Beyond GWAS MA

Sarah Medland
Genetic Epidemiology
QIMR

Candidate genes vs GW approaches

- Conceptual division
  - Similar to Confirmatory vs Exploratory factor analysis?
- Complementary approaches
  - Address different questions
- WHAT is the desired outcome
  - Easier to answer in Medical Research

Behav Genet
DOI 10.1007/s10519-011-9504-z

BRIEF COMMUNICATION

Editorial Policy on Candidate Gene Association and Candidate Gene-by-Environment Interaction Studies of Complex Traits

John K. Hewitt

The literature on candidate gene associations is full of reports that have not stood up to rigorous replication. This is the case both for straightforward main effects and for candidate gene-by-environment interactions (Duncan and Keller 2011). As a result, the psychiatric and behavior genetics literature has become confusing and it now seems likely that many of the published findings of the last decade are wrong or misleading and have not contributed to real advances in knowledge. The reasons for this are complex, but include the likelihood that effect sizes of individual polymorphisms are small, that studies have therefore been underpowered, and that multiple hypotheses and methods of analysis have been explored; these conditions will result in an unacceptably high proportion of false findings (Ioannidis 2005).

What we do can have consequences...

- Science & Funding
- Treatment
  - Personalised medicine
    - Tailor treatment to genotype
    - Flip-side make access to certain treatments more difficult
- Legal
  - Bradley Waldroup
    - Charged with murder, attempted murder, kidnapping
    - Defence – not who but why - MAO-A * childhood mistreatment
    - Sentenced with voluntary manslaughter, attempted 2nd degree murder and kidnapping

Behav Genet
DOI 10.1007/s10519-011-9504-z

BRIEF COMMUNICATION

Editorial Policy on Candidate Gene Association and Candidate Gene-by-Environment Interaction Studies of Complex Traits

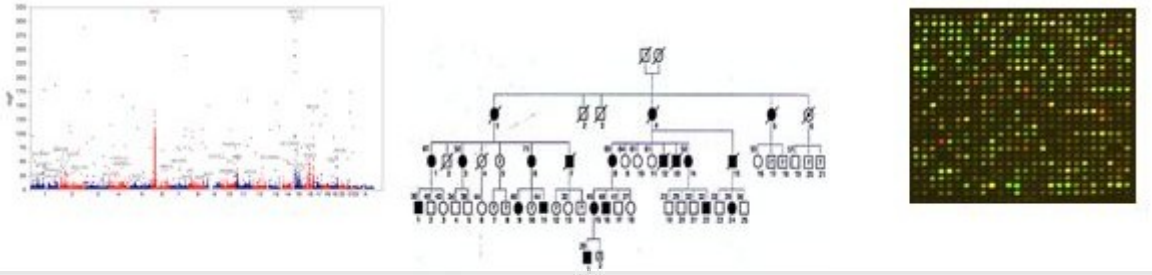
John K. Hewitt

- It is a rigorously conducted, adequately powered, direct replication study of a previously reported result; for well conducted replication studies, there is no editorial preference in *Behavior Genetics* for or against null, positive, or contradictory findings.
- It was an exploratory study or test of a novel hypothesis, but with an adequately powered, direct replication study reported in the same paper.
- It was an exploratory analysis or test of a novel hypothesis in the context of an adequately powered study, and the finding meets the statistical criteria for genome wide significance taking into account all sources of multiple testing (e.g. phenotypes, genotypes, environments, covariates, subgroups).
- It is a meta-analysis of several or many studies addressing the same genetic variant and/or environmental variable and the same behavioral outcome.

Candidate Gene Study Approaches

Less problematic

- Fine mapping as a form of candidate gene studies. Following up previous studies.



- Related traits esp. multivariate analyses
  - Depression, Anxiety, Bipolar
- Literature based selection

More problematic

- Legacy/Publically available data

The usual subjects...

