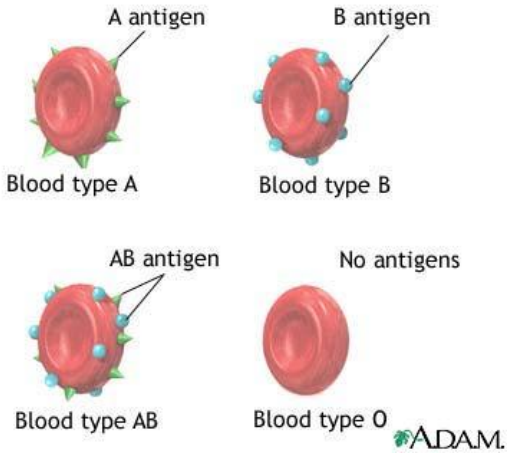
Psych 3102
Lecture 5
Extensions of Mendel
- continued

Multiple alleles

- where more than two alleles are present for the trait in the population

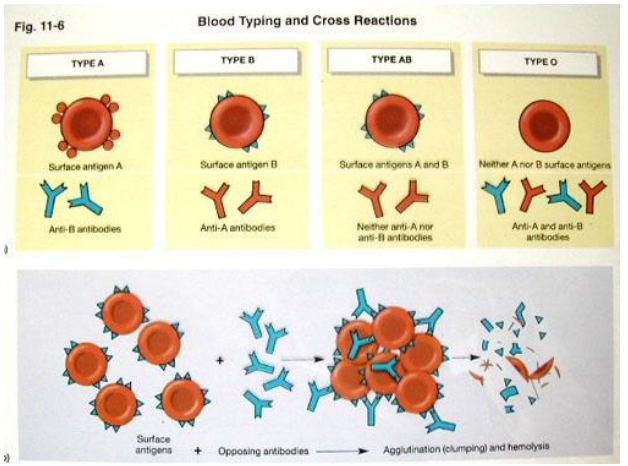


Example: ABO blood group system in humans

antigen = on surface of red blood cells

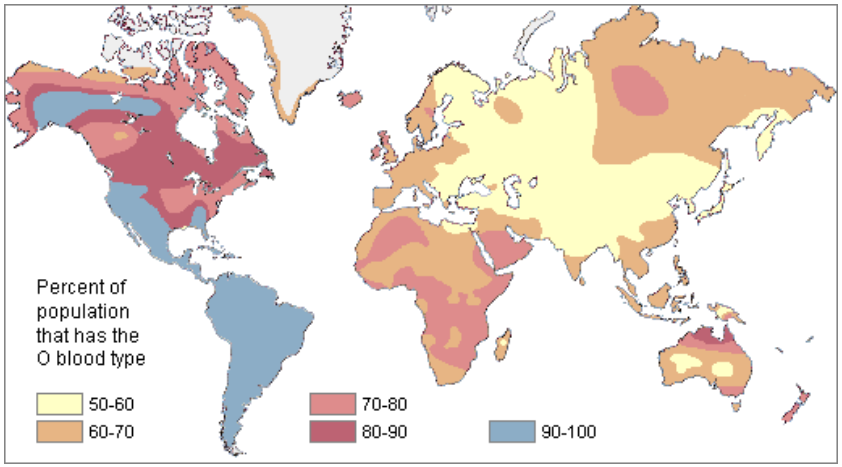
antibody = present in blood plasma

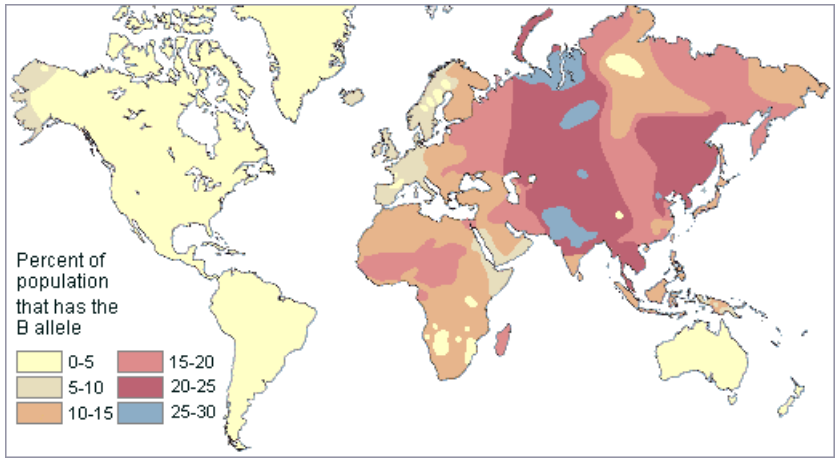
antigen A
+ antibody A → agglutination (clumping of red cells)

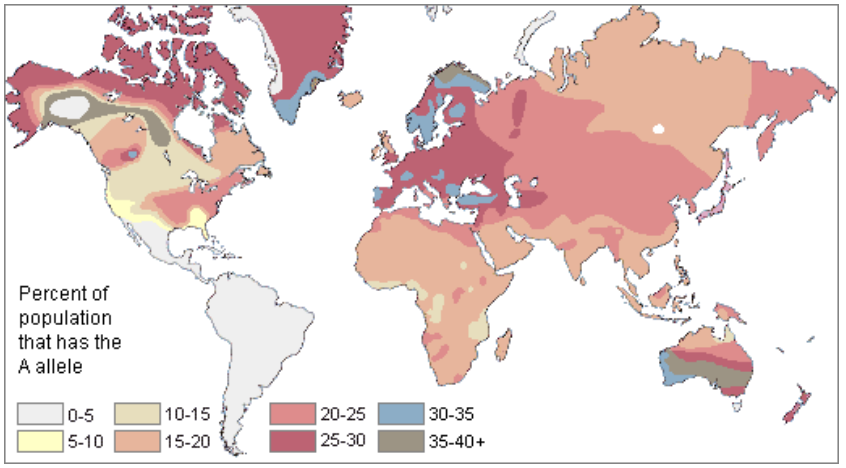


Frequencies of blood group alleles vary across populations. Why?

- resistance to disease eg. cholera







I = blood group locus

A,B,O are alleles at that locus

The ABO Blood System

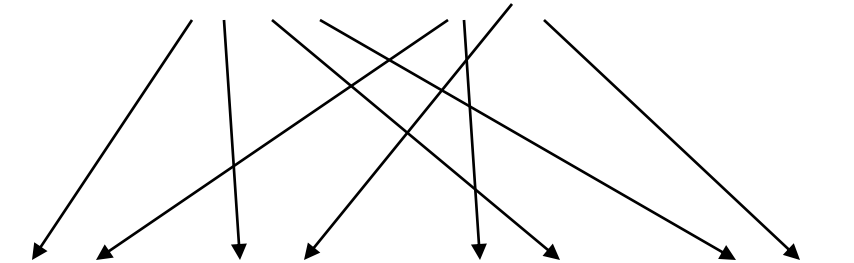
BLOOD TYPE	GENOTYPE	REACTION WITH ANTI-A SERUM	REACTION WITH ANTI-B SERUM	TYPE OF DONOR BLOOD ACCEPTED
A	I^AI^A or I^AI^O	Clumping of red blood cells	No clumping	A or O
B	I^BI^B or I^BI^O	No clumping	Clumping of red blood cells	B or O
AB	I^AI^B	Clumping of red blood cells	Clumping of red blood cells	A, B, AB, or O
O	I^OI^O	No clumping	No clumping	O

I^A and I^B are co-dominant

I^O is recessive to both I^A and I^B

Can a group A mother have a group O child with a group B father?

