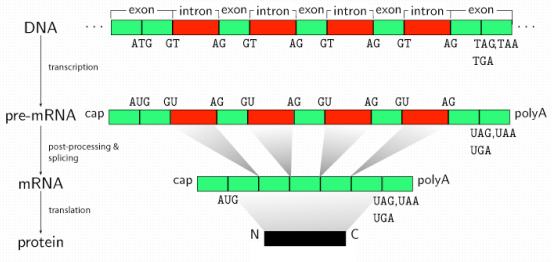
- It might be thought that this terminology is simply a convenient short- hand means of expressing the finding that genes play some contributory role in influencing individual variations in the liability for these traits.
- The genetic effects on the liability to psychiatric disorders are both much weaker and much more indirect than the 'genes for' terminology conveys. However, even beyond that, the terminology is misleading because of the wrong impression conveyed of how genes operate.



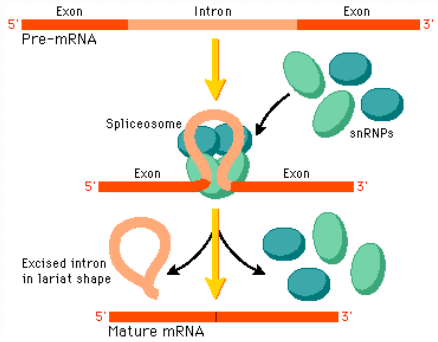
A key feature of gene expression is that it is modulated by extra-cellular signals and by environmental influences

- Sequence of AGCT in DNA is only the first phase of the process
- DNA specifies mRNA and transcription is influenced by a whole range of other factors both genetic and environmental
- Concept of cis- and trans- acting factors
- Concept of promoter
- Enhancers and silencers of gene transcription
- And then the complexities of translation which takes place in the cytoplasm

- By implication genes or gene regions that are inherited and that are important in contributing to complex traits are not necessarily in the coding region of the genes themselves
- Example – splicing sequences in the intron



The vast majority of splice sites are characterized by the presence of specific dimers on the intronic side of the splice site: **GT** for donor and **AG** for acceptor sites.



Rather than talking about there being direct effects of a single gene on a single outcome, it is more appropriate to think of a dynamic process in which the effects of a single gene are influenced by multiple inherited DNA elements and by the actions of environments.

Susceptibility genes

- The susceptibility genes that have been found differ from the pathogenic abnormal mutations in two ways;
- they are very common and they do not prevent vital functions.
- These genes that have been found to be involved in susceptibility to mental disorders have several important features.
- First, the allelic variations that carry risk are mostly very common, affecting, say, a third of the population even though the disorders for which they provide susceptibility are much rarer than that. Second, the allelic variations that carry risk do so at only a quite low probability level.
- Thus, as Kendler (2005b) has noted, the odds ratios are usually substantially less than two. Accordingly, it makes no sense to describe these as genes 'for' any particular mental disorder. They are implicated in the causal processes leading to the mental disorder, but only along with other genes and with a range of environmental influences. In short, they constitute part of multifactorial causation and not any direct genetic effect.

Dimensional measures

- In most cases, the susceptibilities are dimensional, rather than categorical. That is to say, the risks need to be viewed as operating on a continuum rather than on a present/absent basis.
- It should be emphasized, incidentally, that this applies to the genes involved in multifactorial somatic disorders such as coronary artery disease, hypertension, asthma and diabetes just as much as psychiatric disorder.
- It is a general phenomenon, not one that is in any way specific to psychopathology.

Indirect action

- Genes appear to operate indirectly in the sense that they predispose to disorder only through their effects on exposure to risk environments, rather than through a risk mechanism that might be viewed in any sense as directly leading to psychopathology.
- **Concept of neither necessary nor sufficient cause**

ApoE4 and Alzheimer's disease

- Carriers of ApoE4 carry a much increased risk of AD
- Many people with ApoE4 do not develop AD and many people without ApoE4 develop AD
- ApoE4 carries increased risk to adverse consequences viz., AD, as a result of head trauma
- Risk varies by ethnicity
- Enriched environment in transgenic mice is protective of adverse effects of ApoE4.

Effects of environment on genes

- Michael Meaney's studies
- Petronis DRD2 and methylation in LBC
- See Cancedda et al 2004 BDNF in the visual cortex
- Environment cannot influence gene sequence (barring natural selection and evolutionary forces over long time periods) but can influence gene expression

Variations in genetic influence according to environmental circumstances

- From the outset, geneticists have emphasized that heritability is a statistic that applies to population variance and not to individuals or to traits as a fixed feature.
- A high heritability means that genetic factors account for much of the variation in the liability to show a particular trait in a particular population at a particular point in time.
- It does not mean that genetic factors play a major role in the causation of that trait in any one individual.

- If genetic conditions change, or if environmental circumstances alter, the heritability will not remain the same.
- Marlow et al 2004
- Heritability of non-verbal cognitive performance was heritable and genetic factors accounted for 25% of the variance but no heritability for this phenotype in extremely premature babies.
- Similar results were observed for problem behavior – heritability was significantly lower in children with low birth weight.

- The findings of this group of studies are best conceptualized as representing a demonstration of the basic feature of heritability;
- namely, that the population variance attributable to genetic factors may be expected to be lower in any subsection of the population exposed to a major adverse environmental influence known to impact on the trait being investigated.
- The evidence, albeit based on a tiny number of studies, confirms that this does indeed happen.

Societal moderators of heritability

- The question here is whether heritability changes over time when environmental circumstances alter in some major way or vary across segments of the population that differ in their constraints or opportunity for expression of individual differences.
- First, it has been proposed that when there are widespread social constraints discouraging a behavior, heritability will tend to be relatively low.
- By contrast, when constraints are removed or diminished, genetic effects become more influential

- Heath, Jardine, and Martin (1989) found that the heritability of alcohol consumption was lower in married than in unmarried women, this being so in both younger and older age groups.
- Boomsma, de Geus, van Baal, and Koopmans (1999) found that a religious upbringing was associated with a lower heritability for 'disinhibition' (assessed in terms of drinking, going to parties, and having a variety of sexual partners).
- The interaction was significant in males; indeed, in those with a religious upbringing, genetic influences had no significant effect on individual differences in disinhibition.
- The trend in females was similar but not so strong.
- Koopmans, Slutske, van Baal, and Boomsma (1999) found the same with respect to alcohol use in females, although not in males.

- Dunne et al. (1997) found that women and men born between 1922 and 1952, who would have reached adolescence during an era when social controls inhibiting sexual intercourse were relatively strong, showed a low heritability for the variance in the age of first intercourse (32% in women and 0% in men).
- By contrast, in those born between 1952 and 1965, so reaching adolescence in an era of greater sexual tolerance, the heritabilities were 49% and 72% respectively for women and men.

- The second general notion has been that heritability should increase if opportunities for the expression of a trait become greater.
- Thus, Heath, Kendler, Eaves, and Markell (1985) found an increase in the heritability of educational attainment in Norwegian males during a period in which educational opportunities became more widely available.
- No such effect was found in females. Silventoinen, Kaprio, Lahelma, and Koskenvuo (2000) found an increase in the heritability of height among Finnish men and women over a period of30 years (a birth date before 1928 to a birth date of 1957). The rise was small – from 76% to 81% in men and from 66% to 82% in females – but significant. The change coincided with a time in which there was an overall increase in height of some 5 cm and both the rise in mean height and the increase in heritability were attributed to improved nutrition.

Overview of variations in heritability findings

- Heritability levels are specific to particular populations.
- The levels are likely to go up or down whenever there are major changes in the balance between genetic or environmental effects on phenotypic variation.
- The research into variations in heritability, however, has had the much more ambitious, and potentially valuable, goal of identifying the mechanisms that may be involved.
- if genetic effects operate through influences on sensitivity to the environment, the effect of an increase in environmental risks will be to increase heritability (as a result of the increased effect of the gene–environment interaction, G · E, on population variance in the trait affected by G · E.
- As we discuss in more detail in a later section, there is good evidence that this does happen.

- Also, however, it has been argued that the power of environmental proximal processes to actualize genetic potentials will be greater in advantaged stable environments than in disadvantaged, disor- ganised ones (Bronfenbrenner & Ceci, 1994).
- This leads to an opposite prediction regarding the effects of greater environmental risk on heritability; namely, that it should fall rather than rise.

rGE gene-environment correlation

- Plomin et al. (1977) differentiated among 'passive,' 'active' and 'evocative' rGE and we use the same distinctions.
- The term 'passive' rGE refers to the fact that the genetic influences on individual differences in envir- onmental risk exposure are independent of actions of the individual child.
- The rGE comes about because the kind of rearing environment that parents provide will be influenced by their own behavioral characteristics (with respect, for example, to personality features, mental disorder, and intellectual qualities), and these characteristics are influenced by genetic (as well as environmental) factors.
- Epidemiological evidence is consistent in showing that there are strong associations between parental psychopathology and the family environments that they provide for the upbringing of their children.

'Active' and 'evocative' rGE are different in that they concern the child's genes

- Active rGE refers to the genetic effects on the child's behaviour that serve to select or shape the environments experienced.
- Thus, according to their interests and skills, some children will spend much of their free time reading, others will be out on the football field, some will be practicing the violin or piano, and some will be chatting and playing with friends.
- That they choose to spend their time in a particular way will be affected by genetically influenced behaviors, attitudes and propensities.
- 'Evocative' rGE is different only in the sense that it refers to interpersonal effects rather than effects on non-social aspects of the environment. Thus, some children are fun to be with, but others tend to irritate or annoy and these tendencies will serve to shape how they are treated by other people.

- rGE is sometimes used by behavioral geneticists in the more restrictive sense of a shared genetic liability that impinges on both the environmental risk factors and the phenotype being studied.
- Thapar, Harold, and McGuffin (1998) showed that the co-occurrence of life events and depression in young people in part reflected shared genetic liability
- Kendler and Karkowski-Shuman (1997) similarly showed that there was a shared genetic liability between major depression and liability to negative life events in adults.

x E G

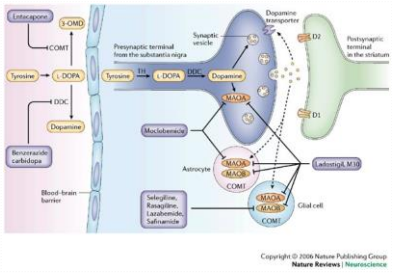
- During the era of the 1980s to early 1990s, the generally accepted view in behavioral and psychiatric genetics was that gene–environment interactions (G X E) were rare, and of such limited importance that they could be ignored in most circumstances.
- In summary, the traditional notion that strictly additive, non-interactive, effects for genetic and environmental influences would constitute the norm must now be rejected.
- That is not to say that in some instances (perhaps many instances) the environ- mental influences on psychopathology will operate through entirely different causal pathways than those involved in genetic effects, but it does not seem probable that that will generally be the case.

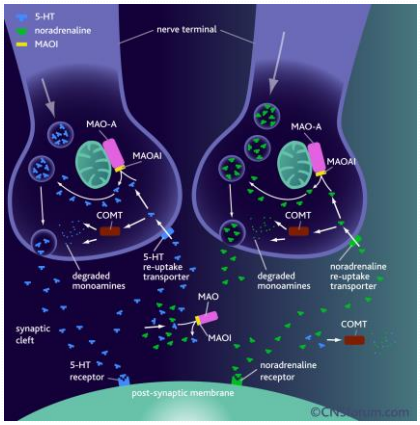
Gene-environment interaction

Avshalom Caspi

G x E

Monoamine oxidase A (MAOA)





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Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder

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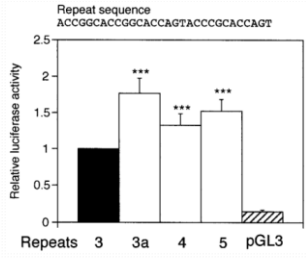

Role of Genotype in the Cycle of Violence in Maltreated Children

Avshalom Caspi,[1,2] Joseph McClay,[1] Terrie E. Moffitt,[1,2]* Jonathan Mill,[1] Judy Martin,[3] Ian W. Craig,[1] Alan Taylor,[1] Richie Poulton[3]

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Brief summary

- A functional polymorphism in the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (*MAOA*) was found to moderate the effect of maltreatment.
- Maltreated children with a genotype conferring high levels of *MAOA* expression were less likely to develop antisocial problems.

- These findings may partly explain why not all victims of maltreatment grow up to victimize others, and they provide epidemiological evidence that genotypes can moderate children's sensitivity to environmental insults.

Background

- Childhood maltreatment is a universal risk factor for antisocial behavior. Boys who experience abuse--and, more generally, those exposed to erratic, coercive, and punitive parenting--are at risk of developing conduct disorder, antisocial personality symptoms, and of becoming violent offenders.

- The earlier children experience maltreatment, the more likely they are to develop these problems. **But there are large differences between children in their response to maltreatment.**

- Although maltreatment increases the risk of later criminality by about 50%, most maltreated children do not become delinquents or adult criminals
- The reason for this variability in response is largely unknown, but it may be that vulnerability to adversities is conditional, depending on genetic susceptibility factors

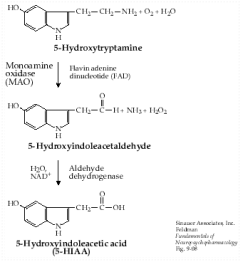
- In this study, individual differences at a functional polymorphism in the promoter of the monoamine oxidase A (*MAOA*) gene were used to characterize genetic susceptibility to maltreatment and to test whether the *MAOA* gene modifies the influence of maltreatment on children's development of antisocial behavior.

Monoamine oxidase MAO A gene

- The *MAOA* gene is located on the X chromosome (Xp11.23-11.4). It encodes the *MAOA* enzyme, which metabolizes neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA), rendering them inactive
- Genetic deficiencies in *MAOA* activity have been linked with aggression in mice and humans.

- Increased aggression and increased levels of brain NE, 5-HT, and DA were observed in a transgenic mouse line in which the gene encoding *MAOA* was deleted, and aggression was normalized by restoring *MAOA* expression.

Serotonin catabolism



- In humans, a null allele at the *MAOA* locus was linked with male antisocial behavior in a Dutch kindred.
- Because *MAOA* is an X-linked gene, affected males with a single copy produced no *MAOA* enzyme--effectively, a human knockout.
- However, this mutation is extremely rare. Evidence for an association between *MAOA* and aggressive behavior in the human general population remains inconclusive

Circumstantial evidence

- Circumstantial evidence suggests the hypothesis that childhood maltreatment predisposes most strongly to adult violence among children whose *MAOA* is insufficient to constrain maltreatment-induced changes to neurotransmitter systems.
- Animal studies document that maltreatment stress (e.g., maternal deprivation, peer rearing) in early life alters NE, 5-HT, and DA neurotransmitter systems in ways that can persist into adulthood and can influence aggressive behaviors.

- In humans, altered NE and 5-HT activity is linked to aggressive behavior.
- Maltreatment has lasting neurochemical correlates in human children, and although no study has ascertained whether *MAOA* plays a role, it exerts an effect on all aforementioned neurotransmitter systems.

MAO A promoter region polymorphism

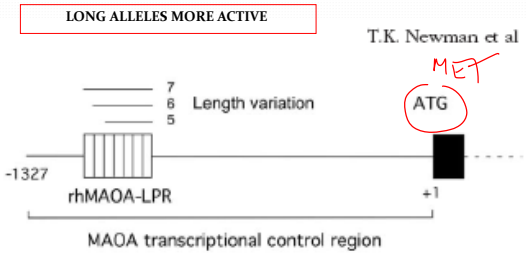
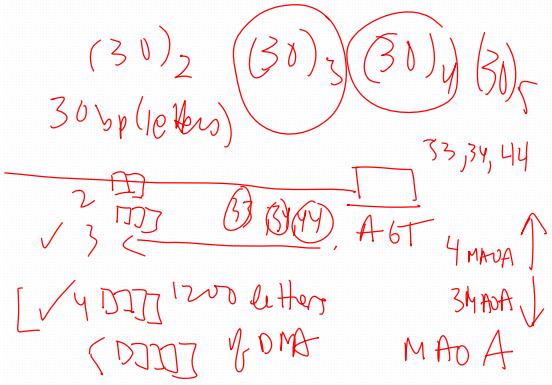


Figure 1. Map of the transcriptional control region of the rhesus monkey monoamine oxidase A (rhMAOA) gene-linked (EMBL-GenBank accession number AJ544234) polymorphic region. The rhMAOA-linked polymorphic region comprises an 18-bp repetitive sequence with length variation of 5–7 repeats.