- Serotonin – 5-HTTLPR
- MAOA - MAOA-uVNTR
- COMT - *Val*158*Met*
- Dopamine – DRD2 DRD4
- XAR

However, these initial results based on twin studies beg the question "which genes?" The natural place to start the search for such genes is among those that have already been shown to account for variation in social behavior. And among these, MAOA and 5HTT are prime candidates. These two genes tran-

JAMES H. FOWLER AND CHRISTOPHER T. DAWES
The Journal of Politics, Vol. 70, No. 3, July 2008, Pp. 579–594

Expressed in the brain...

Citation: Ramsköld D, Wang ET, Burge CB, Sandberg R (2009) An Abundance of Ubiquitously Expressed Genes Revealed by Tissue Transcriptome Sequence Data. PLoS Comput Biol 5(12): e1000598. doi:10.1371/journal.pcbi.1000598

Tissue/Cell	Number of genes*	Fraction of genes*	Ensembl genes†
Skeletal muscle[1]	11,276	0.61	11,953
Liver[1,3]	11,392	0.61	12,191
BT474[4]	11,844	0.64	12,808
MB435[4]	11,847	0.64	12,726
HME[5]	12,084	0.65	12,920
T47D[4]	12,205	0.66	12,983
Heart	12,209	0.66	13,159
MCF7[4]	12,281	0.66	13,216
Adipose tissue	12,553	0.68	13,503
Colon	13,016	0.70	14,052
Cerebellum[2,3]	13,132	0.70	14,043
Kidney	13,235	0.71	14,177
Brain[1]	13,298	0.71	14,107
Breast	13,406	0.72	14,537
Lymph node	13,534	0.73	14,686
Testes	15,518	0.84	16,869

Tissue/Cell	Fraction ubiquitous
Liver[2]	0.31
Heart	0.66
Brain	0.74
HME[4]	0.75
Breast	0.75
Skeletal muscle	0.76
Cerebellum[1,2]	0.76
Testes	0.77
Kidney	0.78
Adipose tissue	0.81
Colon	0.82
Lymph node	0.84
T47D[3]	0.87
MB435[3]	0.89
MCF7[3]	0.89
BT474[3]	0.90

For example 5-HTTLPR...

The 5HTTLPR is defined by a length variation of a repetitive sequence with a short (484 base pairs, 14 repeat units) and a long allele (528 base pairs, 16 repeat units) on chromosome 17. The basal activity of the long allele transcript is about threefold higher than that of the short allele, resulting in induced expression and function of the SLC6A4 gene.[1]

THE SEROTONIN TRANSporter gene (5-HTT, SERT, *SLC6A4*) is arguably both the most and least loved gene in psychiatric genetics. Fifteen years ago, the discovery of a common, functional promoter polymorphism (5-HTTLPR) that modulates SERT expression[1] launched innumerable association studies. In part,

It is time to move beyond observational studies of single variants, particularly in *DSM*-defined neuropsychiatric disorders, including everyone's favorite whipping boy, the 5-HTTLPR.

Randy D. Blakely, PhD
Jeremy Veenstra-VanderWeele, MD

ARCH GEN PSYCHIATRY/VOL 68 (NO. 5), MAY 2011

Tip of the iceberg

Showing Results 1 - 10 of 170 | Next 10

Pathway: Serotonin clearance from the synaptic cleft from Reactome [19 molecules]
Reviewed: Restituito, S, 2008-11-27 12:38:49.

- ... **Serotonin** clearance from the synaptic cleft ...
- Clearance of **serotonin** ...
- ... such key regulator is the **serotonin** transporter (5-HTT), which is observed to remove **serotonin**

Pathway: serotonin degradation from HumanCyc [29 molecules]
General Background The neurotransmitter and neuron
mammalian central nervous system including learning,

- ... **serotonin** degradation ...
- ... bGeneral Background/b The neurotransmitter and neuromodu
SEROTONIN| levels is associated with a variety of neurological
focus of |FRAME: **SEROTONIN**| research, only a small percentag
serotonergic neurons of the central nervous ...

