

Workshop on “Theoretical, Empirical and Policy Inputs to Modeling Healthy Ageing”

The role of genes and environment in complex diseases and the biology of ageing

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The study of disease has provided many insights into the causal mechanisms for abnormal biological phenomena. Disease has often been thought of as being of either genetic or environmental origin. However investigation into the most prevalent diseases that affect large proportions of the population such as cardiovascular disease, type II diabetes and mental illness has shown that most of these disorders are complex disorders, with both a genetic and an environmental component. In addition, most common diseases are not caused by a single gene mutation, but rather result from the effect of multiple genetic variants, each contributing towards the overall risk of illness.

While the focus of much research is on disease, the role of genes and environment are equally as important in normal biological processes. The challenge for ageing research is to better define the specific genetic and environmental effects that impact this key biological process.

For disorders that arise from an environmental cause, it is not usual to consider genetic impacts, but there are clear examples where a genetic predisposition can alter the impact of the environmental factor. One such example viral infection by HIV which is the causal agent of AIDS. The chemokine receptor CCR5 is a co-receptor needed for the cellular internalization of the HIV virus and is therefore required for HIV infection. A genetic variant in the chemokine receptor referred to as $\Delta 32$ is unable to internalize the HIV virus, thus those individuals who carry the $\Delta 32$ variant are resistant to HIV infection. Similarly, it is usually considered that Mendelian genetic disorders have no environmental component. However, mutations in the enzyme phenylalanine hydroxylase, which converts the amino acid phenylalanine to tyrosine, cause the recessively inherited disorder phenylketonuria. Phenylketonuria leads to an accumulation of phenylalanine and phenylketones in the brain, resulting in progressive mental retardation and seizures. But when diagnosed early in life, a simple environmental change, the dietary modification of restricting phenylalanine and supplementing tyrosine in the diet can lead to a nearly complete cure. Thus, we have good examples of genetic disorders that can be controlled by environmental manipulations and environmental disorders that are able to be prevented by genetic variation. If fully genetic or environmental disorders can be manipulated as outlined above, then there should exist even greater prospects for manipulation of environmental or genetic factors in complex disorders.

Two landmark studies published by Caspi and colleagues in *Science* in 2002 and 2003 have shown the role of genes and environment in maltreatment and in depression. In the first study (Caspi et al, 2002), a polymorphism in the promoter region of the monoamine oxidase A gene (MAOA), which results in lower enzymatic activity, was correlated with the degree of adverse childhood maltreatment. MAOA is an enzyme that degrades monoamine neurotransmitters such as norepinephrine, a neurotransmitter involved in sympathetic functions such as arousal and rage. Maltreated children with the low-activity MAOA variant were more likely to develop antisocial conduct disorders than maltreated children with the high-activity variant. The proposed

mechanism by which this gene-environment interaction occurs is via the decreased ability of those with low MAOA activity to degrade norepinephrine leading to a greater propensity to violence. This study shows that genetic susceptibility is not pre-determined at birth, but rather varies with exposure to environmental influences.

Serotonin (5-HT) is a key neurotransmitter which contributes to many physiologic functions, including appetite, sleep, aspects of cognition and expression of depression and anxiety. The serotonin transporter (5-HTT) is central to the regulation of brain and peripheral serotonergic neurotransmission. A functional polymorphism in the promoter region of the serotonin transporter gene, known as 5-HTTLPR, has been inferred to be involved with depression and risk factors to depression (eg. neuroticism), with the short 's' allele reducing transcriptional efficiency of the serotonin promoter and leading to decreased 5-HTT expression (Lesch et al, 1996). However, meta-analysis does not support a direct relationship between the 5-HTTLPR and depression onset.

The second study by Caspi and colleagues (2003) examined the relationship between the 5-HTTLPR polymorphism, multiple adverse life events in a cohort that had been studied longitudinally, and the onset of major depression. Study participants with the s/s genotype at the 5-HTT gene were more likely (than those with s/l or l/l genotypes) to experience major depression, but only if they had experienced multiple adverse life events, demonstrating a gene x environment interaction.