P I^AI^O x I^BI^O



F_1 I^AI^B I^AI^O I^BI^O I^OI^O

Phenotypes AB A B O

Allelic interactions

- between alleles at one locus

- complete dominance
  - allele is expressed in the phenotype when present in heterozygous condition
  example: HD allele

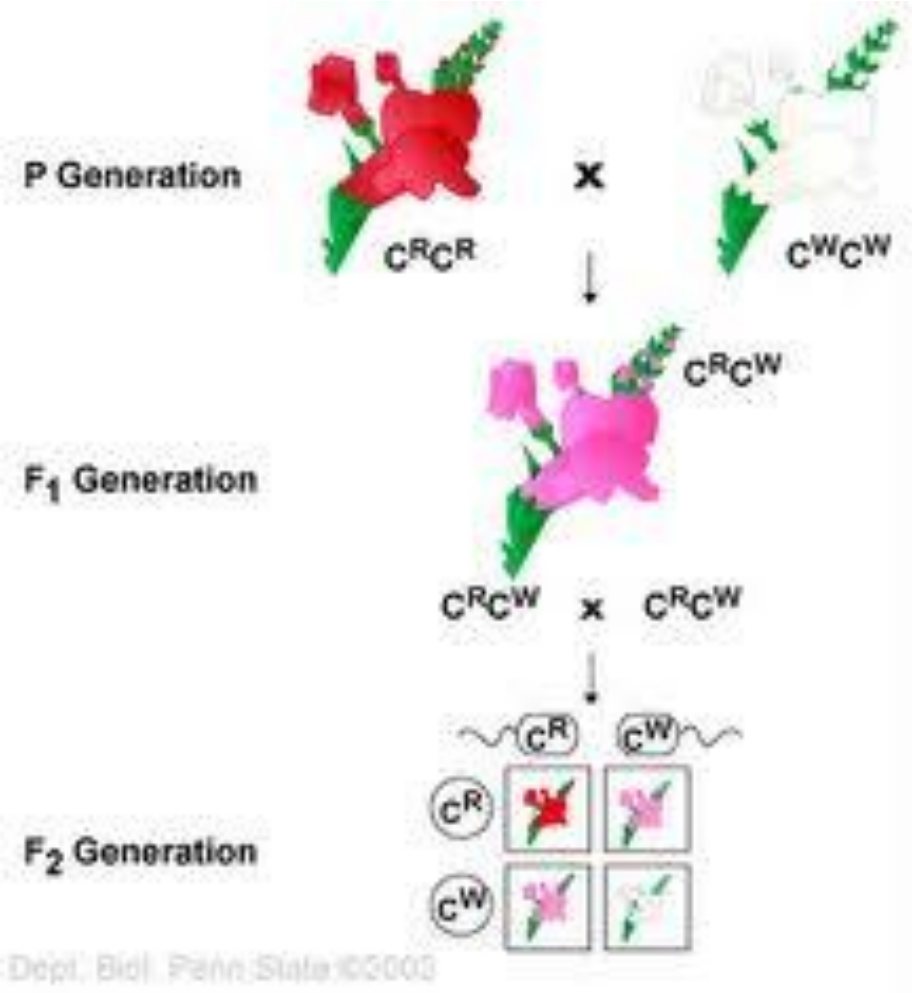
- recessive
  - allele has be present in homozygous condition to show phenotypic expression
  example: PKU allele

- codominance
  - both alleles at a locus are expressed in the phenotype
  example: AB blood group alleles

- incomplete dominance
  - heterozygote shows intermediate phenotype, full effects of 'dominant' allele are not shown
  example: chickens 'Andalusian blue' phenotype
  horses 'Palamino' phenotype
  humans familial hypercholesterolemia

haploinsufficiency – 1 copy of wild type allele not enough to produce wildtype phenotype

Incomplete dominance in snapdragons



Gene interactions - nonallelic interactions

Phenotype is result of complex, integrated pattern of reactions under control of more than one gene and the environment.

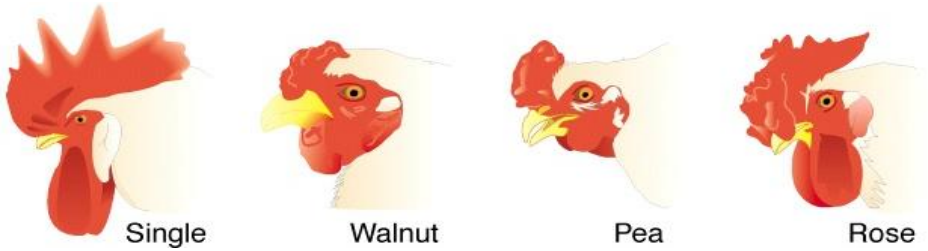
1. Epistasis (true non-allelic interaction)

- expression of a single trait depends on interaction between 2 or more genes
- why is this different from describing the trait as being polygenic?

examples:

comb shape in chickens depends on genotype at 2 unlinked loci (P and R)

Phenotypes



Genotypes rrpp R-P- rrP- R-pp (1 : 9 : 3 : 3 in F2)

behavioral example:

anorexia nervosa (AN)

allelic variants of MAOA 5HTT NET genes

Genotype	Risk of AN
MAOA risk allele	slight increase
5HTT alleles	no effect
NET risk allele	x 2 increase
MAOA + 5HTT risk alleles	x 8 increase epistasis (**non-additive effect**)
MAOA + NET risk alleles	slightly more than x 2 no interaction (additive effect)

Important note: results like this often fail to replicate, so whether this is a real effect or not is uncertain

European Journal of Human Genetics (2005) 13, 370–375

www.nature.com/ejhg

ARTICLE

Epistatic interaction between the monoamine oxidase A and serotonin transporter genes in anorexia nervosa

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The serotonin (5-HT) and norepinephrine (NE) systems are likely involved in the aetiology of anorexia nervosa (AN) as sufferers are premorbidly anxious. Specifically, we hypothesize that genes encoding proteins, which clear 5-HT and NE from the synapse, are prime candidates for affecting susceptibility to AN. Supporting our hypothesis, we earlier showed that the NE transporter (NET) and monoamine oxidase A (MAOA) genes appear to contribute additively to increased risk of developing restricting AN (AN-R). With regard to the *MAOA* gene, a sequence variant that increases MAOA activity and has suggested association with the anxiety condition, panic disorder was preferentially transmitted from parents to affected children. Here we provide evidence in support of interaction between the *MAOA* and serotonin transporter (SERT) genes in 114 AN nuclear families (patient with AN plus biological parents). A *SERT* gene genotype with no apparent individual effect on risk and known to be associated with anxiety is preferentially transmitted to children with AN (χ^2 trend = 9.457, 1 df, $P = 0.0021$) and AN-R alone (χ^2 trend = 7.477, 1 df, $P = 0.0063$) when the 'more active' *MAOA* gene variant is also transmitted. The increased risk of developing the disorder is up to eight times greater than the risk imposed by the *MAOA* gene variant alone – an example of synergistic epistatic interaction. If independently replicated, our findings to date suggest that we may have identified three genes affecting susceptibility to AN, particularly AN-R: the *MAOA*, *SERT*, and *NET* genes.