- 1.2 kb upstream of the MAOA coding sequences, consists of a 30-bp repeated sequence present in 2 (291 bp), 3 (321 bp), 3.5 (336), 4 (351 bp), or 5 (381 bp) copies. The polymorphism is in linkage disequilibrium with other MAOA and MAOB gene markers and displays significant variations in allele frequencies across ethnic groups.
- Sabol, S. Z., Hu, S., and Hamer, D., 1998, A functional polymorphism in the monoamine oxidase A gene promoter, *Hum Genet* **103**(3):273-9.
- Since Deckert and his colleagues (14), and replicated by Sayagailo et al (16), showed that the 3.5, 4 and 5 alleles are more active than the 3 alleles, subjects were grouped into two genotype classes, 3 repeats (short) and 4 & 5 repeats (long). 3 & 4 are by far the most common alleles.
- Deckert, J., M. Catalano, et al. (1999). "Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder." Hum Mol Genet **8**(4): 621-4.



Experimental Design

- We genotyped this polymorphism in members of the Dunedin Multidisciplinary Health and Development Study, a sample without population stratification confounds.
- This birth cohort of 1,037 children (52% male) has been assessed at ages 3, 5, 7, 9, 11, 13, 15, 18, and 21 and was virtually intact (96%) at age 26 years.

The study offers three advantages for testing gene-environment (*G* × *E*) interactions.

- The sample has well-characterized environmental adversity histories. Between the ages of 3 and 11 years, 8% of the study children experienced "severe" maltreatment, 28% experienced "probable" maltreatment, and 64% experienced no maltreatment.

Gene x Environment correlation rGE

- Maltreatment groups did not differ on *MAOA* activity, chi-square= 0.38, $P = 0.82$, suggesting that genotype did not influence exposure to maltreatment.

Third, the study has ascertained antisocial outcomes rigorously.

- Antisocial behavior is a complicated phenotype, and each method and data source used to measure it (e.g., clinical diagnoses, personality checklists, official conviction records) is characterized by different strengths and limitations.
- Using information from independent sources appropriate to different stages of development, we examined four outcome measures.

- Adolescent conduct disorder was assessed according to criteria DSM-IV);
- convictions for violent crimes were identified via the Australian and New Zealand police;
- a personality disposition toward violence was measured as part of a psychological assessment at age 26;

- symptoms of antisocial personality disorder were ascertained at age 26 by collecting information about the study members from people they nominated as "someone who knows you well."
- A common-factor model fit the four measures of antisocial behavior well, with factor loadings ranging from 0.64 to 0.74, showing that all four measures index liability to antisocial behavior.

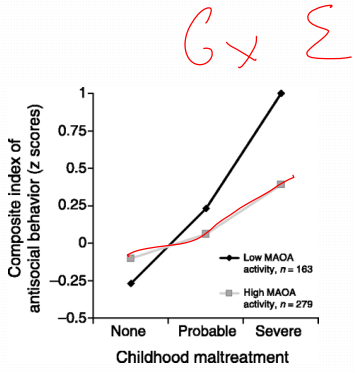
Results

- Using moderated regression analysis, we predicted scores on a composite antisocial index comprising the four measures of antisocial behavior
- The main effect of *MAOA* activity on the composite index of antisocial behavior was not significant:

- b = 0.01, SE = 0.09, t = 0.13, P = 0.89, whereas the main effect of maltreatment was significant: (b = -0.36, SE = 0.14, t = 2.53, P = 0.01).

Interaction

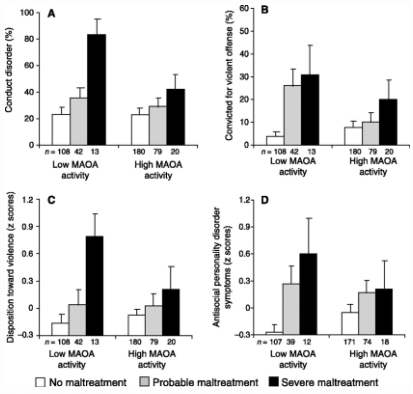
- A test of the interaction between *MAOA* activity and maltreatment revealed a significant $G \times E$ interaction: b = -0.36, E = 0.14, t = 2.53, P = 0.01.
- This interaction within each genotype group showed that the **effect of childhood maltreatment on antisocial behavior was significantly weaker among males with high *MAOA* activity** (b = 0.24, SE = 0.11, t = 2.15, P = 0.03) than among males with low *MAOA* activity (b = 0.68, SE = 0.12, t = 5.54, P < 0.001).



GxE is robust across all 4 measures of antisocial behavior

- We conducted further analyses to test if the $G \times E$ interaction was robust across each of the four measures of antisocial behavior that made up the composite index.
- For all four antisocial outcomes, the pattern of findings was consistent with the hypothesis that the association between maltreatment and antisocial behavior is conditional, depending on the child's *MAOA* genotype ($G \times E$ interaction P = 0.06, 0.05, 0.10, and 0.04, respectively).

- For adolescent conduct disorder (Fig. 2A), maltreated males (including probable and severe cases) with the low-*MAOA* activity genotype were more likely than nonmaltreated males with this genotype to develop conduct disorder by a significant odds ratio (OR) of 2.8 [95% confidence interval (CI): 1.42 to 5.74]. In contrast, among males with high *MAOA* activity, maltreatment did not confer significant risk for conduct disorder (OR = 1.54, 95% CI: 0.89 to 2.68).
- For adult violent conviction (Fig. 2B), maltreated males with the low-*MAOA* activity genotype were more likely than nonmaltreated males with this genotype to be convicted of a violent crime by a significant odds ratio of 9.8 (95% CI: 3.10 to 31.15). In contrast, among males with high *MAOA* activity, maltreatment did not confer significant risk for violent conviction (OR = 1.63, 95% CI = 0.72 to 3.68).
- For self-reported disposition toward violence (Fig. 2C) and informant-reports of antisocial personality disorder symptoms (Fig. 2D), males with the low-*MAOA* activity genotype who were maltreated in childhood had significantly elevated antisocial scores relative to their low-*MAOA* counterparts who were not maltreated. In contrast, males with high *MAOA* activity did not have elevated antisocial scores, even when they had experienced childhood maltreatment.



Conclusions

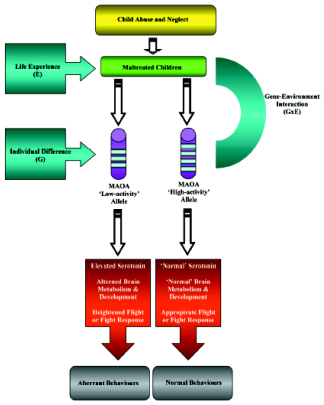
- These findings provide initial evidence that a functional polymorphism in the *MAOA* gene moderates the impact of early childhood maltreatment on the development of antisocial behavior in males. Replications of this $G \times E$ interaction are now needed.
- Replication studies should use valid and reliable ascertainments of maltreatment history and should obtain multiple measures of antisocial outcomes, in large samples of males and females (30).

Implications

- If replicated, the findings have implications for research and clinical practice.
- With regard to research in psychiatric genetics, knowledge about environmental context might help gene-hunters refine their phenotypes.
- Genetic effects in the population may be diluted across all individuals in a given sample, if the effect is apparent only among individuals exposed to specific environmental risks.

- Numerous biological and psychological processes have been put forward to explain why and how experiences of maltreatment are converted into antisocial behavior toward others, but there is no conclusive evidence that any of these processes can account for the progression from childhood maltreatment to later criminal violence.
- Moreover, some youngsters make the progression, but others do not, and researchers have sought to understand why.
- The search has focused on social experiences that may protect some children, overlooking a potential protective role of genes.

- Genes are assumed to create vulnerability to disease, but from an evolutionary perspective they are equally likely to protect against environmental insult.
- Maltreatment studies may benefit from ascertaining genotypes associated with sensitivity to stress, and the known functional properties of *MAOA* may point toward hypotheses, based on neurotransmitter system development, about how stressful experiences are converted into antisocial behavior toward others in some, but not all, victims of maltreatment.



Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene

Avshalom Caspi,[1,2] Karen Sugden,[1] Terrie E. Moffitt,[1,2*] Alan Taylor,[1] Ian W. Craig,[1] HonaLee Harrington,[2] Joseph McClay,[1] Jonathan Mill,[1] Judy Martin,[3] Antony Braithwaite,[4] Richie Poulton[3]

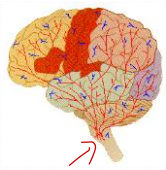
Why depression?

- Depression is among the top five leading causes of disability and disease burden throughout the world.
- Across the life span, stressful life events that involve threat, loss, humiliation, or defeat influence the onset and course of depression
- However, not all people who encounter a stressful life experience succumb to its depressogenic effect.
- Diathesis-stress theories of depression predict that individuals' sensitivity to stressful events depends on their genetic makeup

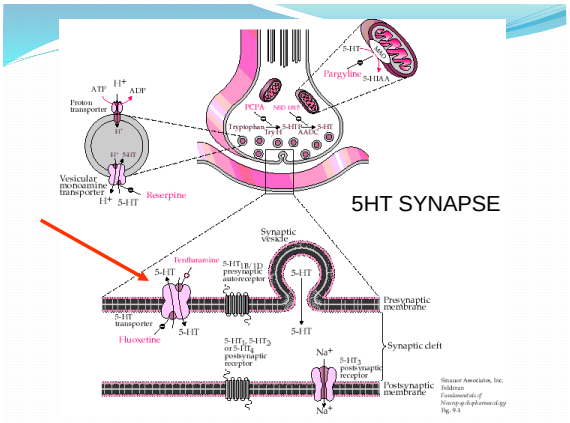
- Behavioral genetics research supports this prediction, documenting that the risk of depression after a stressful event is elevated among people who are at high genetic risk and diminished among those at low genetic risk (8).
- However, whether specific genes exacerbate or buffer the effect of stressful life events on depression is unknown.

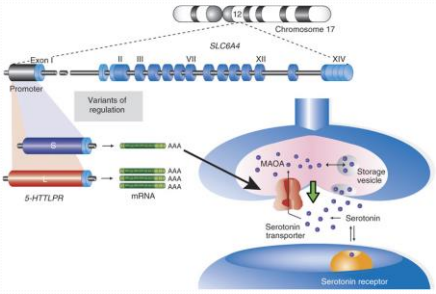
- In this study, a functional polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) was used to characterize genetic vulnerability to depression and to test whether 5-HTT gene variation moderates the influence of life stress on depression.

Serotonin

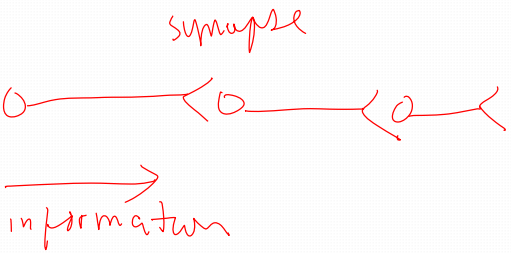


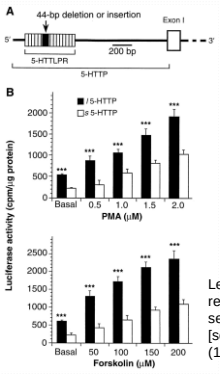
Most serotonin cells (in red) begin in a specific area of the the brain stem called the **"raphe nuclei."** Their dendrites and cell body's are located here, and they have very long axons that extend into every other part of the brain. Serotonin axons are much denser and have many more tree-like branches than we were able to show in this drawing. They are also much longer than any diagram can easily depict. If you were to stretch out a serotonin neuron on a table in front of you, it might be a foot long, but you still wouldn't be able to see it because it would be so thin. Most people think of brain cells as shorter and confined to particular brain regions (in blue). While some brain cells are like this, this is not the case with seronoin cells. No wonder serotonin plays such an important role in so many brain functions, such as the regulation of mood, heart-rate, sleep, appetite, pain and other things.





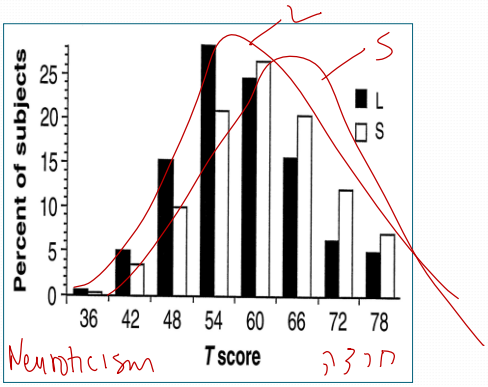
The short (S) 5-HTTLPR variant (purple) of the 5-HTT gene (SLC6A4) produces significantly less 5-HTT mRNA and protein, as indicated by the green arrow, than the long (L) variant (red), leading to higher concentrations of serotonin in the synaptic cleft. The short variant is associated with anxiety-related personality traits such as neuroticism, which are risk factors for affective spectrum disorders. MAOA, monoamine oxidase A; SSRI, selective serotonin





PROMOTER REGION POLYMORPHISM HTTLPR

Lesch, K.P. et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region [see comments]. *Science* **274**, 1527-31 (1996).



SSRI's

- The serotonin system provides a logical source of candidate genes for depression, because this system is the target of selective serotonin reuptake–inhibitor drugs that are effective in treating depression.

HTTLPR

- The serotonin transporter has received particular attention because it is involved in the reuptake of serotonin at brain synapses.
- The promoter activity of the 5-HTT gene, located on 17q11.2, is modified by sequence elements within the proximal 5′ regulatory region, designated the 5-HTT gene-linked polymorphic region (5-HTTLPR).
- The short ("s") allele in the 5-HTTLPR is associated with lower transcriptional efficiency of the promoter compared with the long ("l") allele.

- Evidence for an association between the short promoter variant and depression is inconclusive.
- Although the 5-HTT gene may not be directly associated with depression, it could moderate the serotonergic response to stress. Three lines of experimental research suggest this hypothesis of a gene-by-environment (G x E) interaction.

mice

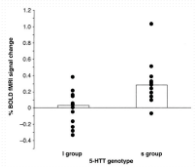
- First, in mice with disrupted 5-HTT, homozygous and heterozygous (5-HTT –/– and +/–) strains exhibited more fearful behavior and greater increases in the stress hormone (plasma) adrenocorticotropin in response to stress compared to homozygous (5-HTT +/+) controls, but in the absence of stress no differences related to genotype were observed (13).

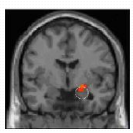
Rhesus monkeys

- Second, in rhesus macaques, whose length variation of the 5-HTTLPR is analogous to that of humans, the short allele is associated with decreased serotonergic function [lower cerebro-spinal fluid (CSF) 5-hydroxyindoleacetic acid concentrations] among monkeys reared in stressful conditions but not among normally reared monkeys (14).

Imaging

- Third, human neuroimaging research suggests that the stress response is mediated by variations in the 5-HTTLPR. Humans with one or two copies of the s allele exhibit greater amygdala neuronal activity to fearful stimuli compared to individuals homozygous for the l allele (15).
- Taken together, these findings suggest the hypothesis that variations in the 5-HTT gene moderate psycho-pathological reactions to stressful experiences.





Study design

- We tested this G x E hypothesis among members of the Dunedin Multidisciplinary Health and Development Study (16). This representative birth cohort of 1037 children (52% male) has been assessed at ages 3, 5, 7, 9, 11, 13, 15, 18, and 21 and was virtually intact (96%) at the age of 26 years.

New Zealand caucasians

- A total of 847 Caucasian non-Maori study members, without stratification confounds, were divided into three groups on the basis of their 5-HTTLPR genotype (11): those with two copies of the s allele (s/s homozygotes; *n* = 147; 17%), those with one copy of the s allele (s/l heterozygotes; *n* = 435; 51%), and those with two copies of the l allele (l/l homozygotes; *n* = 265; 31%).

25% SS 50% SL 25% LL

Stressful life events

- The 14 events included employment, financial, housing, health, and relationship stressors.
- Thirty percent of the study members experienced no stressful life events; 25% experienced one event; 20%, two events; 11%, three events; and 15%, four or more events.

rG E correlation

- There were no significant differences between the three genotype groups in the number of life events they experienced, $F(2,846) = 0.56$, $P = 0.59$, suggesting that 5-HTTLPR genotype did not influence exposure to stressful life events.

Assessing depression

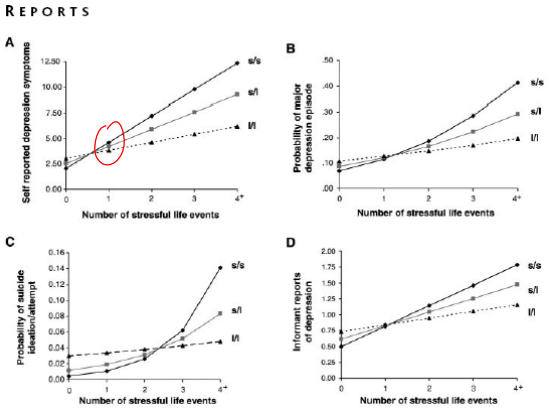
- Study members were assessed for past-year depression at age 26 with the use of the Diagnostic Interview Schedule, which yields a quantitative measure of depressive symptoms and a categorical diagnosis of a major depressive episode according to *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria.

- 17% of study members (58% female versus 42% male; odds ratio = 1.6; 95% confidence interval from 1.1 to 2.2) met criteria for a past-year major depressive episode, which is comparable to age and sex prevalence rates observed in U.S. epidemiological studies.