Our own study by Wilhelm and colleagues (2006) initially sought to replicate the reported gene x environment interaction in a cohort with individualised longitudinal data for adverse life events, serotonin transporter genotype and major depression onset. We did not observe a relationship between 5-HTTLPR genotype and risk factors to depression or with positive life events. However, we were able to demonstrate a gene x environment effect between multiple adverse life events and those with the s/s genotype which led to a significantly greater incidence of depression onset.

In a follow on study, (Willem et al, 2007) we examined whether the strategies people use to cope with stress, as occurs during adverse life events, were associated with differing 5-HTT genotypes. Coping behaviours were assessed within the abovementioned longitudinal study in 1993. Using regression analysis, associations were then examined between the coping scales and the 5-HTT genotype and gender. Four coping dimensions emerged from the factor analysis: problem solving, anger/tension release, emotional regulation and distraction. The 's' variant of the 5-HTT promoter polymorphism was found to be associated with the use of fewer problem-solving strategies. Our results raise the possibility that a gene-related disposition to greater emotional reactivity may preclude those with the 's' variant of the 5-HTT promoter polymorphism from drawing on problem-solving strategies to deal with stress.

The results of our studies raise the distinct possibility that it may be possible to undertake genotype relevant interventions to address the greater emotional reactivity in those with at risk genotypes (ie s/s genotypes) and at risk phenotypes (ie multiple adverse life events) to effectively ameliorate the risk for developing depression. While this hypothesis will need to be tested using controlled clinical trials, the observations of the impact of gene x environment interactions raise that distinct possibility that a range of complex disorders may be able to be treated by novel therapeutic approaches.

Genetic analysis has begun to identify risk factors for complex diseases. Such approaches are also starting to identify genetic factors that may underpin complex biological processes such as

ageing. Epidemiological studies have long been used to examine the environmental exposures that may impact on disease and ageing. The case studies detailed above have demonstrated that the combination of genetics and epidemiological studies may now give rise the very real prospect of identifying and harnessing gene x environment interactions to lead to improved health and ageing outcomes.

References

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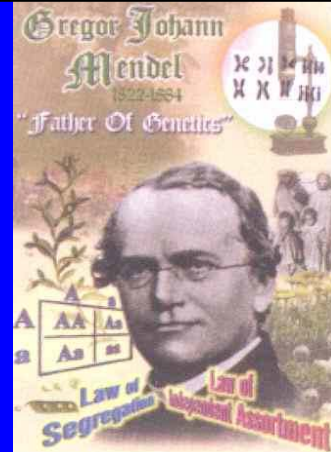
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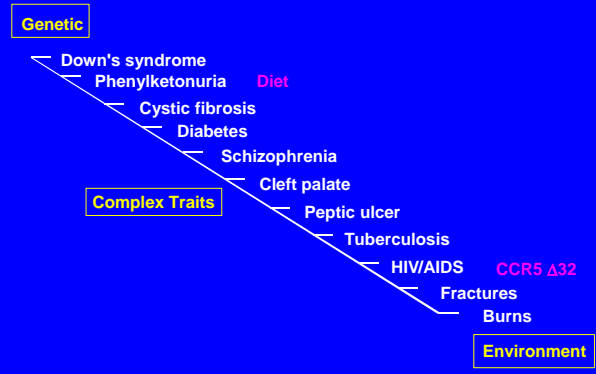
The role of genes and environment in complex diseases and the biology of ageing

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Most diseases have a genetic component



Environmental enrichment delays HD onset

Delaying the onset of Huntington's in mice
 "This unremitting disease develops later in animals stimulated by their environment"

QuickTime™ and a None decompressor are needed to see this picture.

Van Dellen et al. Nature 404, 721, 2000

Environmental enrichment delays HD onset



Van Dellen et al. Nature 404, 721, 2000

Positive impact of environmental enrichment & physical activity on neurological disorders

QuickTime™ and a None decompressor are needed to see this picture.