European Journal of Human Genetics (2005) **13**, 370–375. doi:10.1038/sj.ejhg.5201328
Published online 3 November 2004

Keywords: eating disorders; genetic epistasis; amino-acid oxidoreductases; biogenic amine neurotransmitters; tryptophan; tyrosine

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Introduction

Individuals suffering from anorexia nervosa (AN) perceive themselves as fat even when emaciated, and pursue thinness through food restriction (AN-R) or purging (AN-BP). 'Anxiety' genes likely contribute to the suggested genetic component in AN as sufferers are usually anxious prior to developing the disorder.[1,2] The 'anxiety' neurotransmitters serotonin (5-HT) and norepinephrine (NE) are

Epistasis between neurochemical gene polymorphisms and risk for ADHD

European Journal of Human Genetics (2011) 19, 577–582

www.nature.com/ejhg

ARTICLE

Epistasis between neurochemical gene polymorphisms and risk for ADHD

Ricardo Segurado*,[1], Mark A Bellgrove[2], Francesca Manconi[3], Michael Gill[1] and Ziarah Hawi[1,2]

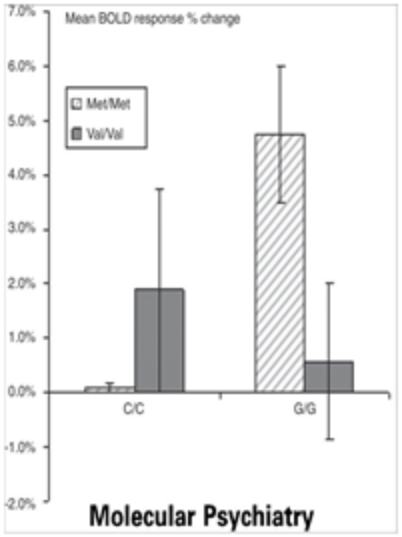
A number of genes with function related to synaptic neurochemistry have been genetically associated with attention deficit/hyperactivity disorder. However, susceptibility to the development of common psychiatric disorders by single variants acting alone, can so far only explain a small proportion of the heritability of the phenotype. It has been postulated that the unexplained 'dark heritability' may at least in part be due to epistatic effects, which may account for the small observed marginal associations, and the difficulties with replication of positive findings. We undertook a comprehensive exploration of pair-wise interactions between genetic variants in 24 candidate genic regions involved in monoaminergic catabolism, anabolism, release, re-uptake and signal transmission in a sample of 177 parent-affected child trios using a case-only design and a case–pseudocontrol design using conditional logistic regression. Marker-pairs thresholded on interaction odds ratio (OR) and *P*-value are presented. We detected a number of interaction ORs >4.0, including an interesting correlation between markers in the *ADRA1B* and *DBH* genes in affected individuals, and several further interesting but smaller effects. These effects are no larger than you would expect by chance under the assumption of independence of all pair-wise relations; however, independence is unlikely. Furthermore, the size of these effects is of interest and attempts to replicate these results in other samples are anticipated.
European Journal of Human Genetics (2011) **19**, 577–582; doi:10.1038/ejhg.2010.250; published online 2 February 2011

Keywords: ADHD; epistasis; candidate gene

INTRODUCTION

The heritability of attention deficit/hyperactivity disorder (ADHD) is well established, and genetic association data have been reviewed and meta-analysed recently by Gizer *et al*.[1] The detection of DNA variants, which increase risk for this disorder is important for biochemical and pharmacological research into this disorder, and may permit the facilitation of diagnosis, or refinement of the phenotype on the basis of a biological marker. Specifically, association between monoaminergic genes and ADHD is an active area of investigation, stimulated principally by the mode of action of current pharmaco-

are related directly via protein–protein interaction or indirectly via regulatory pathways. Therefore, there are grounds for prior supposition that common genetic variants in several risk genes may act synergistically to influence disease risk. Methods for detecting interaction, or epistasis, in case–control samples have received much attention recently,[9,10] particularly in the rapidly developing areas of machine learning. Methods for family-based samples are less well developed – usually adaptations of case–control methods (eg, using matched case–pseudocontrol samples). However, in addition to large family-based samples being used in the latest generation of gene-



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doi:10.1038/mp.2011.145

Molecular Psychiatry (2013) 18, 174–182

www.nature.com/mp

ORIGINAL ARTICLE

Genetic modulation of neural response during working memory in healthy individuals: interaction of glucocorticoid receptor and dopaminergic genes

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Suboptimal performance in working memory (WM) tasks and inefficient prefrontal cortex functioning are related to dysregulation of dopaminergic (DA) and hypothalamic-pituitary-adrenal systems. The aim of the present study was to investigate the joint effect of genetic polymorphisms coding for DA catabolism and glucocorticoid receptor (GR, NR3C1) on brain functioning. The study group (90 right-handed white Caucasian healthy individuals) underwent functional magnetic resonance imaging experiments to examine blood oxygenation level dependent (BOLD) response during a WM task with varying cognitive load (1-, 2- and 3-back). We have also examined skin conductance response (SCR) during the WM task and resting-state cerebral blood flow with continuous arterial spin labelling. The genetic markers of interest included Catechol-O-Methyl-Transferase (COMT) (Met^{158}Val) and NR3C1 single-nucleotide polymorphisms (BclI C/G rs41423247, 9β A/G rs6198 and rs1866388 A/G). Haplotype-based analyses showed (i) a significant effect of COMT polymorphism on left anterior cingulate cortex, with greater deactivation in Met carriers than in Val/Val homozygotes; (ii) a significant effect of BclI polymorphism on right dorsolateral prefrontal cortex (DLPFC), with greater activation in G/G carriers than in C carriers and (iii) an interactive effect of BclI (G/G) and COMT (Met/Met) polymorphisms, which was associated with greater activation in right DLPFC. These effects remained significant after controlling for whole-brain resting-state blood flow. SCR amplitude was positively correlated with right DLPFC activation during WM. This study demonstrated that GR and COMT markers exert their separate, as well as interactive, effects on DLPFC function. Epistasis of COMT and BclI minor alleles is associated with higher activation, suggesting lower efficiency, of DLPFC during WM.
Molecular Psychiatry (2013) **18,** 174–182; doi:10.1038/mp.2011.145; published online 15 November 2011

Keywords: COMT; fMRI; glucocorticoid receptor gene; *n*-back; working memory

Introduction

Working memory (WM) is a cognitive mechanism that underlies temporary storage and manipulation of limited amounts of information.[1] The role of the dopaminergic (DA) system in modulating WM processes in dorsolateral prefrontal cortex (DLPFC) is well established.[2] It is known that dopamine levels in DLPFC are modulated mainly by Catechol-O-Methyl Transferase (*COMT*), the gene for which has common Val^{158}Met polymorphisms. The Val allele is 3–4 times more active than the Met allele, resulting in a lower level of dopamine in DLPFC in Val carriers.[3–6] A seminal study demonstrated greater activation, suggesting reduced efficiency, of DLPFC during WM in healthy individuals that was associated with the number of the Val alleles.[4] These authors proposed that the deficit in prefrontal function associated with Val allele may increase susceptibility to schizophrenia.