- In addition, 3% of the study members reported past-year suicide attempts or recurrent thoughts about suicide in the context of a depressive episode.
- We also collected informant reports about symptoms of depression for 96% of study members at age 26 by mailing a brief questionnaire to persons nominated by each study member as "someone who knows you well."

results

- We used a moderated regression framework, with sex as a covariate, to test the association between depression and (i) 5-HTTLPR genotype, (ii) stressful life events, and (iii) their interaction (table S1).
- The interaction between 5-HTTLPR and life events showed that the **effect of life events on self-reports of depression symptoms** at age 26 was significantly stronger ($P = 0.02$) among individuals **carrying an s allele** than among l/l homozygotes (Fig. 1A).



- The G x E interaction also showed that stressful life events predicted a diagnosis of major depression among carriers of an s allele but not among l/l homozygotes ($P = 0.056$, Fig. 1B).

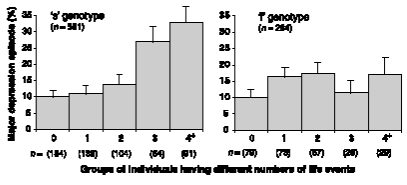


Fig. 3. The percentage of individuals meeting diagnostic criteria for depression at age 26, as a function of 5-HTT genotype and number of stressful life events between the ages of 21 and 26. The figure shows individuals with either one or two copies of the short allele (**left**) and individuals homozygous for the long allele (**right**). In a hierarchical logistic regression model, the main effect of genotype (coded as s group = 0 and l group = 1) was not significant, $b = -0.15$, SE = 0.21, $z = 0.72$, $P = 0.47$; the main effect of number of life events was significant, $b = 0.34$, SE = 0.06, $z = 5.70$, $P < 0.001$; and the interaction between genotype and number of life events was significant, $b = -0.30$, SE = 0.15, $z = 1.97$, $P = 0.05$.

Possible interaction with MAO A?

- We previously showed that variations in the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (MAOA) moderate children's sensitivity to maltreatment.
- MAOA has high affinity for 5-HTT, raising the possibility that the protective effect of the l/l allele on psychiatric morbidity is further augmented by the presence of a genotype conferring high MAOA activity.

- However, we found that the moderation of life stress on depression was specific to a polymorphism in the 5-HTT gene, because this effect was observed regardless of the individual's MAOA gene status (tables S4 and S5).

Discussion

- Until this study's findings are replicated, speculation about clinical implications is premature.
- Nonetheless, although carriers of an s 5-HTTLPR allele who experienced four or more life events constituted only 10% of the birth cohort, they accounted for almost one-quarter (23%) of the 133 cases of diagnosed depression.

- Moreover, among cohort members suffering four or more stressful life events, 33% of individuals with an s allele became depressed, whereas only 17% of the l/l homozygotes developed depression (Fig. 3).

- Thus, the G x E's attributable risk and predictive sensitivity indicate that more knowledge about the functional properties of the 5-HTT gene may lead to better pharmacological treatments for those already depressed.
- Although the short 5-HTTLPR variant is too prevalent for discriminatory screening (over half of the Caucasian population has an s allele), a microarray of genes might eventually identify those needing prophylaxis against life's stressful events.

- Much genetic research has been guided by the assumption that genes cause diseases, but the expectation that direct paths will be found from gene to disease has not proven fruitful for complex psychiatric disorders.
- Our findings of G x E interaction for the 5-HTT gene and another candidate gene, MAOA, point to a different, evolutionary model. This model assumes that genetic variants maintained at high prevalence in the population probably act to promote organisms' resistance to environmental pathogens.

Environmental pathogens

- We extend the concept of environmental "pathogens" to include traumatic, stressful life experiences and propose that the effects of genes may be uncovered when such pathogens are measured (in naturalistic studies) or manipulated (in experimental studies).

Implications for future linkage studies

- To date, few linkage studies detect genes, many candidate gene studies fail consistent replication, and genes that replicate account for little variation in the phenotype.
- If replicated, our G x E findings will have implications for improving research in psychiatric genetics. Incomplete gene penetrance, a major source of error in linkage pedigrees, can be explained if a gene's effects are expressed only among family members exposed to environmental risk.

- If risk exposure differs between samples, candidate genes may fail replication. If risk exposure differs among participants within a sample, genes may account for little variation in the phenotype.
- We speculate that some multifactorial disorders, instead of resulting from variations in many genes of small effect, may result from variations in fewer genes whose effects are conditional on exposure to environmental risks.

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Meta-analysis of gene–environment interactions in developmental psychopathology

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Abstract

As studies of measured gene–environment interactions (G × E) in developmental psychopathology gain momentum, methods for systematically and quantitatively summarizing effects across multiple studies are urgently needed. Meta-analyses of G × E findings are critical for evaluating the overall statistical and theoretical significance of any given G×E based on cumulative and systematically combined knowledge. Although meta-analytic methods for the combination of study findings based on single effect measures such as odds ratios and mean differences are well established, equivalent methods for the meta-analysis of studies investigating interactions are not well developed. This article describes one simple approach to the meta-analysis of G × E effects using, as a contemporaneous example, the interaction of the monoamine oxidase A (*MAOA*) gene and the impact of childhood maltreatment on risk for developing antisocial behavior.

Metaanalysis

- A meta-analysis consists of the following seven broad steps:
- (a) define the research question to be analyzed, such as the effectiveness of a drug treatment or the existence and magnitude of a G × E;
- (b) identify relevant research reports, including unpublished manuscripts where applicable;
- (c) extract measures of effect size and a description of salient study characteristics from each report based on a predefined protocol;
- (d) select the studies that are to be included in the final meta-analysis (these should meet defined inclusion criteria, such as having comparable definitions of the study outcome);
- (e) convert all effect measures to a common metric, for example, translating all measures to odds ratios or correlations;

- (f) combine the individual study estimates using standard meta-analytic techniques that weight effect measures by an estimate of their precision to obtain an overall or combined effect size and confidence interval (CI); and
- (g) carry out sensitivity analyses of the results to study specific confounders and investigate publication bias.

Clinical trials

- Meta-analysis has become particularly well developed in the assessment of clinical trials, which provides a "gold standard" of methods and procedures for the application of meta-analytic methods.
- At the initial stage of trial publication, the Consolidated Standards for Reporting Trials Checklist guidelines for clinical trials ensures that study analyses are reported with sufficient detail to provide the necessary building blocks for systematic reviews based on meta-analytic methods.
- The Cochran Collaboration (provides guidelines for the appropriate conduct of a meta-analysis and the Quality of Reporting of Meta-Analysis Statement recommendations set out best practice reporting standards for the completed analysis.

G x E

- G × E studies often use epidemiological cohort studies where reporting standards are not well developed and where the quality of study reporting can be highly variable
- Inconsistency in reporting is partly because of the complexity of the phenomena under study and the variety of study designs and analysis techniques used.
- This influences the quality and ease of carrying out a meta-analysis as variable reporting impacts the ability to extract and validly combine results across studies.
- There are now some recommendations on the reporting of meta-analyses based on epidemiological studies, which should help these analyses attain the standards set by the meta-analysis of clinical trials.

Metaanalysis MAOA & aggression

- In this meta-analysis update, studies were included if they fulfilled four criteria.
- First, the study had to be published in a peer-reviewed journal.
- Second, the study had to include genotypic information on the variable number tandem repeat polymorphism in the promoter region of the *MAOA* gene.
- Third, the study had to include a measure of serious familial adversity in childhood that was significantly associated in a main effect fashion with the outcome measure.
- Fourth, the sample had to include males drawn from a nonclinical population.
- There are now eight studies that meet all of these inclusion criteria

- Metaanalysis using differences in correlations as the effect measure.
- As a first step, the association between the environmental risk and the phenotype of interest is converted to a correlation for each genotype group.
- These correlations can then be included in a meta-analysis by genotype group to investigate the consistency of the association across studies by genotype.

- A crucial component of any meta-analysis, but particularly for those based on observational data, is to investigate the level of heterogeneity of effect sizes across studies and the impact of publication bias.
- Heterogeneity across studies can be assessed using the chi-square based Q statistic, as well as the I^2 measure of the percentage of across-study variation attributable to heterogeneity
- Given that the first published finding is generally larger than subsequent replications (Ioannidis, 2005), a simple sensitivity analysis should be carried out by reestimating the pooled effect size excluding the first published study. To determine if any given study is having an excessively large effect on the pooled estimate,

- For example, Figure 1 displays each study's effect size, together with CIs and the weight given to each study in the combined estimate.
- Squares are used to denote the size or importance of each study in the overall estimate.
- The overall combined estimate, together with its CI, is also plotted.

- The forest plot and results in Figure 1 show the meta-analysis of the correlations between maltreatment and antisocial behavior by *MAOA*activity group for all studies.
- This gives an overview of the interaction effect as it shows the across-study effect size within each *MAOA* genotype group, although it does not provide a formal test of the significance of the interaction effect

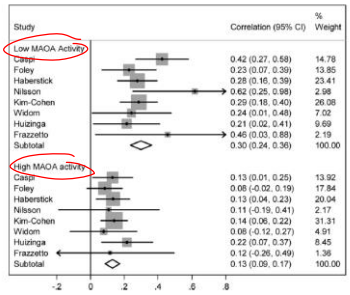


Figure 1. A forest plot of the meta-analysis of the correlations between antisocial behavior and childhood maltreatment for each monoamine oxidase A (*MAOA*) genotype group. Weights are from random effects analysis.

- The results for the meta-analysis of the interaction effect, using the differences in correlations by *MAOA* activity group, are shown in Figure 2.
- No significant heterogeneity was detected across the studies, χ^2 (7) = 6.07, p = .532, I^2 = 0.0%.
- The pooled random effects estimate of the difference in correlations across the low versus high *MAOA* groups indicated a significant difference in correlations of .17 (95% CI = .09 and .24,p < .001).

- Two additional sensitivity analyses of the meta-analysis results were conducted.
- First, the initial published study by Caspi and colleagues (2002) was removed and the results reestimated to rule out any potential bias contributed by the first published study of this interaction
- This resulted in a significant but reduced pooled difference in effect of .15 (95% CI = .07 and .23, p < .001).
- Second, the two studies that reported an effect size larger in magnitude than the original study were also removed, resulting in a pooled effect of .13 (95% CI = .05, .21, p < .002).

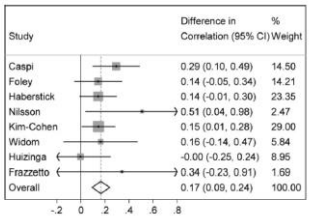
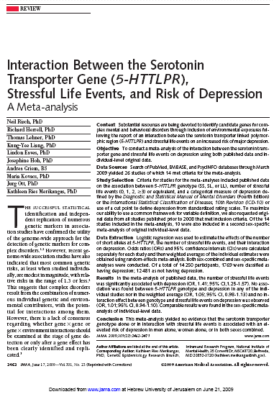


Figure 2. A forest plot of the differences in the correlations between antisocial behavior and childhood maltreatment across monoamine oxidase A (*MAOA*) genotype groups. Weights are from random effects analysis.

- The cumulative evidence across studies shows a small but significant combined effect of .17, and indicates that the effect of the interaction between *MAOA* and childhood maltreatment on conduct problems and aggression is robust.



REVIEW

Interaction Between the Serotonin Transporter Gene (*5-HTTLPR*), Stressful Life Events, and Risk of Depression

A Meta-analysis

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Context Substantial resources are being devoted to identify candidate genes for complex mental and behavioral disorders through inclusion of environmental exposures following the report of an interaction between the serotonin transporter linked polymorphic region (*5-HTTLPR*) and stressful life events on an increased risk of major depression.

Objective To conduct a meta-analysis of the interaction between the serotonin transporter gene and stressful life events on depression using both published data and individual-level original data.

Data Sources Search of PubMed, EMBASE, and PsycINFO databases through March 2009 yielded 26 studies of which 14 met criteria for the meta-analysis.

Study Selection Criteria for studies for the meta-analyses included published data on the association between *5-HTTLPR* genotype (SS, SL, or LL), number of stressful life events (0, 1, 2, ≥3) or equivalent, and a categorical measure of depression defined by the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) or the *International Statistical Classification of Diseases, 10th Revision (ICD-10)* or use of a cut point to define depression from standardized rating scales. To maximize our ability to use a common framework for variable definition, we also requested original data from all studies published prior to 2008 that met inclusion criteria. Of the 14 studies included in the meta-analysis, 10 were also included in a second sex-specific meta-analysis of original individual-level data.

Data Extraction Logistic regression was used to estimate the effects of the number of short alleles at *5-HTTLPR*, the number of stressful life events, and their interaction on depression. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated separately for each study and then weighted averages of the individual estimates were obtained using random-effects meta-analysis. Both sex-combined and sex-specific meta-analyses were conducted. Of a total of 14 250 participants, 1769 were classified as having depression; 12 481 as not having depression.

Results In the meta-analysis of published data, the number of stressful life events was significantly associated with depression (OR, 1.41; 95% CI, 1.25-1.57). No association was found between *5-HTTLPR* genotype and depression in any of the individual studies nor in the weighted average (OR, 1.05; 95% CI, 0.98-1.13) and no interaction effect between genotype and stressful life events on depression was observed (OR, 1.01; 95% CI, 0.94-1.10). Comparable results were found in the sex-specific meta-analysis of individual-level data.

Conclusion This meta-analysis yielded no evidence that the serotonin transporter genotype alone or in interaction with stressful life events is associated with an elevated risk of depression in men alone, women alone, or in both sexes combined.

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The successful statistical identification and independent replication of numerous genetic markers in association studies have confirmed the utility of the genome-wide approach for the detection of genetic markers for complex disorders.[1,2] However, recent genome-wide association studies have also indicated that most common genetic risks, at least when studied individually, are modest in magnitude, with relative risks in the range of 1.3 or less.[2] This suggests that complex disorders result from the combination of numerous individual genetic and environmental contributors, with the potential for interactions among them. However, there is a lack of consensus regarding whether gene × gene or gene × environment interactions should be examined at the stage of gene detection or only after a gene effect has been clearly identified and replicated.[3]

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Common genetic risk

- However, recent genome-wide association studies have also indicated that most common genetic risks, at least when studied individually, are modest in magnitude, with relative risks in the range of 1.3 or less.[2]
- This suggests that complex disorders result from the combination of numerous individual genetic and environmental contributors, with the potential for interactions among them.
- However, there is a lack of consensus regarding whether gene x gene or gene x environment interactions should be examined at the stage of gene detection or only after a gene effect has been clearly identified and replicated.

Psychiatric disorders

- Despite progress in risk gene identification for several complex diseases, few disorders have proven as resistant to robust gene finding as psychiatric illnesses.
- The slow rate of progressin psychiatry and behavioral sciences partly reflects a still-evolving classification system, absence of valid pathognomonic diagnostic markers, and lack of well-defined etiologic pathways.

- In terms of environmental factors, however, stressful life events have been well-established as a risk factor for a range of mental disorders, most commonly major depression.
- It is therefore appropriate to consider gene identification studies in the context of this environmental risk factor and in particular the simultaneous consideration of both genetic and environmental agents.

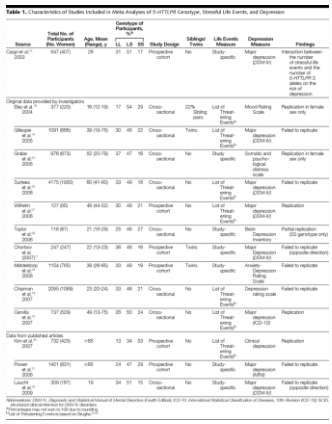
NIH inititative

- Because of the increasing interest in incorporating environmental risk factors in gene discovery studies, the National Institute of Mental Health convened a workshop in 2006[14] to review the status of research on gene-environment interaction in mental and behavioral disorders and to provide input on future directions of this work.
- Because there was a lack of consensus on the status of replication of gene-environment interaction studies, a comprehensive review and meta-analysis of this research was proposed.
- A subset of the workshop participants and a team of other experts in statistical genetics, epidemiology, and behavior genetics followed up by conducting a comprehensive review of the literature and conceptualizing and implementing the meta-analysis.

Method

- A search of the PubMed, EMBASE, and PsycINFO databases was conducted through March 2009 using the search terms life stress, life event, depression, depress, serotonin transporter, 5-HTTLPR, interaction, and moderation. Bibliographies of studies were also reviewed.
- A total of 26 articles published or set for publication were identified through this search strategy

- We first examined the association between the number of stressful life events compared with none as the explanatory variable with depression as the response variable.
- For each number of stressful life events, we calculated an odds ratio (OR) and its 95% confidence interval (CI).
- Second, we investigated the associations between stressful life events alone (entered as an ordinal variable) and the number of *S* alleles alone on depression as the response variable.
- Third, we examined the joint effect of number of stressful life events and number of *S* alleles on the risk of depression to test whether there is an interaction in their influence on the risk of depression, as indicated by a significant OR for their joint effect on the response variable of depression.
- Fourth, to determine whether gene-environment correlation exists, we evaluated the association between the proportion of *S* alleles (dependent variable) and number of stressful life events (independent variable) among those without depression by logistic regression.