Nithianantharajah & Hannan, Nat Rev Neuro 7, 697, 2006

Effects of environmental enrichment and enhanced physical activity on animal models of CNS disorders

Disorder	EE/PA	Behavioural	Cellular	Molecular
Huntington's disease	EE PA	x x	x x	x x
Alzheimer's disease	EE PA	x x	x x	x x
Parkinson's disease	EE PA	x x	x x	x x
Amyotrophic lateral sclerosis	EE PA	x x		
Epilepsy	EE	x	x	x
Stroke	EE	x	x	x
Traumatic brain injury	EE	x	x	x
Fragile X syndrome	EE	x	x	x
Down syndrome	EE	x	x	

Nithianantharajah & Hannan, Nat Rev Neuro 7, 697, 2006

Susceptibility Genes

- Variants of normal genes
- Risk or protection variants
- Neither necessary nor sufficient
- Interaction with other genes
- Interaction with environment



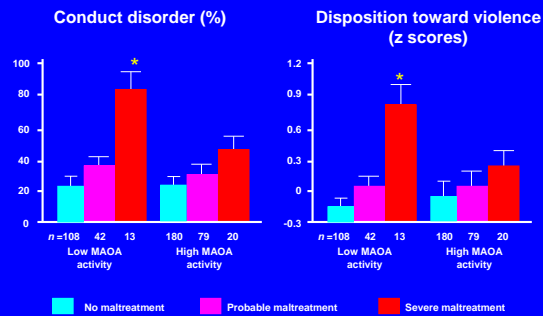
Cunningham Dax Collection

“Role of genotype in the cycle of violence in maltreated children”



Caspi et al. Science 297, 851, 2002

High MAOA levels are socially protective



Caspi et al. Science 297, 851, 2002

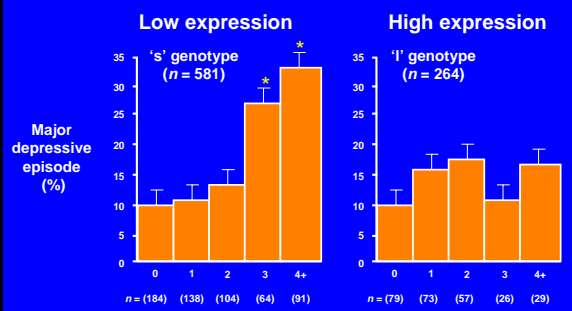
“Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene”



Cunningham Dax Collection

Caspi et al. Science 301, 386, 2003

Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene



Caspi et al. Science 301, 386, 2003

Serotonin Transporter Gene

- *SLC6A4*: serotonin transporter (5HTT)
- Terminates 5HT action by uptake into presynaptic neuron
- 5HTTLPR - a 43 bp insertion/deletion ~1 kb upstream



- s/s and s/l genotypes show less than 50% of the expression 5HTT compared to l/l genotype (Lesch et al. Science 1996)
- Individuals with the "s" allele (reduced 5HTT expression and function) exhibit greater amygdala activity as assessed by fMRI in response to fearful stimuli (Hariri et al. Science 2002)
- *This may contribute to increased fear and anxiety in people with "s" allele*

The Teachers Study - A longitudinal cohort

1978 Baseline assessments on 170 teachers
 Mean age 23.3 +/- 4.3 years
 Assessed longitudinally each 5 years
 85% retention at 20 yrs

- History of depression and anxiety
- Positive and negative life events
- Trait depression, neuroticism, self esteem
- Parental bonding and intimate bonds
- Coping styles and social support
- Drug, alcohol and medical history

2003 DNA obtained from 128 participants (75%)

The Teachers Study

Logistic Regression Analysis on 127 participants

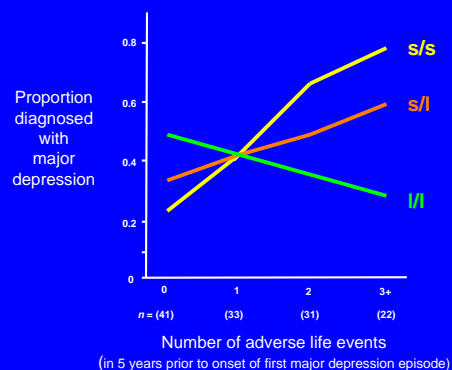
Main effect associated with onset of major depression