Another line of research in WM shows an impact of the Hypothalamic-Pituitary Axis (HPA) on prefrontal cortical functioning. Animal studies have shown that deficits in executive performance and WM may be associated with stress-related dysregulation of the HPA.[6,7] Moreover, there is strong evidence supporting the role of HPA dysregulation as a risk factor for depression and psychosis,[8] which is related to maladaptive responses to stress and glucocorticoids. This is in line with the reported association of less

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2. Pleiotropy

- a single allele has multiple, correlated phenotypic effects

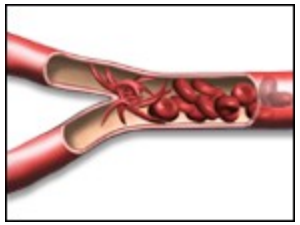
Sometimes produces heterozygous advantage
(hybrid vigor,overdominance)

- enables an otherwise deleterious recessive allele to survive in the population at unexpectedly high levels

examples: cystic fibrosis (cholera)
sickle cell disease (malaria)
congenital deafness (dysentery)

- Sickle cell disease

  pleiotropic effects across body



point mutation: 6th amino acid in 146 amino acid chain

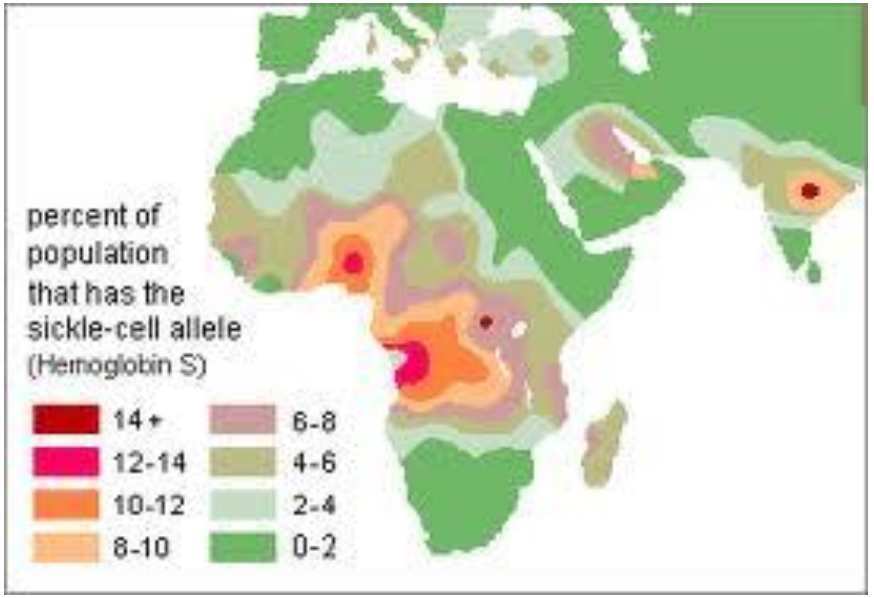
glu → val - causes red cells to be misshapen

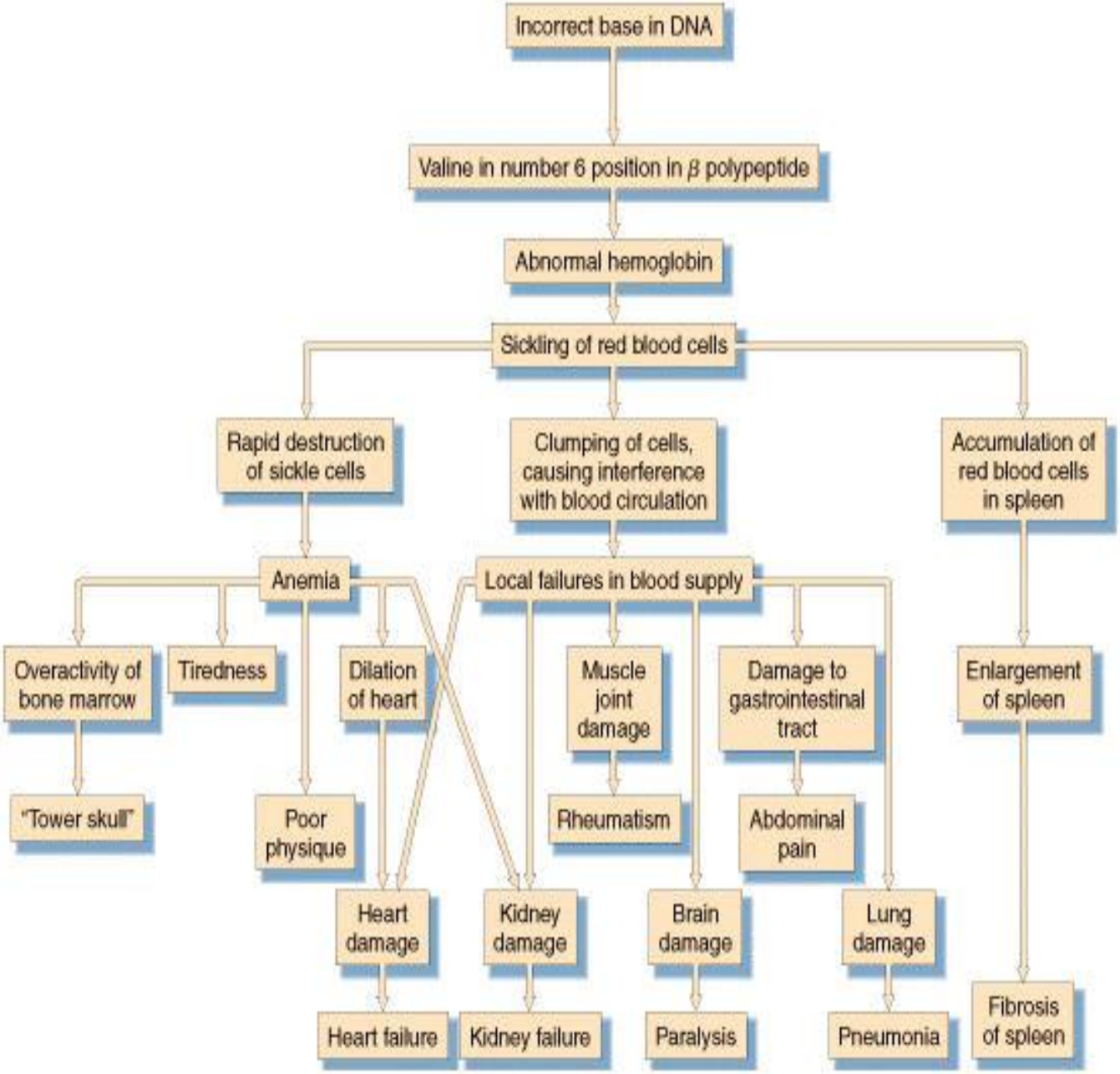
Heterozygous advantage:

resistance to malaria

Sickle cell allele is usually indicated as recessive

Can the sickle cell allele be completely recessive?