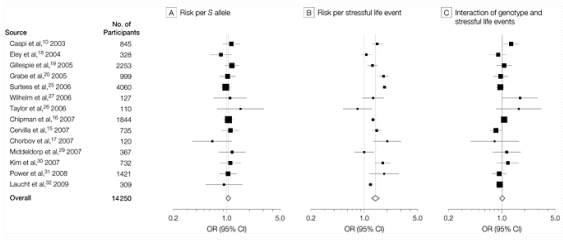
- There were a total of 14 250 participants of whom 1769 were classified as having depression, and 12 481 did not have depression. Published data were used from 4 studies and original data were used from 10 studies.
- There was wide variation in the demographic characteristics (e.g., proportion of women, age distribution) and sample sizes, which ranged from 118 to 4175 participants. Most of the participants were white except for a multiethnic sample in one study and an Asian sample in another study.
- Genotype distributions were similar among white participants; however, Asian individuals had higher *S* allele frequency than white individuals.
- Nine of the studies used a structured interview to assess either *DSM-IV* or *ICD-10* major depression, whereas the other 5 assessed depressive symptoms via self-rated symptoms scales.

- Stressful life events measures were assessed consistently across most of the studies using the Brugha List of Threatening Experiences,[42,43] whereas a few studies included measures of somatic illness, unemployment, and social stressors.

Stressful life events and depression

- Table 2 presents the results of the meta-analysis of the relationship between depression and number of stressful life events for each study for both sexes combined (n=12 520). Although the estimates for the individual studies were highly variable, the results of the meta-analysis across all studies reveals a direct association between the number of life events and depression (1 vs 0 stressful life event:
- OR, 1.31; 95% CI, 0.98-1.75; 2 vs 0 stressful life events: OR, 1.95; 95% CI, 1.33-2.87; and 3 vs 0, stressful life events: **OR, 3.21; 95% CI, 2.07-4.99).** Sex-specific estimates show a similar pattern.

- The analysis shows no significant allele frequency difference between those with and without depression in any of the single studies or in the meta-analysis of all studies combined (OR,1.05; 95% CI, 0.98-1.13; Figure 2A). Thus, the genotype alone did not predict depression.



- Finally, in the analysis of the interaction between genotype and number of life events on risk of depression, the regression coefficient was not significant for females in any of the studies and was nominally significant for males in 2 of the 10 studies.
- The meta-analysis showed no interaction effect for either females (OR, 0.95; 95% CI, 0.86- 1.04), males (OR, 1.02; 95% CI, 0.86-1.20), or both sexes combined (OR, 0.98; 95% CI, 0.90-1.07).
- **Thus, there was no evidence that the serotonin transporter genotype alone or in interaction with stressful life events is associated with an elevated risk of depression in males alone, females alone, or both sexes combined.**
- The only significant finding across studies was the potent association of stressful life events with the risk of depression.

Discussion

- This work highlights several pertinent issues for interpreting reports of replication and for conducting future meta-analyses of genetic association studies.
- First, it was not possible to conduct a traditional meta-analysis using the standards of randomized clinical trials because few of the studies included sufficient descriptive data to conduct a standard meta-analysis.
- This explains why a previous report included only 5 studies that dichotomized the exposure.
- To supplement our analysis of published data, we also requested original data from many of the authors in order to classify the data in the same way as those of the original study.
- Second, as highlighted in earlier reviews of this topic and a recent critical review of life stressors across these studies, the samples, study designs, measures, and analyses were highly divergent across studies, thereby limiting the comparability of the studies and their evidence regarding replication.

Future of G x E studies

- The results of this meta-analysis should not deter investigators from including environmental risk factor information in their studies, once robust marginal gene associations have been identified.
- Characterization of gene-environment interaction has been most successful for diseases or traits that allow the study of a single gene with a major effect in the context of a relevant environmental exposure of varying magnitude, and also when the environmental exposure has a strong effect.
- For example, the identification of the protective influence of the 32 mutation in the CCR5 chemokine receptor against human immunodeficiency virus infection was based on evaluation of unaffected controls with high exposure to the virus.[34]

- On the other hand, few examples of gene-environment interaction exist for modest gene effects or small environmental impacts, most likely due to lack of power to characterize such an interaction.
- Unless the statistical relationship between genotype and environmental exposure on disease risk strongly deviates from a multiplicative (or log-additive) model, the power to detect an interaction will be low, and thus weak positive results should be interpreted carefully.
- Attempts to rescue an unsuccessful candidate gene disease association, no matter how strong the candidate, by invoking an interaction with a common environmental exposure (such as life events) may be fraught with similar rates of false-positive associations as the original marginal gene association studies themselves.

- Despite the lack of valid confirmation of the Caspi et al[10] results, the approach to implicate candidate genes that had failed previous direct association studies through inclusion of an environmental exposure has been rapidly embraced, and substantial resources have been devoted to subsequent research.[5] The widespread acceptance of these findings is likely to have been in part attributable to the acclaim the original articlereceived, as well as a field that was eager for a new approach due to the frustrating lack of progress in gene identification for mental disorders despite intensive efforts for more than a decade.

- The disciplines of behavioral and social sciences have rapidly adopted this approach as seen by an increasing number of reports that attempt to link candidate genes with a wide range of human behaviors that may not even be under strong genetic influence such as number of sexual partners[64] and delinquency.
- A more serious concern, however, is that the findings of this and other nonreplicated genetic associations are now being translated to a range of clinical, legal, research, and social settings such forensics, diagnostic testing, study participants, and the general public.
- It is critical that health practitioners and scientists in other disciplines recognize the importance of replication of such findings before they can serve as valid indicators of disease risk or have utility for translation into clinical and public health practice.

ARTICLE IN PRESS

ARCHIVAL REPORT

Gene × Environment Interactions at the Serotonin Transporter Locus

Marcus R. Munafò, Caroline Durrant, Glyn Lewis, and Jonathan Flint

Background: Although it is universally accepted that human disease and behavior depend upon both environmental and genetic variation, a view supported by family and twin studies, examples of environmental interactions with genes identified at the molecular level (G × E) are not so well established.

Methods: We carried out a systematic review and meta-analysis of the serotonin transporter (5-HTTLPR) polymorphic region × stressful life event (SLE) literature and investigated to what extent the main effects reported in this literature are consistent with a number of G × E hypotheses. Our aim was to provide a framework in which to assess the robustness of the claim for the presence of an interaction.

Results: The results from our systematic review and meta-analysis indicate that the main effect of 5-HTTLPR genotype and the interaction effect between 5-HTTLPR and SLE on risk of depression are negligible. We found that only a minority of studies report a replication that is qualitatively comparable to that in the original report.

Conclusions: Given reasonable assumptions regarding likely genetic and environmental effect sizes, our simulations indicate that published studies are underpowered. This, together with other aspects of the literature, leads us to suggest that the positive results for the 5-HTTLPR × SLE interactions in logistic regression models are compatible with chance findings.

Molecular Psychiatry (2010) 15, 18–22

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PERSPECTIVE

The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update

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An updated review of 34 human observational studies indicates that the length polymorphism of the serotonin transporter gene moderates the effect of environmental adversity in the development of depression. This finding depends on the use of contextual or objective methods to assess environmental adversity and is attenuated when self-report instruments are used. Inconsistent findings in male adolescents suggest a developmental stage and sex-specific protective mechanism. These systematic relationships between method and results should be followed up to specify causal mechanisms leading to depression.
Molecular Psychiatry (2010) **15**, 18–22; doi:10.1038/mp.2009.123

Keywords: gene–environment interactions; serotonin transporter; depression; stressful life events; adversity; adolescence

- An updated review of 34 human observational studies indicates that the length polymorphism of the serotonin transporter gene moderates the effect of environmental adversity in the development of depression.
- This finding depends on the use of contextual or objective methods to assess environmental adversity and is attenuated when self-report instruments are used.
- Inconsistent findings in male adolescents suggest a developmental stage and sex-specific protective mechanism. These systematic relationships between method and results should be followed up to specify causal mechanisms leading to depression.

Assessment of adversity

- We classified the assessment of adversity according to the level of objectivity: self-report questionnaires are classified as the most subjective method, as they rely entirely on the memory and judgment of study participants;
- interviews are still based on subjective verbal report of participants, but the interviewer is trained to introduce a level of objectivity by systematically probing and making a judgment about the presence and severity of reported stressors;
- measures of adversity were classified as 'objective' if they were collected independently of participants' report and of researchers (for example, social services record of child abuse, natural disaster or physical illness established by objective examina- tion) or if they were facts very unlikely to be influenced by any reporting bias (for example, grow- ing up in a single parent family).

- all studies using objective indicators or structured interviews to assess stress replicated the gene–environment interaction fully or partially, whereas all non-replications relied on brief self-report measures of adversity.
- This relationship between the method used to assess environmental adversity and results was statistically significant($w2(1)$testfortrend = 8.51,P = 0.004).

EPIGENETICS

EPIGENETICS IN ACTION

Epigenetic and pharmacoepigenomic studies of major psychoses and potentials for therapeutics

Individuals with neuropsychiatric diseases have epigenetic programming disturbances, both in the brain, which is the primary affected organ, and in secondary tissues. Epigenetic modulations are molecular modifications made to DNA, RNA and proteins that fine-tune genotype into phenotype and do not include DNA base changes. For instance, gene-expression modulation is linked to epigenetic codes in chromatin that consist of post-replication DNA methylation and histone protein modifications (e.g., methylation, acetylation and so on), particularly in gene-promoter regions. Epigenetic coding is modulated globally, and in a gene-specific manner by environmental exposures that include nutrition, toxins, drugs and so on. Analysis of epigenetic aberrations in diseases helps to identify dysfunctional genes and pathways, establish more robust cause–effect relationships than genetic studies alone, and identify new pharmaceutical targets and drugs, including nucleic acid reagents such as inhibitory RNAs. The emerging science of pharmacoepigenomics can impact the treatment of psychiatric and other complex diseases. In fact, some therapeutics now in use target epigenetic programming. In the near future, epigenetic interventions should help stabilize affected individuals and lead to prevention strategies.

KEYWORDS: bipolar disorder, brain, chromatin, DNA methylation, epigenetics, nutrition, pharmacoepigenomics, pharmacogenomics, psychiatry, schizophrenia

Hamid Mostafavi Abdolmaleky

EPIGENETIC PROGRAMMING

- In 1939, Waddington linked genetics, development and the environment together through the concept of epigenetic programming.
- Epigenetic programming refers to codes that are 'epi', imposed on top of genetic (DNA sequence) that allows short-term adaptation to the environment.
- By contrast, the genomic base sequence could be responsible for long-term adaptation and evolution.
- Today, epigenetics refers to reversible molecular changes to DNA, RNA or proteins (e.g., histones) that may be heritable but do not involve DNA base changes, and that regulate gene-expression, or RNA or protein function.

Variety of modifications

- Chromatin changes include post-synthetic covalent modification of histone proteins and DNA.
- RNA changes include covalent modification (i.e., addition of bases and or methyl groups), splicing, editing (base changes), and RNA interference of transcribed RNA.
- Post-translation protein modifications are numerous and include methylation, acetylation, phosphorylation, sumoylation, ubiquitination and so on.

Epigenetic programming of chromatin

- Epigenetic changes to chromatin fine-tune gene expression temporally in each cell during different developmental periods, and involve closely coordinated modification to both histone proteins and DNA.
- Although chromatin modification is initially modified shortly after DNA synthesis, subsequent alterations may occur in response to diverse ordinary or pathological environmental or biological factors.
- Note, it is not clear whether DNA or the histone code is the primary driver of chromatin epigenetic state, although it is likely to be some combinations and interactions.

- Generally, genomic chromatin codes are preserved through mitosis. However, during meiosis and the early development complex, differential global chromatin reprogramming occurs, some specific for male or female germline or somatic cells.
- Histones condense cellular DNA (2 m in length) to fit within the nucleus. In sperm, but not eggs, protamine (a small molecule) replaces histones increasing DNA condensation for packaging the haploid genome into the sperm heads [25].
- This means that there are no paternal histone modifications introduced into the fertilized eggs, and argues, at least for the paternal genome, that epigenetic DNA codes directly the formation of histone codes.

Imprinting

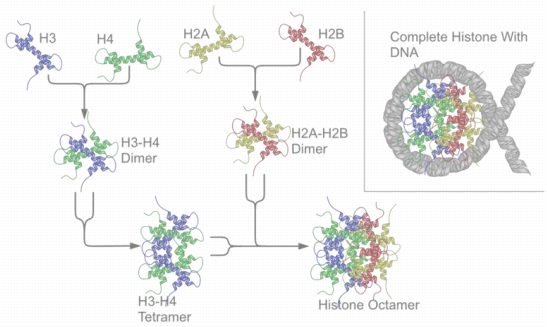
- Epigenetic programming is responsible for imprinting some genes with parental origin so that expression in progeny is parental origin dependent. Gene imprinting is proven for approximately 80 genes, and predicted for approximately 200 genes.
- In most cases, imprinting appears to be regional: approximately 20 regions for proven cases, and approximately 50 regions total for predicted genes.
- Curiously, in some regions, differential parental imprinting appears to occur. Many imprinted genes are linked to growth and development.

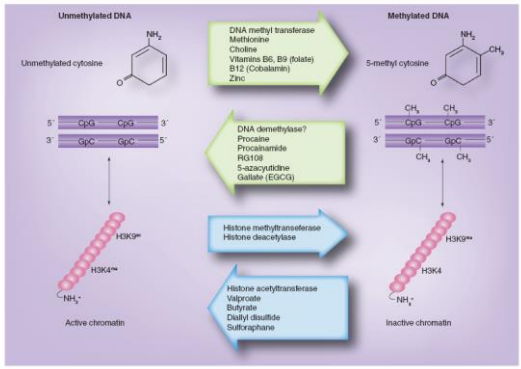
X inactivation

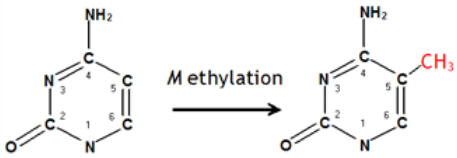
- In female cells, epigenetic changes turn off all gene expression from one of the X chromosomes early during embryogenesis. This insures that chromosome X gene-expression levels are similar for female (XX) and male (XY) cells; an attribute important for the developmental programs in multicellular sexual organisms.
- Usually, inactivation is random for each cell; thus phenotypically, females have an additional layer of complexity due to X expression mosaicism.
- Female cells have other layers of epigenetic complexity; the inactive X chromosome can be reactivated with age, and in some individuals there is a preferential inactivation of one X chromosome.

Chromatin structure & epigenetic codes

- The **nucleosome** is the basic structural unit of chromatin and is composed of 146 base pairs (bp) of DNA wrapped around four core histones, H2A, H2B, H3 and H4.
- Histone 1 binds to the 20–80 bp linker DNA at nucleosome entry and exit sites [20].
- The amino-terminal amino acids ('histone tails') are the major targets for various modifications.
- Histone 3 lysine 9 acetylation (H3K9ac) and histone 3 lysine 4 methylation (H3K4me) associate with unmethylated promoter DNA and transcriptionally active genes **(Figure 1).**







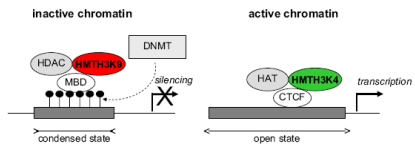
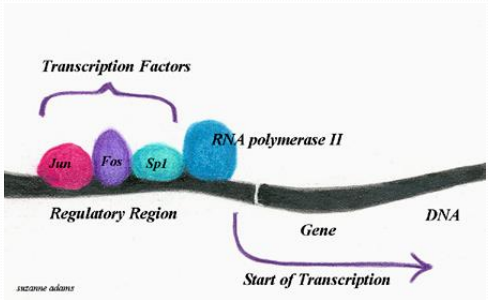


Figure 1. Epigenetic regulation of gene expression. In transcriptionally inactive regions of DNA, the CpG island recruits DNA methyltransferases (DNMT), methyl-CpG-binding proteins (MBD), histone methyltransferases (HMT) and histone deacetylases (HDAC), causing chromatin condensation and blocking transcriptional initiation. DNMTs promote DNA methylation (black lollipops). HMTs generate repressive histone methylation marks, such as lysine-9 and lysine-27 methylation on histone H3. In transcriptionally active regions of DNA, the CpG island recruits histone acetyltransferases (HAT) and HMT generating permissive histone methylation marks (such as lysine-4 methylation on histone H3). This recruitment, together with the binding of insulator proteins (such as CTCF), results in chromatin decondensation and transcriptional activity. At imprinted loci, one parental allele carries repressive epigenetic marks and the other has epigenetic modifications favouring transcription.