Adverse life events	p=0.01 *
5-HTT promoter polymorphism	p=0.15
Gene x Environment interaction	p=0.03 *

Covariates

Gender	p=0.43
Age on onset	p=0.65

Teachers Study: Life events and depression onset



Four coping styles for managing stress

In 1993, the cohort members were asked what coping strategies they used when 'stressed'

- Problem Solving
 - Distraction/Changing Focus
 - Anger Expression *
 - Internal Emotional Regulation *
- * Both more common in women

Factor analysis showed the four coping styles account for 44.9% of total variance

A history of one or more episodes of major depression during one's lifetime predicted the use of more anger or tension release strategies

Hypothesis: Is the 5HTTLPR associated with depression or with stress management?

To determine whether those with the 's' or 'l' alleles of the 5-HTTLPR utilise different coping strategies to deal with stressful events likely to lead to depression

We predict that those with the 's' allele would use more coping styles aimed to decrease emotional arousal when stressed.

Analysis

- When stressed, subjects with 's' allele used fewer problem solving strategies than the 'l/l' genotype group
- No differences for anger/tension release, distraction and internal emotional regulation

Implications

- Coping is influenced by 5-HTT genotype and gender
- Gene-related disposition to greater emotional reactivity may preclude those with the 's' allele from using problem solving strategies to deal with stress
- Is problem solving a universally applicable coping strategy?
- Do more emotionally reactive people require emotion regulation techniques prior to problem solving?

A stress management intervention trial

- Study on patients with type I and II diabetes
- Tertiary centres, GP clinics, Diabetes Australia
- A highly stressed population with daily stress due to illness
 - an increase in adverse life events
- Clinical interview
- Block Randomization and crossover design
 - Emotional regulation
 - Problem focused
 - Control (expressive writing)
- Intervention package explained by RAs
- Telephone follow-up by RAs
- Questionnaires mailed out at 6 wks, 3 and 6 months

Stress management interventions

Problem Focused Strategies

- i) Structured Problem Solving - worksheets guide participants through the stages of structured problem solving, from identification of problem to execution.
- ii) Goal-setting - leads participants through the importance of making changes in their lives and then setting appropriate goals eg self, relationships and work

2) Emotional Regulation Strategies

- i) Mindfulness and Meditation - the practice of being in the present; examples include focusing on breathing for 1 minute, mindful eating or walking
- ii) Relaxation Exercises - eg progressive muscle relaxation, guided imagery, etc

3) Expressive Writing

Outcomes

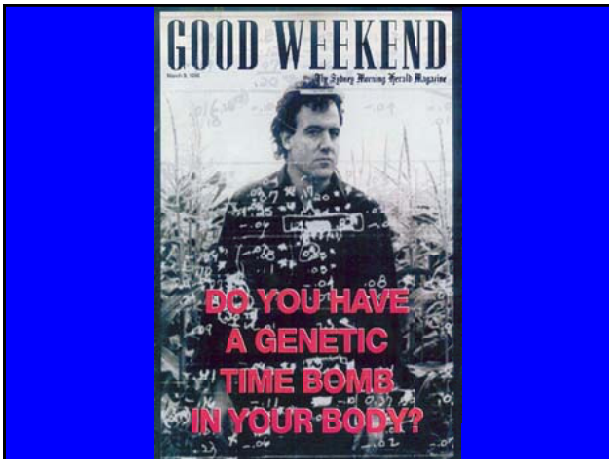
- What did you like the most?
Patients will be asked which intervention they preferred and used the most
- Patient choice important in determining which psychological intervention works best
- Genotype based diagnosis
- Genotype based interventions
 - Pharmacogenomic?
 - Cognitive?