Incorrect base in DNA
Valine in number 6 position in β polypeptide
Abnormal hemoglobin
Sickling of red blood cells
Rapid destruction of sickle cells
Clumping of cells, causing interference with blood circulation
Accumulation of red blood cells in spleen
Anemia
Local failures in blood supply
Overactivity of bone marrow
Tiredness
Dilation of heart
Muscle joint damage
Damage to gastrointestinal tract
Enlargement of spleen
"Tower skull"
Poor physique
Rheumatism
Abdominal pain
Heart damage
Kidney damage
Brain damage
Lung damage
Fibrosis of spleen
Heart failure
Kidney failure
Paralysis
Pneumonia

Analysis of Hb S/haplotype combinations indicates that the mutation must have occurred at least five times. Four are found predominantly in Africa and are designated Bantu, Benin, Senegal, and Cameroon. A fifth is found in the populations of India and Saudi Arabia in which the **sickle cell** gene occurs. The Hb S allele in Sicily and other Mediterranean areas occurs with the Benin haplotype, which is otherwise very rare there. This suggests an African origin for the Mediterranean alleles.

There is some difference in severity of sickle cell disease also associated with haplotypes. Persons homozygous for the Arabian haplotype are least severely affected and those with the Bantu haplotype are the most severely affected. Because the Hb S mutation does not differ among the haplotypes, it is likely that regulatory elements in the b-globin complex vary, perhaps resulting in more or less fetal hemoglobin that interferes with sickling.

The diagnosis of homozygosity for Hb S is readily accomplished by gel electrophoresis of red **cell** lysates from blood of newborns or adults. Examination of Hb in fetal red **cell**s cannot be used routinely for diagnosis because of the low production of b-globin during this period. Direct examination of DNA has made prenatal diagnosis possible, however. Most earlier procedures were based on the fact that the DNA sequence for codons 5 to 7 is 5′-CCTGAGGAG-3′ in the case of Hb A and 5′ -CCTGTGGAG-3′ for Hb S. The restriction enzyme MstII cleaves at the sequence CCTNAGG, which is present in Hb A but not in Hb S. Thus the restriction fragment patterns will differ for the two alleles. The difference can be observed using a Southern blot with an appropriate b-globin probe. Several variations on polymerase chain reaction (PCR) amplification of the altered DNA segment have also been developed. These have the advantage of requiring minute quantities of DNA, such as single **cell**s from an in vitro-fertilized 8-**cell**embryo or from rare fetal **cell**s in the maternal circulation.

Effective treatment of sickle cell disease has yet to be developed. Current approaches have been directed toward increasing the level of fetal Hb, which interferes with sickling. Hydroxyurea increases fetal Hb and has some beneficial effect on sickling, but the levels required and the uncertain long term effects have been problems. Nevertheless, it is the treatment of choice at present. Bone marrow replacement should be effective but generates its own major problems.

PKU allele shows pleiotropic effects

point mutation causes non-functional enzyme and inability to metabolize phenylalanine, subsequent brain damage

tyrosine normally produced from phenylalanine

tyrosine used to produce thyroxine epinephrin melanin

pleiotropic effects of PKU allele :

fair hair blue eyes

3. Penetrance

not everyone with a particular genotype shows the expected phenotype

dominant allele – penetrance = frequency with which it expresses itself in the phenotype, as percentage

Anything less than 100% = low (or incomplete) penetrance

Examples:

fragile-X mutation - X-linked dominant with 50% penetrance in females

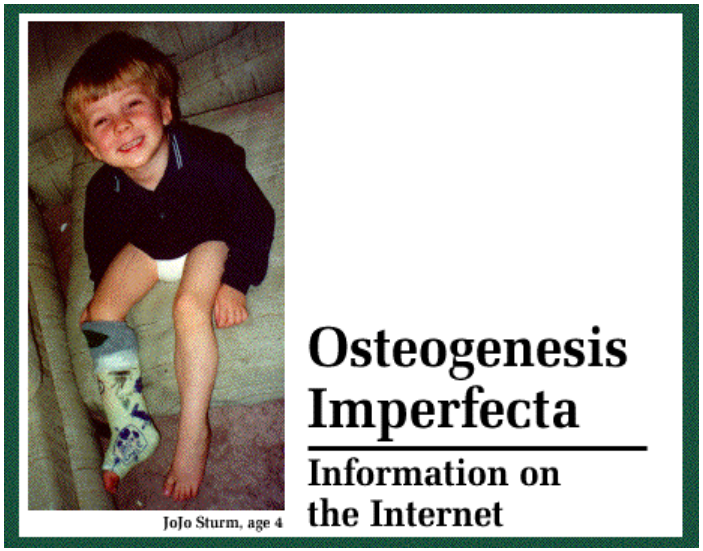
Huntington allele – penetrance is age-dependent

BRCA-1 - major risk factor allele for breast cancer, age-dependent penetrance

37% by age 40	w/out allele = 0.4%
66% by age 55	3%
85 % by age 80	8%

4. Expressivity

degree to which penetrant allele expresses itself in phenotype

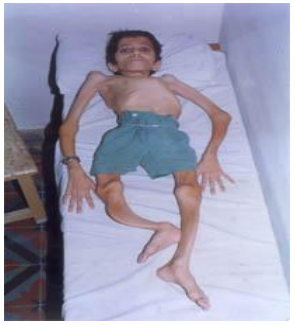


Examples:

osteogenesis imperfecta

autosomal dominant

100% penetrant since all who carry allele show blue sclera phenotype, other effects vary



fragile-X syndrome

X-linked dominant, 50% penetrance

males more severely effected than females, but expression varies in both sexes

5. Internal environment

factors that can change expression of genes:

age

Huntington allele Duchenne muscular dystrophy
male-pattern baldness

gender

sex-linked traits alleles on X or Y chromosome

sex-limited traits alleles NOT on sex chromosome but affected by genes on sex chromosomes (epistasis)

Baldness sex-limited trait

50% male population, small number of women

androgenic alopecia = male-pattern baldness, most common cause

2 major genes

androgen receptor on X (X-linked) x 3.3 risk

transcription factor region on 20p x 1.6 risk

both risk alleles (14% of men) x 7 risk

epistasis

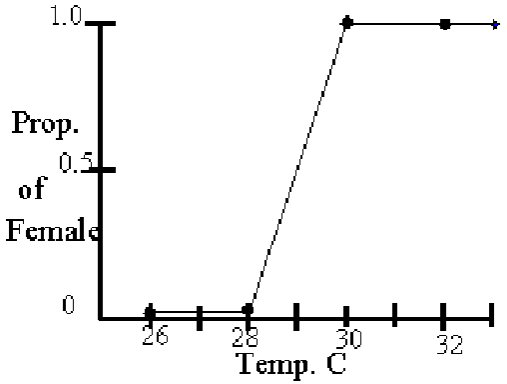
6. External environment

environmental factors that can change gene expression:

temperature

coat color in Himalayan rabbits

sex-determination in crocodilians



environmental chemicals

phenocopies non-hereditary phenotypic modifications that mimic the effect of genes

German measles/hereditary deafness

thalidomide/phocomelia

Accutane/congenital deformities

interactions

genotype x environment interactions where effect of genotype depends on environment (& vice-versa)

effect of diet on PKU

effect of smoking/α-1-antitrypsin gene

effect of diet on coat color in mice

Epigenetics possible mechanism for GxE

- gene expression is altered (eg. by methylation)
- phenotype is altered
- genotype is unchanged

Example of an environmental factor changing gene expression : - coat color in agouti mice

pregnant female mice fed diet with supplements of vit B_{12}, folic acid, & choline had offspring with agouti coats

pregnant female mice fed diet without supplements had offspring with yellow coats + offspring had tendency to diabetes, heart disease, obesity

extra nutrients turned down expression of agouti gene, which has pleiotropic effects on appetite and metabolism as well as effecting coat color.