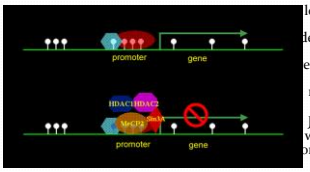
- DNMTs add **a** methyl group to the cytosine in promoter CpG islands to create a reversible modification of the DNA. HMT, HAT and other enzymes covalently modify histones.
- Chromatin modifications are closely coordinated in, an as yet, unknown fashion. For instance, methylated promoter DNA is linked to H_3K_4 in histone 3 and deacetylated lysine-9 and methylated lysine-9 (H_3K_9me).
- Usually, these modifications are associated with unexpressed genes. Unmethylated DNA is accompanied by acetylation of lysines including lysine-9 (H_3K_9ac) and methylation of lysine-4 of the histone-3 (H_3K_4me) tail.
- Enzymes, environmental factors (dietary components) and drugs impact the epigenomic state of chromatin. Aberrant chromatin modifications are linked to psychiatric diseases.
- The chromatin epigenetic state can be modulated and provides a promising platform for the development of novel therapeutics, even dietary manipulation for the prevention and treatment of psychiatric disease.
- Different chromatin states occur at genes linked to disease; hence, targeted epigenetic therapies (pharmacoepigenomics) may be needed to treat individuals. DNMTs: DNA methyl transferases; H_3K_4: Demethylated lysine-4; HAT: Histone acetyltransferases; HMT: Histone methyl transferases

DNA methylation

- Dense DNA methylation is associated with irreversible silencing of gene expression [5,27,28].
- Partial DNA methylation marks genes that may become unmethylated and be expressed, allowing for re-adaptation to a changing micro- or macro-environment (e.g., seasons, ecological conditions, nutritional habits and the demand of different developmental periods [see below]).
- Usually, DNA methylation targets promoter regions rather than specific dinucleotide sequences.
- However, methylation of 5′CpG3′ dinucleotides within the recognition sequences (e.g., GGGCGG and TGACGTCA) block binding of the cognate transcription factors, stimulatory protein 1 (SP1) and cAMP response element binding protein (CREB), decreasing gene expression (see next section)



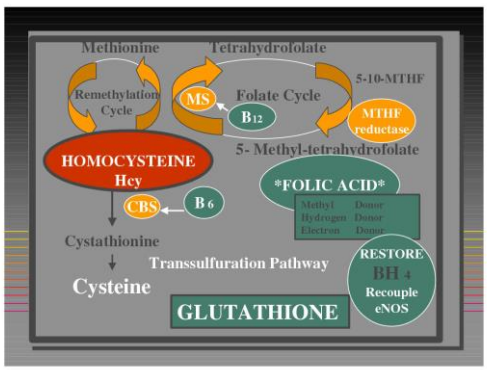
Rett syndrome



- Sometime between the age of 6 and 18 months, after a period of seemingly normal development, girls affected with Rett Syndrome lose interest in play; they gradually become withdrawn and anxious, develop autistic-like behaviors, and acquire specific symptoms like repetitive teeth-grinding and hand-wringing. This devastating neurological disease affects one in 15,000 female children
- Just five years ago, Rett Syndrome was tracked to mutations in a gene on the X chromosome, *MECP2*. But how this gene, not previously associated with the brain or nervous system, could cause a neurological developmental disorder remained a puzzle

Table 1. Potential MeCP2 target genes and their function

Potential MeCP2 target genes	Function	Ref.
LINE-1 (Long interspersed nuclear elements)	retrotransposons	46
Leukosialin	major sialoglycoprotein on the surfaces of hematopoietic cells; role in signal transduction and cell adhesion	47
BDNF (brain-derived neurotrophic factor)	neural plasticity, learning and memory	48, 49
DLX5 (distal-less homeobox 5)	production of enzymes that synthesize γ-aminobutyric acid (GABA)	51
Crh (corticotropin-releasing hormone)	corticotropin-releasing hormone production	53
FXYD1 (FXYD domain-containing transport regulator 1)	Phospholemman production (single spanning membrane protein that controls cell excitability by modulating Na^+/K^+-ATPase activity)	54



Dietary folate

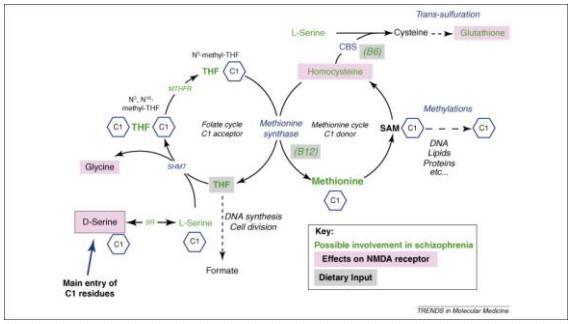
- S-adenosyl methionine is formed in the methionine cycle from adenosine and dietary methionine. Demethylated SAM (i.e., S-adenosyl homocysteine) is split into homocysteine and adenosine.
- Then, methionine is resynthesized through the addition of a methyl group from a **folate** (vitamin B9) derivative (5-methyl tetrahydrofolate), or betaine (a metabolic product of choline oxidation).

Environmental modulation of epigenetically important metabolites & disease

- Butyrate [36], tea polyphenols [37] and alcohol [38,39] change gene expression through epigenetic mechanisms.
- Environmental contaminants such as bisphenol A (used in plastic manufacturing, for instance in baby bottles, [40]), and heavy metals (e.g., lead, mercury, cadmium, iron) and other elements (e.g., arsenic, aluminum), [41,42] interfere with DNA methylation.
- In any event, it is clear that the impact of nutrition and environmental exposures on the epigenome will contribute to the disease and modulate the action of therapeutics.

Malnutrition

- Malnutrition can interfere with SAM synthesis and decrease methylation globally, especially in replicating cells.
- However, it is difficult to predict what will happen at a specific DNA sequence because the affinity of methylases for target cytosine residues will vary with sequence context, number of CpGs within a region, and the presence of proteins that promote/prevent enzyme binding.
- Several, but not all, studies have detected hyperhomocysteinemia in mood disorder and SCZ patients. Homocysteine-reducing and SAM promoting strategies (e.g., folic acid, cobalamin or pyridoxine nutritional supplementation) improved symptoms in SCZ patients with hyperhomocysteinemia.



Then, in 1966 Smythies formulated the hypothesis that transmethylation and the C1 cycle [in which methionine, S-adenosylmethionine (SAM) and folic acid are components] might be involved in schizophrenia [19]. SAM, the active form of methionine, is the main methyl donor for the majority of methyltransferases that modify DNA, RNA, histones and other proteins [20]. The interplay between folate, SAM, methionine, homocysteine and other molecules in these metabolic regulation loops is summarized in Box 1 and Figure 1.

- The methionine cycle allows the net transfer of a methyl group from N5-methyl-THF to methyl acceptors via methionine and the regeneration of SAM.
- Demethylation of SAM produces the potentially toxic compound homocysteine (Hcy) that can be remethylated into methionine by MS and vitamin B12 (MS transfers a methyl group from N5-methyl-THF to homocysteine).
- Folate or vitamin B12 deficiencies impair MS activity and result in reduced methionine and increased homocysteine levels. Homocysteine can also enter the trans-sulfuration pathway that leads to the production of cysteine and GSH.

- The prevailing hypothesis regarding schizophrenia is that a combination of genetic and environmental factors during critical periods of brain development increases the risk for this illness
- The so-called developmental hypothesis is supported by a wide range of studies. Various obstetric complications increase the risk of schizophrenia in offspring.
- Among these, there is convincing evidence that both folate deficiencies and high homocysteine levels during pregnancy are risk factors for schizophrenia.
- Secondly, other studies support a role for abnormal DNA methylation in schizophrenia
- Epigenetic regulation, in addition to mediating gene–environment interactions at the genome level, might account for variability in symptom severity, disease course and heritability of this illness

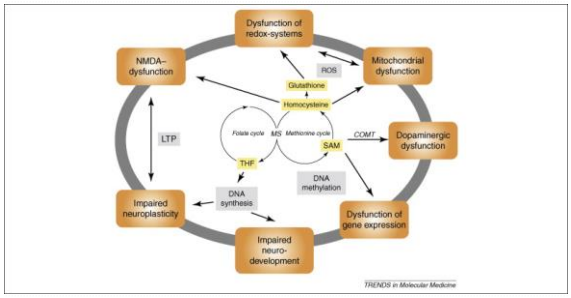
C1 HYPOTHESIS

- Thirdly, mounting evidence also supports the role of glutamate in the pathophysiology of schizophrenia.
- Postmortem brain studies have revealed alterations in pre- and postsynaptic markers for glutamatergic neurons, and pharmacological studies indicate that the dysfunction of N-methyl-D-aspartic acid (NMDA) glutamate receptors, in particular, might contribute to the symptomatology of this disease
- Lastly, the presence of mitochondrial anomalies in patients with schizophrenia has recently been reported.

Introducing the players: C1 metabolism, the methyl and folate cycles

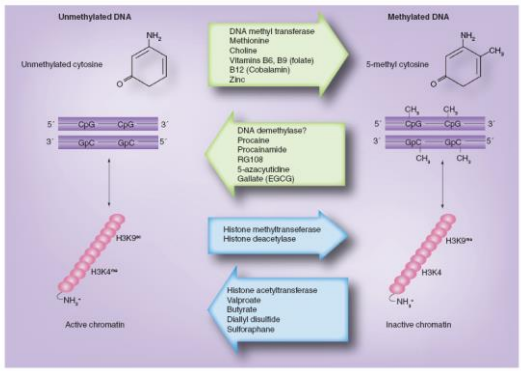
- Many biosynthetic processes involve the addition of a methyl group or an oxidated derivative (collectively a C1 unit) to a metabolic precursor.
- Methionine is the primary source for methyl residues, but methyl residues also originate from the conversion of serine to glycine.
- These processes occur via two metabolic cycles, the methionine and folate cycles, which converge on SAM, the ubiquitous methyl donor for transmethylation
- The methionine cycle refers to the methionine/SAM regeneration cycle and allows the net transfer of a methyl group via the regeneration of SAM.
- Upon demethylation, SAM is converted to homocysteine. This compound can, when accumulated, lead to neural damage and cognitive dysfunction.

- Firstly, remethylation into methionine is instrumental in keeping homocysteine levels down. This reaction is catalyzed by methionine synthase (MS) a rate-limiting enzyme dependent on folate and vitamin B12.
- MS is the link between the methyl and folate cycles.
- Nutritional, epidemiological and biological studies have shown that, in cases of folate deficiency, MS activity is low and, in turn, homocysteine accumulates.
- Secondly, homocysteine can also enter a secondary pathway that leads to the production of GSH (Box 2).
- In summary, methionine overload and folate depletion result in homocysteine accumulation through distinct mechanisms



Recent brain activity modulates epigenetic codes

- Several studies demonstrated that recent neuronal activation alters gene-expression and chromatin epigenetic codes.
- For example, neuronal depolarization induces the expression of the brain derived neurotrophic factor (BDNF) gene
- BDNF is a complex gene and some research indicates that there are nine promoters and 11 splicing variants for BDNF; at least one promoter is regulated by miRNA, and expression from at least two promoters is linked to DNA methylation and histone acetylation.



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Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, *RORA*, whose protein product is reduced in autistic brain

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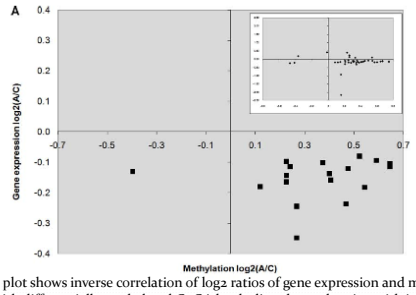
LBC cells MZ & DZ TWINS

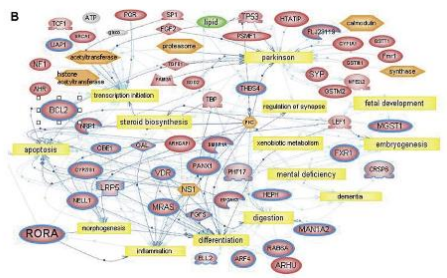
- Large-scale methylation profiling by CpG island microarray analysis of lymphoblastoid cell lines derived from monozygotic twins discordant for diagnosis of autism and their nonautistic siblings.
- Methylation profiling revealed many candidate genes differentially methylated between discordant MZ twins as well as between both twins and nonautistic siblings.

- The methylation status of 2 of these candidate genes, BCL-2 and retinoic acid-related orphan receptor alpha (RORA), was further confirmed by bisulfite sequencing and methylation-specific PCR, respectively.
- Immunohistochemical analyses of tissue arrays containing slices of the cerebellum and frontal cortex of autistic and age- and sex-matched control subjects revealed decreased expression of RORA and BCL-2 proteins in the autistic brain.

- The CpG island microarray analysis identified 73 CpG is- lands as differentially methylated between discordant monozygotic twins.
- Network analyses of the differentially methylated genes immediately at the 3′ end, or overlapping, the CpG islands revealed enrichment of high-level functions biologically relevant to the autism phenotype and included numerous genes involved in neurological disorders as well as nervous system development and function.



A) Scatter plot shows inverse correlation of log2 ratios of gene expression and methylation of genes with differentially methylated CpG islands directly overlapping with its 5′ end. Inset: plot of log2 ratios of genes with differentially methylated CpG islands located either upstream or downstream of the CpG island. Interestingly, the majority of genes identified from these analyses were hypermethylated in the twins relative to their respective nonautistic siblings, with a corresponding decrease in gene expression.



B) Network analysis of 25 genes (circumscribed in blue) that were both differentially methylated and expressed. The network was generated using Pathway Studio 5 network prediction software and identified common biological themes, including apoptosis, cellular differentiation, and inflammation. The analysis also revealed neurologically relevant functions and disorders including synaptic regulation, development, and

- Translation of these findings from LCLs to the detection of decreased BCL-2 and RORA protein in post mortem brain tissues of autistic individuals further confirms the feasibility of using LCLs as a surrogate model for autism, particularly when investigating dysregulated genes with systemic functions, such as apoptosis and circadian rhythm.
- In addition to identifying key autism candidate genes, these studies also yield further insight into the patho-biology of this complex disorder by elucidating global epigenomic modifications relevant to the autistic phenotype.

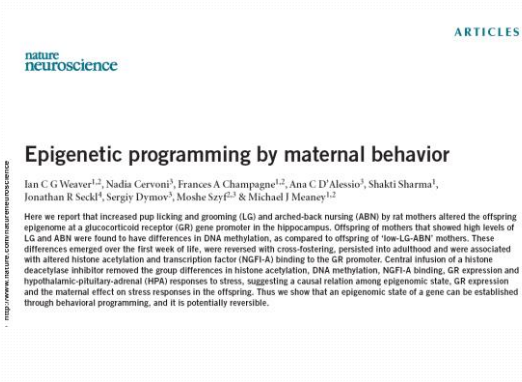
ot therapeutics

- Some psychiatric therapeutics are already in use to target the epigenome. Globally targeting agents include folic acid supplements that are efficacious in refractory major depression and SCZ and valproate, an inhibitor of HDACs widely used in the treatment of Bipolar Disorder.
- In fact, some consider valproate as an 'epigenetic softener'.

- Fluoxetine treatment, used to treat depression, induces expression of *MeCP2 and the MBD1 in* adult rat brains.
- These expression changes were accompanied by an increase in HDAC2 (histone deacetylase) mRNA synthesis, and a decrease in histone H3 acetylation.
- Perhaps, other psychotropic drugs that target the serotoninergic system or even serotonin itself, target the epigenome.

Drugs and DNA modifications

- Animal studies showed that derivatives of benzamide such as sulpiride (that target the dopamine D2 receptor), particularly MS-275, increase histone 3 acetylation at the RELN and glutamic acid decarboxylase 67 (GAD67) promoters in the frontal cortex.
- Haloperidol treatment alters global DNA methylation levels in rats
- Also, clozapine (an atypical antipsychotic drug) modulates histone 4 lysine 3 methylation (H4K3me) of GABAergic gene promoters in mice and in human brains by increasing the MLL1 gene expression (see above).