Depression is one of the most common of all mental health problems

One in five people will experience depression during their lifetime

A clinical vignette of the future



Acknowledgements

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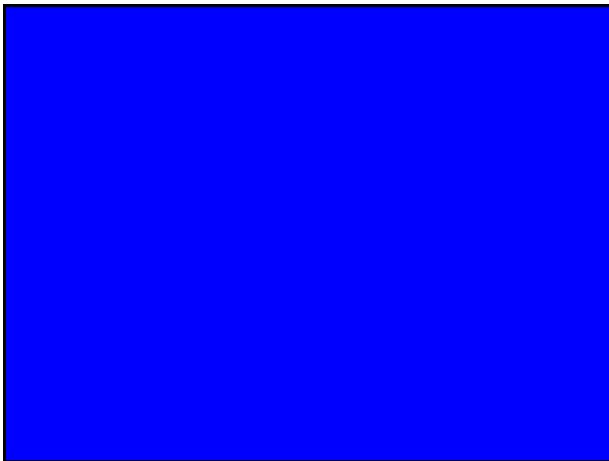
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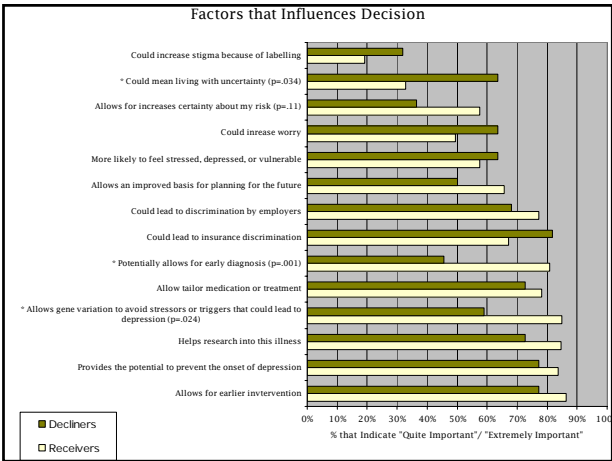
Evaluation of Impact of Genetic Test Results: Participators versus Decliners

- Decliners more influenced by fear of increased anxiety/negative effects on mental health, career
- Receivers may have higher neuroticism and anxiety scores and be more prone anxiety of not knowing
- Receivers more likely to say 'yes' to a test regardless of gene effect

Genetic Test Results

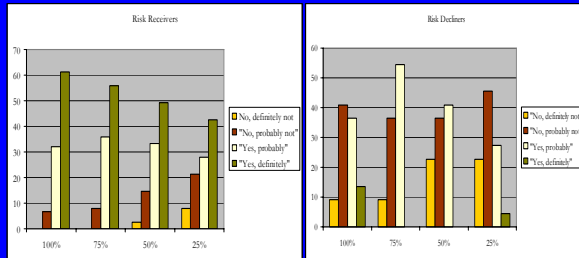
- 116 returned questionnaires
- 71% Receivers* (n=82)
- 19% Decliners (n=22)
- 10% Non-Participators (n=12)
 - * Previous studies around 20% receiver rate

No difference in age, perceived lifetime risk, PANAS score, Anxiety levels, Neuroticism, or caseness of major depression



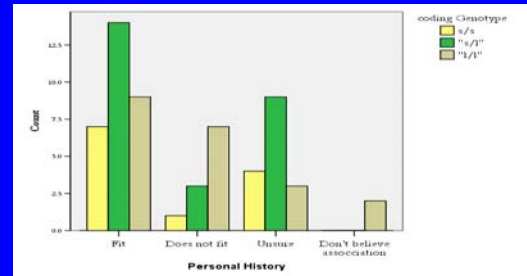
Genetic Test Results for Susceptibility Genes

Receivers more likely to opt for testing regardless of level of risk ($p < .001$)



Genetic Test Results: Self Interpretation

Most individuals overestimated their chance of having the at-risk (s/s) genotype.



Outcomes: Genetic Test Results

- Most had overestimated their chance of being in high risk group and were pleased with knowledge
- Those in high risk group who wanted to know were pleased to have the knowledge
- Participants remember their results well
- Results from this group may not be applicable to younger, less involved subjects