Research Highlight
Nature Reviews Genetics 12, 80 (February 2011) | doi:10.1038/nrg2941

Epigenetics: Dad's diet lives on

Two recent studies in rodents show that unhealthy paternal diets can reprogramme gene expression in offspring, implicating epigenetics in these transgenerational effects.

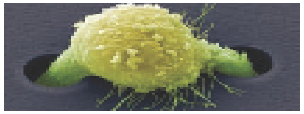
Ng and colleagues fed male rats a high-fat diet and looked for effects in their adult female offspring, which were fed a normal diet. These daughters had normal body fat but showed signs of pancreatic β-cell impairment and altered expression (as compared to controls) of 642 genes that are involved in pathways related to insulin regulation and glucose metabolism. The gene with the greatest alteration in expression was interleukin-13 receptor-α2 (Il13ra2), which is implicated in regulating pancreatic cell function. Interestingly, DNA methylation at a cytosine residue close to the Il13ra2 transcriptional start site was reduced in these females.

Carone and colleagues looked at the effect of a paternal low-protein diet in mice. Offspring of both sexes showed altered gene expression compared to controls, including genes involved in fat and cholesterol biosynthesis, consistent with physiological differences in these mice. Modest changes in DNA methylation were seen at many sites, including a reproducible change close to the Ppara gene, which encodes peroxisome proliferator-activated receptor-α, a regulator of lipid metabolism.

Although there is increasing evidence for effects of parental environment in offspring, these studies add to just a handful of cases in which the molecular basis has been at least partly elucidated. Clearly, the role of epigenetics in such transgenerational effects will be an important focus of future studies.

EPIGENETIC EFFECTS
A few disease studies in the NIH Roadmap Epigenomics Project.

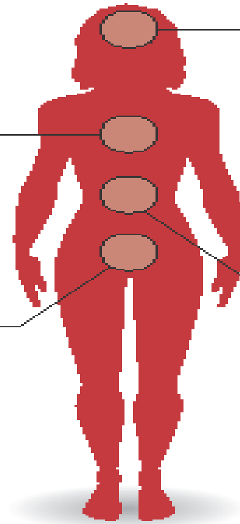
CANCER



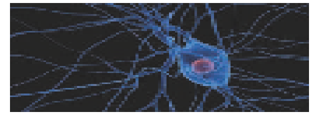
Control of gene expression by epigenetic modification could have a role in tumour formation, and could explain how environmental factors trigger cancer.

PRENATAL CHANGES

Molecular modifications to fetal and maternal DNA before birth could later make people susceptible to type 2 diabetes or cardiovascular disease.

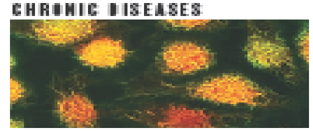


BRAIN DISORDERS



Epigenetic changes have been implicated in brain health, from cognitive decline in normal ageing to conditions such as Alzheimer's disease, schizophrenia, bipolar disorder and autism.

CHRONIC DISEASES



Complex chronic conditions such as systemic lupus erythematosus, asthma and insulin resistance in obesity and diabetes are thought to have an environmental component. Studies aim to identify how this can cause epigenetic changes that might affect disease progression.

GENE EXPRESSION

Epigenome effort makes its mark

Major release of maps charting non-genetic modifications goes beyond DNA in a bid to beat complex human disease.

BY ALLA KATSNELSON

When the human genome was first fully sequenced, it was often described as the recipe for making a person. In reality, the genome is more like an entire cookbook that can produce hundreds of different cell types and a staggering range of cell functions depending on which genes are switched on and off. That switching is accomplished using a vast suite of epigenetic marks — molecular and structural modifications to DNA that do not change the underlying sequence but ensure that the right genes are expressed at the right time.

This week, the Roadmap Epigenomics Project, a US$170-million effort to identify and map those marks — known collectively as the human epigenome — begins its first comprehensive data release. Although it is not the only such effort worldwide (see *Nature* 463, 596–597; 2010), the US National Institutes of Health (NIH) epigenomics project is one of the most ambitious. The newly released data include more than 300 maps of epigenetic changes in 56 cell and tissue types, and represent a significant step towards the complete epigenome — the full picture of all the ways in which DNA can be modified, thus revealing the influence of epigenetics on cell development and its role in complex diseases (see graphic).

Various epigenetic mechanisms regulate gene expression. These include different types of modification on the histone proteins around which genomic DNA winds; attachment of methyl groups to the nucleotide cytosine in DNA, an alteration that is thought to switch off genes; sites of high sensitivity to an enzyme called DNase I, which cleaves accessible DNA and marks the location of gene regulatory regions; and RNA transcription, which, although not a DNA mark, is one measure of the global epigenetic state, revealing how much protein a particular gene makes in different cells. The NIH project developed a standardized protocol for measuring these four factors, and four designated centres around the United States have been charged with making reference maps of each type of modification in embryonic stem cells, induced pluripotent cells and in hundreds of primary adult and fetal tissues.

The project, slated to run for another five years, aims to produce maps for "a broad swathe of cell types that would be useful to disease research, fund work on specific diseases and develop novel technologies," says John Satterlee, a behavioural geneticist at the National Institute on Drug Abuse in Rockville, Maryland, and one of the coordinators of the project.

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A VARIABLE RESPONSE

Some scientists have been wary of the mapping component's 'big science' approach, fearing it will churn out data without ties to the biological questions it is meant to address. Others have questioned the idea that reference maps can be useful to scientists who study specific diseases. Researchers would still have to make their own maps using cells from people without disease, because most studies compare patients to healthy controls who are matched for factors such as age or sex, says John Greally at the Albert Einstein College of Medicine in New York. His projects on epigenomic factors that affect the developing fetus and that cause kidney disease receive funding from the NIH initiative.

Moreover, adds Greally, whereas the four US mapping centres use highly sophisticated techniques to produce their reference maps, individual labs mostly use simpler, cheaper methods to determine epigenetic marks, so comparing their data to the reference maps may be tricky. "Having the [mapping] information is valuable in itself," he says, "but the focus has got to be on how you use this to understand disease."

So far, the wider community of researchers has largely been unaware of the effort's existence. "We've sort of been in stealth mode," says Joseph Ecker, a plant and molecular biologist at the Salk Institute for Biological Studies in La Jolla, California, whose lab is working with a mapping centre to produce reference maps of DNA methylation. The newly released data should push the project to a point where the information can be widely used by researchers in different fields, he says.