ARTICLES

nature neuroscience

Epigenetic programming by maternal behavior

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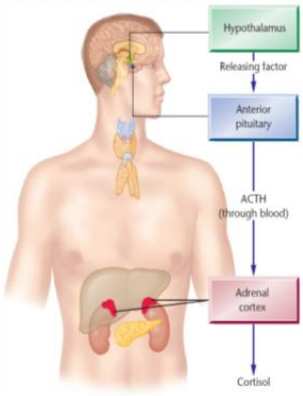
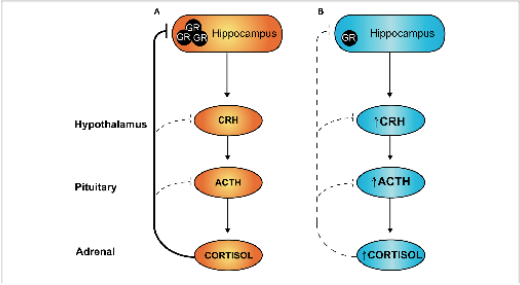
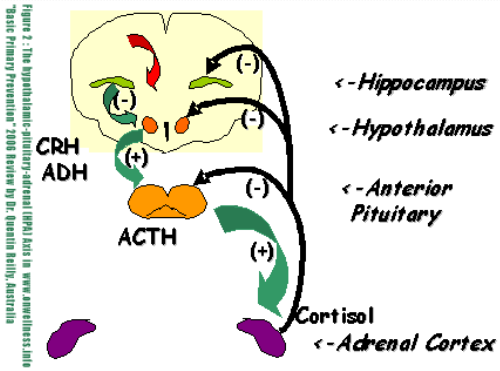
Here we report that increased pup licking and grooming (LG) and arched-back nursing (ABN) by rat mothers altered the offspring epigenome at a glucocorticoid receptor (GR) gene promoter in the hippocampus. Offspring of mothers that showed high levels of LG and ABN were found to have differences in DNA methylation, as compared to offspring of 'low-LG-ABN' mothers. These differences emerged over the first week of life, were reversed with cross-fostering, persisted into adulthood and were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter. Central infusion of a histone deacetylase inhibitor removed the group differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal (HPA) responses to stress, suggesting a causal relation among epigenomic state, GR expression and the maternal effect on stress responses in the offspring. Thus we show that an epigenomic state of a gene can be established through behavioral programming, and it is potentially reversible.







Maternal effects

- maternal effects influence the development of defensive responses to threat in organisms ranging from plants to mammals
- In the rat, such effects are mediated by variations in maternal behavior, which serve as the basis for the transmission of individual differences in stress responses from mother to offspring
- Mother-pup contact in the rat primarily occurs within the context of a nest-bout, which begins when the mother approaches the litter, **licks and grooms** her pups, and nurses while occasionally licking and grooming the pups.

Mechanisms by which maternal behavior moderates pup behavior

- There are stable individual differences in two forms of maternal behavior—LG and ABN (arched-back nursing)—over the first week of lactation. Such naturally occurring variations in maternal behavior are associated with the development of individual differences in behavioral and HPA responses to stress in the offspring.
- As adults, the offspring of 'high-LG-ABN' mothers are less fearful and show more modest HPA responses to stress than the offspring of 'low- LG-ABN' mothers.
- These findings suggest that variations in maternal behavior serve as a mechanism for the nongenomic transmission of individual differences in stress reactivity across generations
- The critical question concerns the mechanisms whereby these maternal effects, or other forms of environmental 'programming', are sustained over the lifespan of the animal.

HPA axis

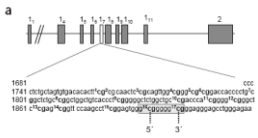
- Maternal behavior in the rat permanently alters the development of HPA responses to stress through tissue-specific effects on gene expression.
- The magnitude of the HPA response to acute stress is a function of hypothalamic corticotropin-releasing factor (CRF) release, which activates the pituitary-adrenal system.
- There are also modulatory influences, such as glucocorticoid negative feedback that inhibits CRF synthesis and release, thus dampening HPA responses to stress
- The adult offspring of high- compared with low-LG-ABN mothers show increased hippocampal GR expression and enhanced glucocorticoid feedback sensitivity.
- Predictably, adult offspring of high-LG-ABN mothers show decreased hypothalamic CRF expression and more modest HPA responses to stress.
- Eliminating the difference in hippocampal GR levels abolishes the effects of early experience on HPA responses to stress in adulthood, suggesting that the difference in hippocampal GR expression serves as a mechanism for the effect of early experience on the development of individual differences in HPA responses to stress5.

GR gene expression

- In vivo and in vitro studies suggest that maternal LG and ABN increase GR gene expression in the offspring through increased serotonin (5-HT) activity at 5-HT7 receptors, and the subsequent activation of cAMP and cAMP-dependent protein kinase activity
- Both the in vitro effect of 5-HT and the in vivo effect of maternal behavior on GR gene expression are accompanied by an increased hippocampal expression of nerve growth factor-inducible protein A (NGFI-A, a transcription factor also known as egr-1, krox-24, zenk and zif-268).

EXON 1(7) NGF1-A

- The non-coding exon 1 region of the hippocampal GR includes a promoter region, exon 17, containing a binding site for NGFI-A16 (Fig. 1a).
- Splice variants of the GR mRNA containing the exon 17 sequence are found predominantly in the brain, and the expression of GR mRNAs containing the exon 17 sequence is increased in the offspring of high-LG-ABN mothers or following manipulations that increase maternal licking and grooming suggesting that the use of this promoter is enhanced as a function of maternal care
- Sequence map of the exon 17 GR promoter including the 17 CpG dinucleotides (bold) and the NGFI-A binding region16 (encircled).



- Although these findings might explain the increased GR expression in the neonate, we are left with the question of how the effect of maternal care might persist into adulthood
- Gene expression is controlled by the epigenome, which is comprised of chromatin structure and DNA methylation.
- We tested the hypothesis that **maternal care alters DNA methylation of the GR exon 17 promoter, and that these changes are stably maintained into adulthood and associated with differences in GR expression and HPA responses to stress.**

Maternal care and methylation of exon 17 promoter

- DNA methylation is a stable, epigenomic mark at CpG dinucleotides often associated with stable variations in gene transcription. Two kinds of changes in DNA methylation are known to affect gene expression: regional, non-site specific DNA methylation around a promoter and site-specific methylation.
- Hypomethylation of CpG dinucleotides of regulatory regions of genes is associated with active chromatin structure and transcriptional activity
- Thus, the methylation pattern is a stable signature of the epigenomic status of a regulatory sequence. We focused on the methylation state of the exon 17 GR promoter, which is activated in the hippocampus in offspring of high-LG-ABN mothers.

- To determine whether DNA methylation of specific target sites on the GR promoter change in response to maternal care, we mapped differences in the methylation status of individual cytosines within the CpG dinucleotides of the exon 17 promoter from hippocampal tissue from the adult offspring of high- and low-LG-ABN mothers.
- We used sodium bisulfite mapping, with a particular interest in the region around the NGFI-A consensus sequence (**Fig. 1a**)

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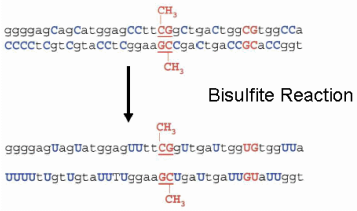
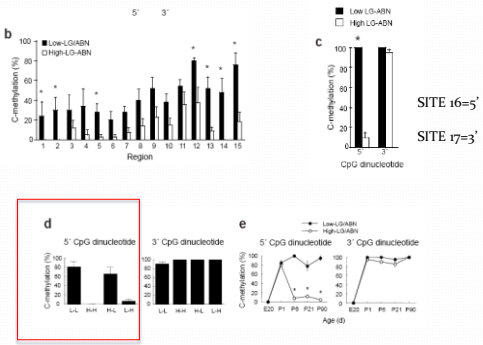
- We used pyrosequencing (23) of bisulfite treated buccal DNA to examine the methylation status of the NR3C1 exon 1F promoter sequence in nonclinical subjects.





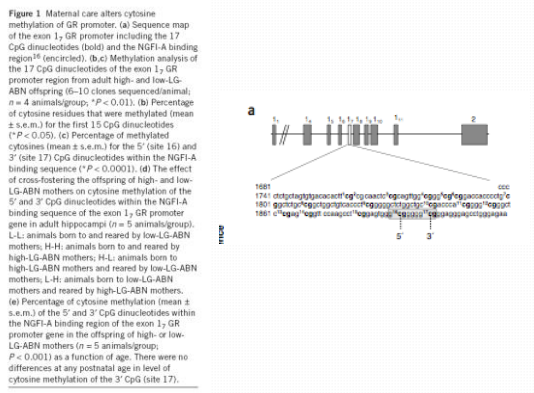


Figure 1 Maternal care alters cytosine methylation of GR promoter. (a) Sequence map of the exon 1_7 GR promoter including the 17 CpG dinucleotides (bold) and the NGFI-A binding region[16] (encircled). (b,c) Methylation analysis of the 17 CpG dinucleotides of the exon 1_7 GR promoter region from adult high- and low-LG-ABN offspring (6–10 clones sequenced/animal; $n = 4$ animals/group; $^*P < 0.01$). (b) Percentage of cytosine residues that were methylated (mean ± s.e.m.) for the first 15 CpG dinucleotides ($^*P < 0.05$). (c) Percentage of methylated cytosines (mean ± s.e.m.) for the 5′ (site 16) and 3′ (site 17) CpG dinucleotides within the NGFI-A binding sequence ($^*P < 0.0001$). (d) The effect of cross-fostering the offspring of high- and low-LG-ABN mothers on cytosine methylation of the 5′ and 3′ CpG dinucleotides within the NGFI-A binding sequence of the exon 1_7 GR promoter gene in adult hippocampi ($n = 5$ animals/group). L-L: animals born to and reared by low-LG-ABN mothers; H-H: animals born to and reared by high-LG-ABN mothers; H-L: animals born to high-LG-ABN mothers and reared by low-LG-ABN mothers; L-H: animals born to low-LG-ABN mothers and reared by high-LG-ABN mothers. (e) Percentage of cytosine methylation (mean ± s.e.m.) of the 5′ and 3′ CpG dinucleotides within the NGFI-A binding region of the exon 1_7 GR promoter gene in the offspring of high- or low-LG-ABN mothers ($n = 5$ animals/group; $P < 0.001$) as a function of age. There were no differences at any postnatal age in level of cytosine methylation of the 3′ CpG (site 17).

Site-specific methylation patters

- The results showed significant differences in the methylation of specific sites of the exon 17 GR promoter sequence (Fig. 1b,c).
- A two-way ANOVA revealed a highly significant effect of Group ($F = 55.9$, $P < '0.0001$) and Region ($F = 27.7$, $P < 0.0001$), as well as a significant Group Region interaction effect ($F = 27.7$, $P < 0.0001$).
- Importantly, the cytosine residue within the 5′ CpG dinucleotide **(site 16)** of the NGFI-A consensus sequence (Fig. 1c) is always methylated in the offspring of low-LG-ABN mothers, and rarely methylated in the offspring of high-LG-ABN dams.
- In contrast, the 3′ CpG dinucleotide **(site 17)** remains methylated, regardless of differences in maternal care.
- Dissected hippocampi inevitably contain glial cells as well as neurons. Considering the pronounced effect of maternal care on the methylation status of the 5′ CpG dinucleotide of the NGFI-A response element (>90%), the effect of maternal care must include neuronal as well as glial cells; both populations express GR23,24 and NGFI-A25 genes.

Cross-fostering reveals epigenetic marking by maternal behavior

- Our findings suggest that specific sites within the exon 17 GR promoter are differentially methylated as a function of maternal behavior, but these findings are merely correlational.
- To directly examine the relation between maternal behavior and DNA methylation within the exon 17 promoter, we performed an adoption study in which the biological offspring of high- or low-LG-ABN mothers were cross-fostered to either high- or low-LG-ABN dams within 12 h of birth.
- Cross-fostering produced a pattern of exon 17 promoter methylation that was **associated with the rearing mother** ($F = 4.8$, $P < 0.05$; Fig. 1d) and **thus reversed the difference in methylation at specific cytosines**, notably at the 5′ CpG dinucleotide (site 16) of the NGFI-A consensus sequence (Fig. 1d, left panel).

- Thus, in the low-LG-ABN offspring that were fostered to high-LG-ABN dams, methylation of this 5′ site within the exon 17 promoter was indistinguishable from that of the biological offspring of high-LG-ABN mothers. Likewise, the methylation of the same 5′ CpG dinucleotide in the biological offspring of high-LG-ABN mothers reared by low- LG-ABN dams was comparable to that of low-LG-ABN offspring.
- There was no effect of cross-fostering at the cytosine within the 3′ CpG dinucleotide (site 17; **Fig. 1d).**

- These findings suggest that variations in maternal care directly alter the methylation status of the exon 17 promoter of the GR gene.
- Thus we have demonstrated that a DNA methylation pattern can be established through a behavioral mode of programming **without germ line transmission**.
- In parental imprinting, a well-established paradigm of inheritance of an epigenomic mark, the paternally and maternally inherited alleles are differentially methylated.
- These methylation patterns are **defined during maturation of spermatocytes and oocytes**, and are transmitted to the offspring through the germ line

Timing of the maternal effect on DNA methylation

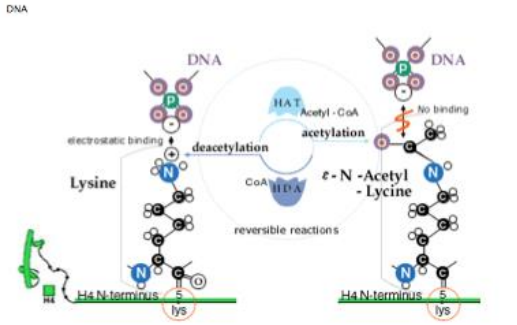
- The maternal care of high- and low-LG-ABN mothers differs only during the first week of life. Thus, we wondered whether this period corresponds to the timing for the appearance of the difference in DNA methylation in the offspring.
- (Fig. 1e). Statistical analysis of the data for the 5′ CpG (site 16) revealed a highly significant effect of Group ($F = 66.7$, $P < 0.0001$) and Age ($F = 21.1$, $P < 0.0001$) as well as a significant interaction effect ($F = 13.7$, $P < 0.0001$).
- Tukey post-hoc analysis revealed that the Group effect on methylation status of the 5′ CpG (site 16) was s**ignificant at P6, P21 and P90 ($P < 0.001$), but not at E20 or P1**.
- Just before birth (embryonic day 20; E20) the entire region was unmethylated in both groups. **Strikingly, one day after birth (postnatal day 1; P1) the exon 17 GR promoter was *de novo* methylated in both groups.**

- The 5′ and 3′ CpG sites of the exon 17 GR NGFI-A response element in the offspring of both high- and low- LG-ABN mothers, which exhibit differential methylation later in life, were de novo methylated to the same extent.
- These data show that both the basal state of methylation and the first wave of de novo methylation after birth occur similarly in both groups.
- Whereas it is generally accepted that DNA methylation patterns are formed prenatally and that de novo methylation occurs early in development, there is at least one documented example of postnatal de novo methylation of the Hoxa5 and Hoxb5 genes.
- Because similar analyses are not documented for other genes, it remains unknown whether changes in methylation are common around birth or **whether they are unique to this GR promoter**.

- The differences in the methylation status of the exon 17 GR promoter between the two groups developed between P1 and P6, the period when differences in the maternal behavior of high- and low- LG-ABN dams are apparent5,8. By P6, the NGFI-A response element 5′ CpG dinucleotide (site 16) was effectively 'demethylated' in the high-, but not in the low-LG-ABN group.
- The group difference in CpG dinucleotide methylation remains consistent through to adulthood (P90; Fig. 1e).
- These findings, together with those of the crossfostering study, suggest that the group difference in DNA methylation occurs as a function of a maternal behavior over the first week of life.
- The results of earlier studies indicate that the first week of postnatal life is a 'critical period' for the effects of early experience on hippocampal GR expression.

Maternal effects on chromatin structure and NGFI-A binding

- The next question concerns the functional importance of such differences in methylation.
- DNA methylation is associated with changes in chromatin activity states. Chromatin gates the accessibility of promoters to transcription factors.
- Histone acetylation at the lysine-9 (K9) residue of H3 and H4 histones is a well-established marker of active chromatin.
- Acetylation of the histone tails neutralizes the positively charged histones, which disrupts histone binding to negatively charged DNA and thus promotes transcription factor binding.

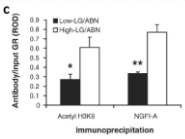


histone acetylation

- We tested the hypothesis that the maternal effect on DNA methylation results in (i) increased histone acetylation at the K9 residue of the H3 histone(s) associated with the exon 17 GR promoter and (ii) increased interaction between NGFI-A and the promoter sequence.
- We performed a chromatin immunoprecipitation (ChIP) analysis of histone H3-K9 acetylation and NGFI-A protein binding to the exon 17 GR promoter in the native chromatin environment in vivo.
- Intact hippocampi from adult offspring of high- and low-LG-ABN mothers were crosslinked in vivo by paraformaldehyde perfusion. We then selectively immunoprecipitated protein-DNA complexes with either an acetylated H3-K9 histone primary antibody or an NGFI-A primary antibody.
- The protein-DNA complexes were uncrosslinked, and the precipitated genomic DNA was subjected to PCR amplification with primers specific for the exon 17 GR promoter sequence.

Maternal programming

- There were significant Group effects for the association of both histone H3-K9 acetylation (*t = 2.1, *P < 0.001) and NGFI-A (t = 3.1, **P < 0.0001) with* the exon 17 GR promoter sequence.
- These results indicated significantly greater histone H3-K9 acetylation association and threefold greater binding of NGFI-A protein to the hippocampal exon 17 GR promoter in the adult offspring of high- compared with low-LGABN mothers (Fig. 2).
- Thus, maternal programming of the exon 17 GR promoter involves DNA methylation, histone H3-K9 acetylation and alterations in NGFI-A binding.

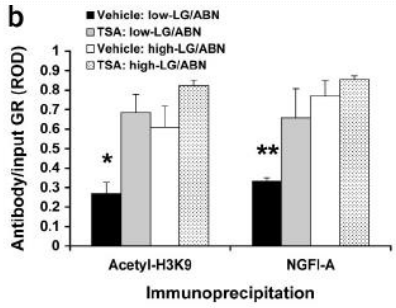


Reversal of maternally mediated epigenetic marking

- A critical question is whether the impact of early experience is reversible and whether epigenetic programming is modifiable in adult, postmitotic tissues? The generally accepted model is that the DNA methylation pattern is an irreversible reaction in adult post-mitotic cells.
- However, recent data from in vitro experiments suggests that in certain instances it is possible to induce replication-independent demethylation of ectopically methylated genes by increasing histone acetylation using the histone deacetylase (HDAC) inhibitor trichostatin A (TSA)

- Cytosine methylation attracts methylated DNA binding proteins and HDACs that prevent histone acetylation and thus transcription factor binding. Activation of chromatin through HDAC inhibition might trigger DNA demethylation by increasing the accessibility of DNA to demethylase activity
- We tested the hypothesis that inhibition of HDACs with TSA would result in increased K9 acetylation of H3-histones associated with the exon 17 GR promoter, DNA demethylation, NGFI-A binding and reversal of maternal programming of stress responses in the adult offspring of low-LG-ABN mothers.

- We first used ChIP analysis to determine whether histone H3-K9 acetylation and NGFI-A protein binding to the exon 17 GR promoter is altered in the offspring of high- and low-LG-ABN mothers through intracerebroventricular (i.c.v.) infusion of the adult offspring with TSA (100 ng/ml) or vehicle
- greater histone H3-K9 acetylation association and more binding (>3 fold) of NGFI-A protein (transcription factor) to the hippocampal exon 17 GR promoter in the adult offspring of TSA-treated low-LG-ABN mothers compared with the vehicle-treated offspring of low-LG-ABN mothers **(Fig. 3);**



- There were no significant differences between TSA treated offspring of low-LG-ABN mothers and either TSA- or vehicle- treated offspring of high-LG-ABN dams.
- As expected, TSA treatment did not change histone H3-K9 acetylation or NGFI-A binding in the adult offspring of high-LG-ABN mothers, because the GR exon 17 promoter region in the offspring of high-LG-ABN mothers is normally associated with acetylated histones and highly bound with NGFI-A.

- To determine whether TSA treatment reverses the maternal effect on methylation within specific CpG dinucleotides on the exon 17 GR promoter, we mapped the differences in methylation using the sodium bisulfite technique, focusing on the NGFI-A consensus sequence within the exon 17 region (Fig. 1a).
- The results again revealed significant differences in the methylation of a number of regions of the exon 17 GR promoter sequence (Fig. 4) with significant differences within the 5′ CpG (site 16) and 3′ CpG (site 17) dinucleotides of the NGFI-A consensus sequence (Fig. 4b).
- Post-hoc analysis revealed that TSA treatment significantly decreased the degree of cytosine methylation within the 5′ **(site 16)** CpG dinucleotide of the NGFI-A binding region of the exon 17 GR promoter in the offspring of low-LG-ABN mothers in comparison to vehicle-treated low-LG-ABN mothers (*$P < 0.001$).

- TSA treatment produced 'demethylation' of the 5′ CpG (site 16) and 3′ CpG (site 17) dinucleotides in the offspring of low-LG-ABN mothers, and hypomethylation of the 3′ CpG (site 17) dinucleotide in the offspring of high-LG-ABN mothers (Fig. 4b).
- These findings suggest that TSA treatment can reverse the hypermethylated status of the exon 17 GR promoter in the offspring of low-LG-ABN mothers.
- TSA treatment resulted in a more extensive change in DNA methylation than maternal care per se, since the 3′ CpG (site 17) dinucleotide, which is unaffected by maternal behavior, is partially 'demethylated' in response to TSA treatment in both cohorts (Fig. 4b).

- Thus, stable DNA methylation marking by maternal behavior is reversible in the adult offspring hippocampus by pharmacological modulation of chromatin structure.
- While TSA altered the methylation of the both the 5′ and 3′ CpG sites within the NGFI-A response element, the former appears to be critical for the effect on NGFI-A binding to the exon 17 promoter.

Reversal of maternal effect on GR expression

- GR gene expression in the hippocampus is increased in the adult offspring of high- compared with low-LG-ABN mothers
- We suggest that such differences are mediated by the differential methylation of the 5′ CpG dinucleotide (site 16) of the NGFI-A consensus sequence in the exon 17 GR promoter and the subsequent alteration of histone acetylation and NGFI-A binding to the exon 17 sequence.
- If the differential epigenetic marking regulates the expression of the exon 17 GR promoter in high- versus low-LG offspring, then reversal of the epigenetic marking should be accompanied by an increase in hippocampal GR expression
- This hypothesis is supported by the results (Fig. 5a) showing that hippocampal GR expression was significantly increased in TSA-treated offspring of low-LG-ABN mothers to levels that were comparable to those of either the vehicle- or TSA-treated offspring of high-LG-ABN mothers.

Reversal of maternal effect on HPA responses to stress

- As adults, the offspring of high-LG-ABN mothers show more modest HPA responses to stress than the offspring of low-LG-ABN mothers.
- The effect of maternal care on HPA responses to stress seems to be, in part, associated with differences in hippocampal GR levels and glucocorticoid negative feedback sensitivity.
- Given that TSA treatment reversed the group difference in hippocampal GR expression, we examined the adrenocortical responses to stress in a separate cohort of vehicle- and TSA-treated animals.
- Central infusion of TSA completely eliminated the maternal effect on HPA responses to acute stress (Fig. 5b).

- In summary, central infusion of the HDAC inhibitor TSA enhanced histone H3-K9 acetylation of the exon 17 GR promoter in the offspring of the low-LG-ABN mothers, increased NGFI-A binding to its cognate sequence, induced hypomethylation of CpG dinucleotide sequences in the promoter and eliminated the maternal effect on hippocampal GR expression and the HPA response to stress.
- These findings are consistent with idea that the maternal effect on GR expression and HPA responses to stress is mediated by alterations in chromatin structure.
- We propose that the reduced binding of NGFI-A to its response element on the hypoacetylated and hypermethylated hypermethylated exon 17 GR promoter contributes to the attenuation of GR expression in low-LG-ABN adult offspring,
- whereas increased NGFIA binding to the hyperacetylated and hypomethylated response element on the exon 17 GR promoter in the offspring of the high-LG-ABN mothers would serve to maintain the differences in gene expression.
- DNA methylation represents a stable epigenetic mark; therefore, our findings provide an explanation for the enduring effect on mother-infant interactions over the first week of postnatallife on HPA responses to stress in the offspring.

Discussion

- Nevertheless, our findings provide the first evidence that maternal behavior produces stable alterations of DNA methylation and chromatin structure, providing a mechanism for the long-term effects of maternal care on gene expression in the offspring.
- These studies offer an opportunity to clearly define the nature of gene- environment interactions during development and how such effects result in the sustained 'environmental programming' of gene expression and function over the lifespan.
- It is important to note that maternal effects on the expression of defensive responses, such as increased HPA activity, are a common theme in biology such that the magnitude of the maternal influence on the development of HPA and behavioral responses to stress in the rat should not be surprising.

- Such effects commonly follow from the exposure of the mother to the same or similar forms of threat and may represent examples whereby the experience of the mother is translated through an epigenetic mechanism of inheritance into phenotypic variation in the offspring. Thus, maternal effects could result in the transmission of adaptive responses across generations
- Indeed, among mammals, natural selection may have shaped offspring to respond to subtle variations in parental behavior as a forecast of the environmental conditions they will ultimately face once they become independent of the parent

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Research Paper

Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (*NR3C1*) and infant cortisol stress responses

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Key words: *NR3C1* methylation, prenatal maternal depression, hypothalamicpituitaryadrenal, HPA, infant stress response

Background: In animal models, variations in early maternal care are associated with differences in hypothalamic-pituitary-adrenal (HPA) stress response in the offspring, mediated via changes in the epigenetic regulation of glucocorticoid receptor (GR) gene (*Nr3c1*) expression.

Objective: To study this in humans, relationships between prenatal exposure to maternal mood and the methylation status of a CpG-rich region in the promoter and exon 1F of the human GR gene (*NR3C1*) in newborns and HPA stress reactivity at age three months were examined.

Introduction

Early life experience can set a course leading to health or disease, often mediated by an altered capacity to regulate responses to stressful events.[1] Among the earliest adverse experience is prenatal exposure to maternal depressed and anxious mood that confers life long risk for behavioral disturbances in childhood and beyond.[2] This process of "fetal programming" is thought to be in part mediated by the impact of prenatal experience on the developing hypothalamic-pituitary-adrenal (HPA) axis stress system.[3,4] The HPA axis is a dynamic metabolic system that regulates homeostatic mechanisms, ...

ABSTRACT

- Objective: To study this in humans, relationships between prenatal exposure to maternal mood and the methylation status of a CpG-rich region in the promoter and exon 1F of the human GR gene (*NR3C1) in newborns and HPA stress reactivity at age three* months were examined.
- Results: Prenatal exposure to increased third trimester maternal depressed/anxious mood was associated with increased methylation of *NR3C1 at a predicted NGFI-A binding site. Increased NR3C1* methylation at this site was also associated with increased salivary cortisol stress responses at 3 months, controlling for prenatal SRI exposure, postnatal age and pre and postnatal maternal mood.
- Methods: The methylation status of a CpG-rich region of the *NR3C1 gene, including exon 1F, in genomic DNA from cord blood* mononuclear cells was quantified by bisulfite pyrosequencing in infants of depressed mothers treated with a serotonin reuptake inhibitor antidepressant (SRI) (n = 33), infants of depressed non treated mothers (n = 13) and infants of non depressed/non treated mothers (n = 36). To study the functional implications of the newborn methylation status of *NR3C1 in newborns, HPA function* was assessed at three months using salivary cortisol obtained before and following a non noxious stressor and at a late afternoon basal time.

Future studies

- Relationships between glucocorticoid receptor expression in a heterogeneous population of **venous cord mononuclear cells**, hippocampal NR3C1 expression and HPA stress responses in newborns needs to be determined.
- Further studies are needed to determine links between the methylation status of this gene in these cell types and methylation status in central and peripheral (i.e., adrenal) regions more directly related to the stress response.
- Studies examining infant HPA reactivity under other provocative challenges (social, emotional, physical), with infants of mothers with a wider range of mood symptoms are also required.
- Finally, studies of links between methylation status of CpG3, human NR3C1 (i.e. mRNA) and links to subsequent HPA function later in infancy are needed to elucidate the functional implications of how early exposure to maternal depressed and anxious mood epigenetically influences infant and child development.

ARTICLES

nature neuroscience

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

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Maternal care influences hypothalamic-pituitary-adrenal (HPA) function in the rat through epigenetic programming of glucocorticoid receptor expression. In humans, childhood abuse alters HPA stress responses and increases the risk of suicide. We examined epigenetic differences in a neuron-specific glucocorticoid receptor (***NR3C1***) promoter between postmortem hippocampus obtained from suicide victims with a history of childhood abuse and those from either suicide victims with no childhood abuse or controls. We found decreased levels of glucocorticoid receptor mRNA, as well as mRNA transcripts bearing the glucocorticoid receptor 1_F splice variant and increased cytosine methylation of an ***NR3C1*** promoter. Patch-methylated ***NR3C1*** promoter constructs that mimicked the methylation state in samples from abused suicide victims showed decreased NGFI-A transcription factor binding and NGFI-A–inducible gene transcription. These findings translate previous results from rat to humans and suggest a common effect of parental care on the epigenetic regulation of hippocampal glucocorticoid receptor expression.

Decreased GR expression

- Familial function and childhood adversity are linked to altered HPA stress responses in humans, which are associated with an increased risk for multiple forms of psychopathology.
- There is evidence for decreased hippocampal glucocorticoid receptor expression in several psychopathological conditions associated with suicide, including schizophrenia and mood disorders.
- Suicide is also strongly associated with a history of childhood abuse and neglect, and this effect is independent of that associated with psychopathology.
- Thus, environmental events that associate with decreased hippocampal glucocorticoid receptor expression and increased HPA activity enhance the risk of suicide.

Post-mortem study of GR

- The effects of maternal care on hippocampal glucocorticoid receptor expression, and therefore HPA responses to stress, in the adult rodent are associated with an epigenetic modification of a neuron-specific exon 1_7 glucocorticoid receptor (*Nr3c1*) promoter.
- We attempted to translate these findings to humans. We examined glucocorticoid receptor expression and *NR3C1* promoter methylation in hippocampal samples obtained from **suicide victims and control subjects** who died suddenly of unrelated causes.

- Suicide victims were either positive or negative for history of childhood abuse (sexual contact, severe physical abuse and/or severe neglect), allowing for the separation of the effects associated with childhood abuse from those associated with suicide *per se*.
- Our controls were all negative for a history of childhood abuse.

expression

- The human glucocorticoid receptor gene *NR3C1* covers a region of more than 80 kb in chromosome 5, containing eight coding exons (exons 2–9) and alternative 5′ noncoding exon 1s.
- The 5′ untranslated region (UTR) of exon 1 of the *NR3C1* gene determines the tissue-specific expression.
- The 5′ UTR of *NR3C1* contains 11 exon 1 splice variants, all of which bear unique splice donor sites and share a common exon 2 splice acceptor site.
- Exon 1_F of *NR3C1* is similar to the rat exon 1_7, which reveals a maternal effect on cytosine methylation and expression.

Hippocampus

- Because individuals with severe forms of major depression show decreased glucocorticoid receptor expression and increased HPA activity, we hypothesized that suicide victims would show decreased expression both of glucocorticoid receptor and glucocorticoid receptor 1_F compared with control subjects.

- We examined the expression of total glucocorticoid receptor and glucocorticoid receptor 1_F using quantitative reverse transcription PCR (qRT-PCR) with RNA extracted from hippocampal tissue of suicide completers with (n = 12) and without (n = 12) a history of childhood abuse and from controls (n = 12).
- There was a significant effect on glucocorticoid receptor expression (F = 3.17, P = 0.05).
- *Post hoc* tests showed that expression of total glucocorticoid receptor mRNA was significantly reduced in suicide victims with a history of childhood abuse relative to nonabused suicide victims or controls (P < 0.05);
- there was no difference between nonabused suicide victims and controls (P > 0.05; Fig. 1a).
- There was also a significant effect on the expression of transcripts containing the exon 1_F*NR3C1* promoter (F = 3.58, P < 0.05).

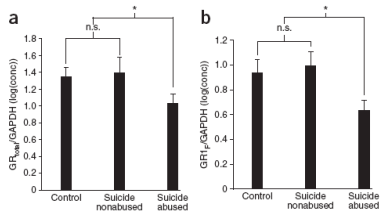

Figure 1 Hippocampal glucocorticoid receptor expression. (**a**,**b**) Mean ± s.e.m. expression levels of total glucocorticoid receptor (GR) mRNA (**a**) and glucocorticoid receptor 1_F (GR1_F) in 12 suicide victims with a history of childhood abuse, 12 nonabused suicide victims and 12 control subjects (**b**). Outliers excluded from analysis included n = 2 control subjects, n = 1 suicide victims with a history of childhood abuse for glucocorticoid receptor 1_F and an additional n = 1 suicide victim with a history of childhood abuse, and n = 3 nonabused suicide victims for overall levels of glucocorticoid receptor. * indicates P < 0.05; n.s. indicates not statistically significant.

Genotyping and methylation analysis

- Because alterations in glucocorticoid receptor 1_F activity could be derived from nucleotide sequence variation and/or epigenetic modifications, we sequenced the *NR3C1* promoter region from each subject.
- No sequence variation was seen among subjects and all of the sequences were identical to those published previously[19].
- Moreover, for each subject, the genomic sequences targeted for binding by the primers used for bisulfite mapping were identical to the published sequence[19], thus eliminating potential primer bias between subjects in sodium bisulfite mapping.

Bisulfite sequencing

- The rat homolog of the exon 1_F*NR3C1* promoter, the exon 1_7 region, is differentially methylated as a function of variations in maternal care[4, 17, 22]. Cytosine methylation is a highly stable epigenetic mark that regulates gene expression via its effects on transcription factor binding[23, 24].
- We used sodium bisulfite mapping[25] to examine the methylation status of individual CpG dinucleotides in the *NR3C1* promoter sequence extracted from the hippocampal tissue of the same subjects used for glucocorticoid receptor expression analysis.
- Sodium bisulfite mapping revealed a significant effect on the percentage of methylated clones (that is, the number of clones with at least one methylated CpG site divided by the total number of clones) between groups ($F = 3.47$, $P < 0.05$).