Satterlee notes that the mapping component is just one arm of the project, making up about $57 million of its total budget. The rest of the money is going to individual investigator grants, 53 of which have already been awarded. Indeed, says cell biologist Benjamin Tycko of Columbia University in New York, whose work on the role of DNA methylation in Alzheimer's disease is funded by the project, "they've actually adopted a small lab approach" by funding research in labs with expertise on different diseases. ■

CANCER: S. GSCHMEISSNER/SPL; BRAIN: INGRAM PUBLISHING/GETTY; CHRONIC: S. MURTI/SPL; PRENATAL: S. GSCHMEISSNER/SPL

bootstrap-based subsampling that should increase robustness and has been successfully applied previously in studies of mice[5]. Of the 1.6 million SNPs, between 203 and 295 (LA traits) and 245 (SLB) were found to be significantly associated to the respective trait. These cies and may also be similar in its genetic architecture. Those working on such species might be reassured by the fact that, with appropriate resources, a large proportion of trait variation may be explained by simple SNP associations. In contrast, an experimental approach based

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The (new) new synthesis and epigenetic capacitors of morphological evolution

Douglas M Ruden

A new study shows that the piRNA-binding protein Piwi interacts with Hsp90 and suppresses phenotypic variation in *Drosophila melanogaster* by preventing the expression of hidden epigenetic variation. This suggests that Hsp90 and Piwi function are dampened in times of stress to increase genetic and epigenetic variability, providing a last-ditch mechanism for a species to survive.

In 1942, Julian Huxley wrote an influential book, *Evolution: The Modern Synthesis*, that influenced generations of geneticists[4]. The basis of the 'modern synthesis', also called the 'new synthesis', is that it reconciles Mendelian genetics with gradual evolution by means of natural selection of existing genetic variation. Now, on page 153 of this issue, Haifan Lin and colleagues report the existence of mechanisms that normally prevent the expression of hidden epigenetic variation[2], suggesting that the new synthesis should be expanded to incorporate both genetic and epigenetic sources of variation on which selection can act.

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Piwi suppresses genetic variation

The Piwi-interacting RNA (piRNA) binding protein Piwi[3], which is in the Argonaut family of small-RNA slicing proteins, was recently found to prevent transposon mobilization in the male germline by 'slicing' (cleaving) transposon RNAs[4,5]. Then, Specchia *et al.*[6] showed that reducing the expression of Hsp90, a chaperone that helps signaling proteins fold properly, disrupts piRNA-mediated silencing and leads to transposon activation. Specchia *et al.*[6] proposed that loss of piRNA can increase genetic variation through transposon mobilization, and this newly induced genetic variation can be genetically assimilated over many generations to increase the fitness of the organism. This work suggests that Piwi is an adaptively inducible 'canalizer'. 'Canalization' is a term proposed by Conrad Waddington in 1942 to explain how assimilation of specific alleles in a population stabilizes a phenotype from environmental stress to produce developmental robustness[7]. The beneficial effect of transposon mobilization is not a new idea; Barbara McClintock proposed in her Nobel Prize seminar in 1983 that transposon mobilization in times of stress can be a last-ditch mechanism of genome reorganization[8].

Analysis of DNA Methylation in a Three-Generation Family Reveals Widespread Genetic Influence on Epigenetic Regulation

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Abstract

The methylation of cytosines in CpG dinucleotides is essential for cellular differentiation and the progression of many cancers, and it plays an important role in gametic imprinting. To assess variation and inheritance of genome-wide patterns of DNA methylation simultaneously in humans, we applied reduced representation bisulfite sequencing (RRBS) to somatic DNA from six members of a three-generation family. We observed that 8.1% of heterozygous SNPs are associated with differential methylation in *cis*, which provides a robust signature for Mendelian transmission and relatedness. The vast majority of differential methylation between homologous chromosomes (>92%) occurs on a particular haplotype as opposed to being associated with the gender of the parent of origin, indicating that genotype affects DNA methylation of far more loci than does gametic imprinting. We found that 75% of genotype-dependent differential methylation events in the family are also seen in unrelated individuals and that overall genotype can explain 80% of the variation in DNA methylation. These events are under-represented in CpG islands, enriched in intergenic regions, and located in regions of low evolutionary conservation. Even though they are generally not in functionally constrained regions, 22% (twice as many as expected by chance) of genes harboring genotype-dependent DNA methylation exhibited allele-specific gene expression as measured by RNA-seq of a lymphoblastoid cell line, indicating that some of these events are associated with gene expression differences. Overall, our results demonstrate that the influence of genotype on patterns of DNA methylation is widespread in the genome and greatly exceeds the influence of imprinting on genome-wide methylation patterns.

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Introduction

Methylation of the 5 carbon of a large number of cytosines in the genome is necessary in mammalian development [1]. Aberrant patterns of DNA methylation have been reported in a wide variety of human diseases, including cancer [2,3], psychiatric disorders [4], autoimmune diseases [5] and diabetes [6]. Some of these patterns are indicative of underlying functional changes that have occurred during disease progression and shed light on genes involved in pathogenesis. One of the most fascinating discoveries surrounding DNA methylation is the observation that two homologous chromosomes can be differentially methylated.

Differential methylation of homologous chromosomes can be the result of epigenetic phenomena such as gametic imprinting [7,8] or X chromosome inactivation [9,10]. DNA sequence, or genotype, may also play a role in establishing differential methylation, as a few well-established cases have been identified in which a locus' DNA methylation state clearly depends on an individual's DNA sequence [11,12]. Recent advances in DNA sequencing technology have opened the door to exploring differential methylation on homologous chromosomes with high accuracy and detail. It is now possible to examine the prevalence of genetic versus epigenetic causes of differential methylation with unprecedented precision and thoroughness.

To distinguish the impact of gametic imprinting vs. genotype on DNA methylation, the inheritance patterns of alleles along with corresponding methylation levels should be observed. Recent studies have suggested that the majority of differential DNA methylation on homologous chromosomes is sequence-dependent and not the result of gametic imprinting, as the same allele has the same influence on DNA methylation in unrelated individuals [13–15]. However, to differentiate definitively between genetic inheritance and imprinting, analysis of DNA methylation in primary tissues from a family is necessary. Analysis of a family allows for the determination of a SNP's parental origin along with inheritance patterns of DNA methylation levels and therefore permits the direct examination of genetic and epigenetic mechanisms of differential methylation. By analyzing DNA methylation in a family, the impact of alleles verses the impact of a chromosome's parental origin on the inheritance of

Behavioral/Systems/Cognitive

Early Prenatal Stress Epigenetically Programs Dysmasculinization in Second-Generation Offspring via the Paternal Lineage

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Studies have linked sex-biased neurodevelopmental disorders, including autism and schizophrenia, with fetal antecedents such as prenatal stress. Further, these outcomes can persist into subsequent generations, raising the possibility that aspects of heritability in these diseases involve epigenetic mechanisms. Utilizing a mouse model in which we previously identified a period in early gestation when stress results in dysmasculinized and stress-sensitive male offspring, we have examined programming effects in second-generation offspring of prenatally stressed (F2-S) or control (F2-C) sires. Examination of gene expression patterns during the perinatal sensitive period, when organizational gonadal hormones establish the sexually dimorphic brain, confirmed dysmasculinization in F2-S males, where genes important in neurodevelopment showed a female-like pattern. Analyses of the epigenomic miRNA environment detected significant reductions in miR-322, miR-574, and miR-873 in the F2-S male brain, levels that were again more similar to those of control females. Increased expression of a common gene target for these three miRNAs, β-glycan, was confirmed in these males. These developmental effects were associated with the transmission of a stress-sensitive phenotype and shortened anogenital distance in adult F2-S males. As confirmation that the miRNA environment is responsive to organizational testosterone, neonatal males administered the aromatase inhibitor formestane exhibited dramatic changes in brain miRNA patterns, suggesting that miRNAs may serve a previously unappreciated role in organizing the sexually dimorphic brain. Overall, these data support the existence of a sensitive period of early gestation when epigenetic programming of the male germline can occur, permitting transmission of specific phenotypes into subsequent generations.