- *Post hoc* tests revealed a significant difference between suicide victims with a history of childhood abuse compared with nonabused suicide victims ($P = 0.05$) or controls ($P < 0.05$).
- There was no difference in the percentage of methylated clones between suicide victims without childhood abuse and controls ($P > 0.05$; Fig. 2a).
- Methylation was limited to specific sites, with no clone showing global methylation (Fig. 2b).
- There were no significant correlations between levels of exon 1_F methylation and age at death ($r = 0.15$, $P > 0.05$), brain pH ($r = 0.08$, $P > 0.05$) or postmortem interval (PMI, $r = 0.24$, $P > 0.05$; Table 1).

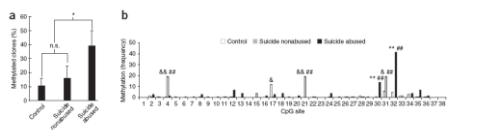

Figure 2 Methylation of the *NR3C1* promoter in the hippocampus. Twenty clones were sequenced for each subject for methylation mapping. (**a**) Mean ± s.e.m. percentage of methylated clones for suicide victims with a history of childhood abuse ($n = 12$), suicide victims without a history of childhood abuse ($n = 12$) and controls ($n = 12$). The methylation percentage was calculated as the number of clones with at least one methylated CpG site divided by the total number of clones (* indicates $P \leq 0.05$; n.s. indicates not statistically significant). (**b**) Methylation of the *NR3C1* promoter region, showing the frequency of methylation observed at each CpG site for suicide victims with a history of childhood abuse, suicide victims with no history of childhood abuse and control subjects (*$P < 0.05$, **$P < 0.001$, abused suicides versus controls; $^{\$}P < 0.05$, $^{\$\$}P < 0.001$, non-abused suicides versus controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$, abused suicides versus non-abused suicides; Bonferroni *post hoc* comparisons).

- These results indicate that methylation attenuates NGFI-A induction of gene expression through the *NR3C1* promoter.
- However, the decreased glucocorticoid receptor transcription observed in suicide victims with a history of childhood abuse was associated with differences in methylation levels occurring only at specific sites in the exon 1_F*NR3C1* promoter (Fig. 2b).
- An ANOVA examining the methylation of CpG dinucleotides across the exon 1_F*NR3C1* promoter revealed a significant effect of CpG site ($F = 13.86$, $P < 0.0001$), a significant effect of group ($F = 17.12$, $P < 0.0001$) and a significant interaction between CpG site and group ($F = 13.44$, $P < 0.0001$). In NGFI-A recognition elements, methylation was observed at CpG sites 12, 13, 30, 31 and 32 (Fig. 2b).

Discussion

- Our findings indicate that hippocampal *NR3C1* gene expression is decreased in samples from suicide victims with a history of childhood abuse compared with controls (victims of sudden, accidental death with no history of abuse).
- In contrast, we found no differences in glucocorticoid receptor expression between suicide victims without a history of childhood abuse and controls.

- The pattern of results for hippocampal expression of the glucocorticoid receptor 1_F variant was identical to that of total glucocorticoid receptor expression.
- Our findings suggest that changes in glucocorticoid receptor expression are closely associated with a developmental history of familial adversity, in this case a history of childhood abuse, than with suicide completion.
- These results are also similar to those of earlier reports in which childhood abuse was associated with an increase in pituitary adrenocorticotropic hormone (ACTH) responses to stress among individuals with or without concurrent major depression[a].
- These findings are particularly relevant, as pituitary ACTH directly reflects central activation of the HPA stress response and **hippocampal glucocorticoid receptor activation dampens HPA activity.**

- Our findings are also consistent with those from studies with rodent and nonhuman primates showing that persistent disruptions of mother-infant interactions are associated with increased hypothalamic corticotrophin-releasing hormone expression and increased HPA responses to stress.
- Variations in maternal care in the rat influence hippocampal glucocorticoid receptor expression, as well as methylation of the rat fetal calf serum *Nr3c1* promoter, the homolog of the human exon 1_F*NR3C1* promoter.

- Hippocampal samples from suicide victims showed increased methylation of the exon 1_F*NR3C1* promoter in comparison with samples from controls, but only in cases with a history of childhood abuse.
- Neither hippocampal glucocorticoid receptor expression nor the methylation status of the exon 1_F*NR3C1* promoter was altered in suicide victims with no history of abuse.
- These findings suggest that variation in the methylation status of the exon 1_F*NR3C1* promoter, similar to that for glucocorticoid receptor 1_F and total glucocorticoid receptor mRNA expression, associates with childhood adversity and not with suicide *per se*.


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Review

Cell

A twin approach to unraveling epigenetics

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Fraga, M.F. et al. (2005) Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. U.S.A. 102,10604–10609

- The first large-scale X-sectional study examined DNA methylation and histone acetylation at multiple genomic regions in 20 x 3-year-old and 20 x 50-year-old Spanish MZ twin pairs and observed that MZ twins have very similar epigenetic profiles, indicative of high epigenetic heritability.
- However, epigenetic variability increased with age across multiple tissues and, interestingly, the greatest differences were observed post hoc in twins who differed most in life style.

- Examined the global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins.
- They found that, although twins are epigenetically indistinguishable during the early years of life, older monozygous twins exhibited remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, affecting their gene-expression portrait.
- These findings indicate how an appreciation of epigenetics is missing from our understanding of how different phenotypes can be originated from the same genotype.

Questionnaires

- All subjects were interviewed by properly trained personnel to complete the questionnaire about their health, nutritional habits, physical activities, pharmacological treatments, and tobacco, alcohol, and drug consumption.
- Weight and height were measured, and a family tree of genetic history was drawn up by the interviewer.
- The data collected in the questionnaires were entered into a database as nominal (natural health history), ordinal (percentage of lifetime shared, nutritional habits, physical activity, and consumption of folates, alcohol, tobacco, and drugs), and numerical (age, weight, and height) variables, which were subsequently used to estimate the phenotypic/environmental distance between twin pairs.

Statistics

- First, a descriptive value was obtained for each individual corresponding to each epigenetic variable (5mC, AcH4, and AcH3).
- The similarity between twin pairs for each variable was estimated as the Euclidean squared distance (ESD) by subtracting their respective descriptive values
- By using these distances, the relationships between the phenotypic-environmental data and the epigenetic variables were examined by categorical principal component analysis, using SPSS software.

Aging and health

- Categorical principal component analysis reduced all of the original variables from the questionnaire to two new uncorrelated components or variables ("aging" and "health").
- The aging principal component included the variables of age, weight, and height and is an indicator of the ontogenic development of the twins.
- The health principal component grouped the variables of diseases and pharmacological treatments

results

- The starting biological material studied was DNA and RNA from peripheral lymphocytes. In all cases, the true MZ twin status was confirmed by microsatellite analysis (Fig. 1A) (7).
- X chromosome inactivation:

We found that among MZ female twins informative for our PCR approach, 81% (13 of 16) had the same X chromosome methylation pattern, and of these, 85% (11 of 13) had an unskewed random distribution of each methylated allele.

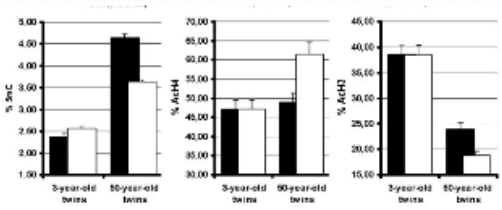
Although the remaining 15% (2 of 13) displayed a skewed X chromosome pattern, this pattern was always concordant between siblings. Among all female MZ twins, only 19% (3 of 16) had a skewed X chromosome methylation pattern that differed between corresponding siblings (Fig. 1B). The presence of a differential X chromosome inactivation pattern in the latter cases was not associated with any epidemiological and clinicopathological feature but raises the possibility that epigenetic discordance can arise early on in the development of some MZ twins.

X-chromosome inactivation

- To control the quantity of gene product, women obligatorily inactivate either the maternal or the paternal gene of the X chromosome.
- Approximately 10% of X chromosome genes are not inactivated, leaving women with some gene products excessive to those of men; those that are inactivated become inactivated in a stochastic manner.
- The result is that some women have **skewing of inactivation very far to one parental side, for some cell lines**.
- Peripheral blood cells for almost 50% scleroderma patients were greater than 90% skewed, compared to only 3% of female controls.

Comparison of 5mC DNA Content and Histone H3 and H4 Acetylation Levels in MZ Twin Pairs

- The epigenetic circumstances of the MZ twins proved to be more remarkable when we took a more global approach to the comparison of the DNA methylation and histone acetylation content of the MZ twins.
- For each pair, we determined the 5mC genomic content and the acetylation levels of histones H3 and H4
- Replicate samples at time-points 0, 2, 4, 6, and 12 weeks for eight MZ twin pairs were assessed to determine short-time fluctuations in the described epigenetic parameters, and no significant changes were observed



Finally, we also found that those twin pairs who, according to the questionnaire, had spent less of their lifetime together and/or had a more different natural health–medical history were those who also showed the greatest differences in levels of 5mC DNA and acetylation of histones H3 and H4 levels (Pearson test, $P < 0.05$).

Genomic Screening and Loci Identification of DNA Methylation Differences in MZ Twin Pairs.

- We next examined **where in the MZ twin genomes** these epigenetic differences arose by using a global methylation DNA fingerprinting technique, AIMS
- Most importantly, the twin pairs with the most differential AIMS bands corresponded to MZ twins who were older, had spent less of their lifetimes together, or had different natural health–medical histories (Pearson test, $P < 0.05$).
- We found that 43% of the clones matched Alu sequences, 9% matched other repetitive sequences (2 LINEs, 2 MER, and 1 MIR), 34% matched ESTs deposited in databases, and 13% of the clones corresponded to identified single-copy genes

Differential expression

- Most importantly, for both Alus and single-copy genes, differential methylation was associated with a different expression of that particular sequence in the MZ twin pair, the presence of DNA methylation being associated with silencing or reduced expression.

- MZ twins who were younger, had similar lifestyles, and had spent more of their lifetimes together displayed minimal DNA methylation changes in all chromosomes, whereas those who were older, had different lifestyles, and had spent less of their lives together had unevenly distributed DNA methylation events (hypermethylation and hypomethylation) throughout the chromosomes described above.

- We observed that the older twin pairs presented 2.5 times as many DNA methylation differences in the CpG islands of single-copy genes as did the younger twin-pairs.
- These DNA methylation differences observed in the older twins also were demonstrated for these particular gene-associated CpG islands

Portraits of Gene Expression in MZ Twin Pairs

- Finally, we addressed the ultimate goal of all major epigenetic modification: global change in gene expression. We examined whether older twin pairs who differed most with respect to DNA methylation and histone acetylation levels in general and at specific loci also displayed the most different gene expression profiles.
- To this end, we extracted RNA from the two most distinct pairs of twins (the 3- and 50-year-old pairs) and performed gene expression microarray analysis.
- The results obtained when each twin expression portrait was confronted with its own sibling demonstrated that although the expression patterns of the 3-year-olds were almost identical, the 50-year-old twins had extremely different expression profiles (Fishers exact test, $P = 0.029$).

Discussion

- Why these epigenetic differences in twins?
- Life style
- Epigenetic drift
- They found that approximately one-third of MZ twins harbored epigenetic differences in DNA methylation and histone modification.
- Our findings also support the role of epigenetic differences in the discordant frequency/onset of diseases in MZ twins

- Other evidence indicates that relatively small differences in epigenetic patterns can have a large impact in phenotype, for instance in cloned animals (4), with MZ twins representing natural human clones.
- Another powerful example is provided by the agouti mouse (19). In this model, diet affects the methylation status of an inserted intracisternal A particle element that changes the animal's coat color: an environmental factor interacting with a single geno- type, mediated by an epigenetic change, to produce a different phenotype.

Summing up

- Why these epigenetic differences in twins?
- Life style
- Epigenetic drift
- They found that approximately one-third of MZ twins harbored epigenetic differences in DNA methylation and histone modification.
- These findings also support the role of epigenetic differences in the discordant frequency/onset of diseases in MZ twins

nature genetics

DNA methylation profiles in monozygotic and dizygotic twins

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Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GH, Wong AH, et al. (2009): DNA methylation profiles in monozygotic and dizygotic twins. *Nat Genet*.

abstract

- Twin studies have provided the basis for genetic and epidemiological studies in human complex traits. As epigenetic factors can contribute to phenotypic outcomes, we conducted a DNA methylation analysis in white blood cells (WBC), buccal epithelial cells and gut biopsies of 114 monozygotic (MZ) twins as well as WBC and buccal epithelial cells of 80 dizygotic (DZ) twins using 12K CpG island microarrays.
- Here we provide the first annotation of epigenetic metastability of approximately 6,000 unique genomic regions in MZ twins.
- An intraclass correlation (ICC)-based comparison of matched MZ and DZ twins showed significantly higher epigenetic difference in buccal cells of DZ co-twins ($P = 1.2 \times 10(-294)$).
- Although such higher epigenetic discordance in DZ twins can result from DNA sequence differences, our in silico SNP analyses and animal studies favor the hypothesis that it is due to **epigenomic differences in the zygotes**, suggesting that molecular mechanisms of heritability may not be limited to DNA sequence differences.

- MZ twins always show some degree of discordance but generally lower than DZ twins
- Looked at white bood cells, buccal cells and gut
- attempted to see if epigenetic cosimilarity is related to functional element which is methylated
- Hence compared the methylation of CpG island to non CpG loci
- Also investigated promoters
- Promoters and CpG were less epigenetically variable in white blood cells but not buccal cells (tissue specificity) compared to non-functional elements

- DNA methylation differences were less in MZ compared to DZ twins (in the buccal cells)

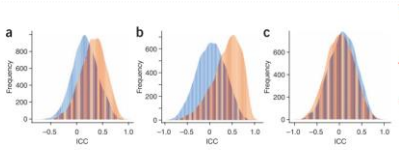

Figure 3 ICC distributions in buccal epithelial cells of MZ and DZ twins. (**a**) All MZ twins ($N = 20$ sets, red) and DZ twins ($N = 20$ sets, blue). (**b**) Dichorionic MZ twins ($N = 10$ sets, red) and matched DZ twins ($N = 10$ sets, blue). (**c**) Monochorionic MZ buccal samples ($N = 10$ sets, red) with matched DZ twins ($N = 10$ sets, blue).

- All differences attributed to dichorionic MZ twins (splitting before 4 days) and not monochorionic (splitting after day 4).

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ORIGINAL ARTICLE

Aberrant DNA methylation associated with bipolar disorder identified from discordant monozygotic twins

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To search DNA methylation difference between monozygotic twins discordant for bipolar disorder, we applied a comprehensive genome scan method, methylation-sensitive representational difference analysis (MS-RDA) to lymphoblastoid cells derived from the twins. MS-RDA isolated 10 DNA fragments derived from 5′ region of known genes/ESTs. Among these 10 regions, four regions showed DNA methylation differences between bipolar twin and control co-twin confirmed by bisulfite sequencing. We performed a case–control study of DNA methylation status of these four regions by pyrosequencing. Two regions, upstream regions of spermine synthase (*SMS*) and peptidylprolyl isomerase E-like (*PPIEL*) (CN265253), showed aberrant DNA methylation status in bipolar disorder. *SMS*, a gene on X chromosome, showed significantly higher DNA methylation level in female patients with bipolar disorder compared with control females. However, there was no difference of mRNA expression. In *PPIEL*, DNA methylation level was significantly lower in patients with bipolar II disorder than in controls. The expression level of *PPIEL* was significantly higher in bipolar II disorder than in controls. We found strong inverse correlation between gene expression and DNA methylation levels of *PPIEL*. These results suggest that altered DNA methylation statuses of *PPIEL* might have some significance in pathophysiology of bipolar disorder.

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Keywords: bipolar disorder; DNA methylation; epigenetics; gene expression; CpG island; lymphoblastoid

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A longitudinal study of epigenetic variation in twins

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Key words: epigenetics, DNA methylation, twin, heritability, dynamic, environment

DNA methylation is a key epigenetic mechanism involved in the developmental regulation of gene expression. Alterations in DNA methylation are established contributors to inter-individual phenotypic variation and have been associated with disease susceptibility. The degree to which changes in loci-specific DNA methylation are under the influence of heritable and environmental factors is largely unknown. In this study, we quantitatively measured DNA methylation across the promoter regions of the dopamine receptor 4 gene (*DRD4*), the serotonin transporter gene (*SLC6A4/SERT*) and the X-linked monoamine oxidase A gene (*MAOA*) using DNA sampled at both ages 5 and 10 years in 46 MZ twin-pairs and 45 DZ twin-pairs (total n = 182). Our data suggest that DNA methylation differences are apparent already in early childhood, even between genetically identical individuals, and that individual differences in methylation are not stable over time. Our longitudinal-developmental study suggests that environmental influences are important factors accounting for interindividual DNA methylation differences, and that these influences differ across the genome. The observation of dynamic changes in DNA methylation over time highlights the importance of longitudinal research designs for epigenetic research.