Introduction

Epidemiological studies have linked prenatal stress to increases in the incidence of neurodevelopmental disorders, including schizophrenia and autism spectrum disorders, associations that are often sex dependent (Huttunen and Niskanen, 1978; van Os and Selten, 1998; Khashan et al., 2008; Kinney et al., 2008). These diseases often display sex differences in prevalence, presentation, or therapeutic outcomes (Bale et al., 2010). While many factors likely contribute to these differences, sex-specific responses to fetal antecedents are likely involved (Weinstock, 2007).

We have previously identified early gestation as a specific window of sensitivity during which male mice were susceptible to the programming effects of maternal stress. These males exhibited physiological and behavioral stress sensitivity and cognitive deficits, endophenotypes associated with human neuropsychiatric disease. In addition, these changes reduced or disrupted established sex differences by dysmasculinizing male offspring measures of stress responsivity (Mueller and Bale, 2007, 2008). Similar disruptions of sex differences in behavior, morphology, and gene expression profiles have previously been reported in studies using prenatal stress paradigms across multiple species (Ward, 1972; Meisel et al., 1979; Reznikov et al., 1999; Kapoor and Matthews, 2005; Biala et al., 2010). The organizational/activational hypothesis of brain development suggests that a surge of gonadal hormones organize the brain in a sexually dimorphic manner during the perinatal sensitive period. Then in adulthood, gonadal hormones can activate this organized neurocircuitry to express appropriate sex-specific behavioral phenotypes, including stress axis responsivity (Phoenix et al., 1959; Arnold and Gorski, 1984; Seale et al., 2005; Bingham and Viau, 2008). The disruption of sex differences identified in our model suggests that early prenatal stress alters the trajectory of neurodevelopment during the perinatal period.

Fetal antecedents likely contribute to adult disease through programming changes in the epigenome. Examples of this phenomenon are emerging in human studies. For example, infants with prenatal exposure to maternal depression or anxious mood exhibited increased glucocorticoid methylation, which was associated with a heightened cortisol response to a mild stressor (Oberlander et al., 2008). Such programming effects may transmit to subsequent generations, predisposing offspring to disease. Animal models have clearly established a role for epigenetics in

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Epigenetic inheritance of a cocaine-resistance phenotype

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We delineated a heritable phenotype resulting from the self-administration of cocaine in rats. We observed delayed acquisition and reduced maintenance of cocaine self-administration in male, but not female, offspring of sires that self-administered cocaine. Brain-derived neurotrophic factor (*Bdnf*) mRNA and BDNF protein were increased in the medial prefrontal cortex (mPFC), and there was an increased association of acetylated histone H3 with *Bdnf* promoters in only the male offspring of cocaine-experienced sires. Administration of a BDNF receptor antagonist (the TrkB receptor antagonist ANA-12) reversed the diminished cocaine self-administration in male cocaine-sired rats. In addition, the association of acetylated histone H3 with *Bdnf* promoters was increased in the sperm of sires that self-administered cocaine. Collectively, these findings indicate that voluntary paternal ingestion of cocaine results in epigenetic reprogramming of the germline, having profound effects on mPFC gene expression and resistance to cocaine reinforcement in male offspring.

A growing body of evidence indicates that ancestral environmental perturbations can influence the physiology and behavior of descendants. Although most studies of this sort focus on maternal effects[1–3], there are examples of paternal phenotype transmission between generations. For example, progeny of male mice fed a low-protein diet show elevated hepatic expression of genes involved in lipid and cholesterol biosynthesis[4], whereas exposing male rats to a high-fat diet results in pancreatic beta cell dysfunction in female offspring[5]. Moreover, early prenatal stress reprograms the male germline, resulting in dysmasculinization in second-generation offspring[6]. Notably, transgenerational influences of ancestral environment are evident in humans—epidemiological data link exposure to a famine in grandfathers to obesity and cardiovascular disease two generations later[7,8].

In terms of drugs of abuse, the adult offspring of female rats exposed to morphine during adolescence show increases in anxiety (female offspring), enhancement of morphine-induced analgesia (male offspring) and augmented behavioral sensitization to morphine (male and female offspring)[9,10]. Maternal exposure to cocaine decreases global DNA methylation in the hippocampus of male offspring[11]; paternal cocaine administration results in impaired working memory in female offspring[12] and causes hyperactivity and increased perseveration in a T-maze among male progeny[13]. The implications of these findings for the descendants of drug-dependent individuals are profound. Thus, we established a rat model to examine the influence of paternal cocaine self-administration on gene expression, chromatin remodeling and cocaine reinforcement in the progeny. We examined paternal transmission to avoid the influence of *in utero* cocaine exposure and the potential influence of previous cocaine experience by dams on maternal behavior.

RESULTS

To develop a model for the intergenerational influence of cocaine self-administration on gene expression, chromatin remodeling and behavior, we allowed male Sprague-Dawley rats to self-administer intravenous cocaine (0.25 mg per infusion, not adjusted for animal weight) for 60 d, the duration of rat spermatogenesis; control rats received yoked intravenous saline injections. The mean (± s.e.m.) number of infusions per day was 23.79 ± 0.5. Rats initially self-administered 3–4 mg (~0.5 mg per kg of body weight per infusion) of cocaine per day, with the daily intake escalating to 7–8 mg per day after 60 d of self-administration (**Fig. 1**). In this experiment, the average cocaine dose was approximately 0.7 mg per kg per infusion. The day after the last self-administration session, the F_0 males were mated with naïve females, resulting in 13 litters from cocaine-experienced sires and 13 litters from saline control sires.

Reduced cocaine intake in male cocaine-sired rats

When they reached approximately 60 d of age, we implanted jugular catheters into 1–3 male and female offspring from each litter. After 7 d of recovery, we assessed the acquisition of cocaine self-administration under a fixed ratio 1 (FR1) schedule of reinforcement. Under an FR1 schedule, all lever presses resulted in cocaine administration. The results indicated no difference in the rate of acquisition or the level of cocaine intake among female offspring of cocaine-experienced males (♀CocSired) relative to controls (♀SalSired) (**Fig. 2a,b**). However, we observed significantly delayed acquisition of 0.5 mg per kg and 1.0 mg per kg cocaine self-administration by male offspring of cocaine-experienced (♂CocSired) rats relative to controls (♂SalSired) (**Fig. 2c,d**). Moreover, when cocaine self-administration reached asymptote, we saw a significant decrease in intake of 1.0 mg

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