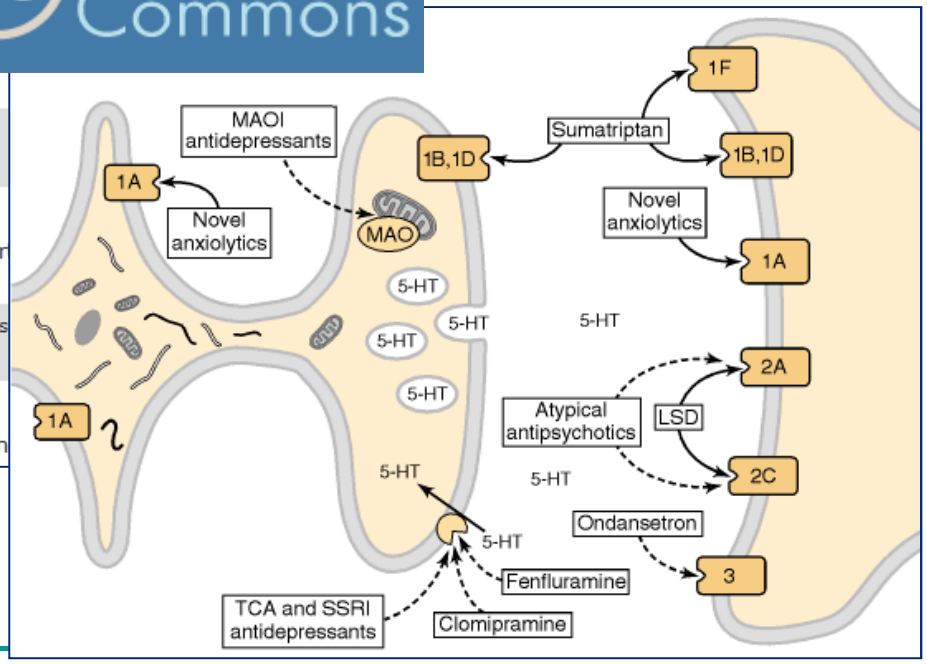
Pathway: Serotonin receptors from Reactome [7 molecules]
Edited: Jassal, B, 2009-02-12 10:33:32.

- ... **Serotonin** receptors ...
- ... **Serotonin** (5-HT) is a monoamine neurotransmitter that plays an impor
classification of receptors for 5-hydroxytryptamine (**Serotonin**) ...

Pathway: Metabolism of serotonin from Reactome [17 molecules
Reviewed: Restituito, S, 2008-11-27 12:38:49.

- Metabolism of **serotonin** ...
- ... **Serotonin** is first metabolized to 5-hydroxyindole acetaldehyde by mon

PC Pathway Commons



That interaction...

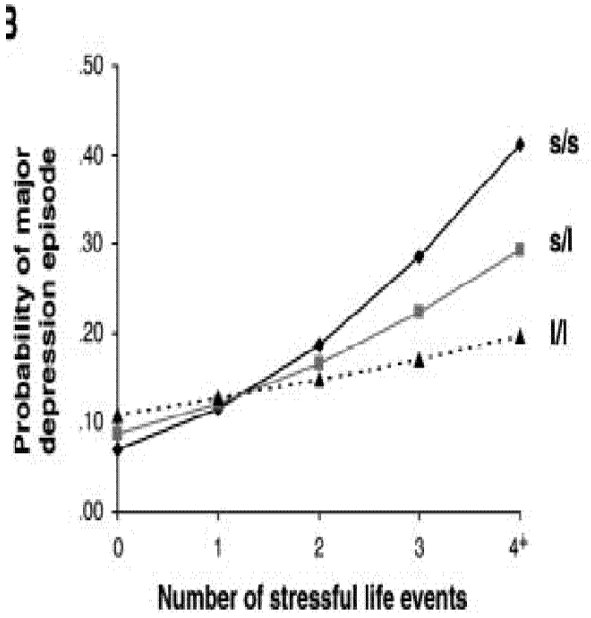
(is the devil in the details?)

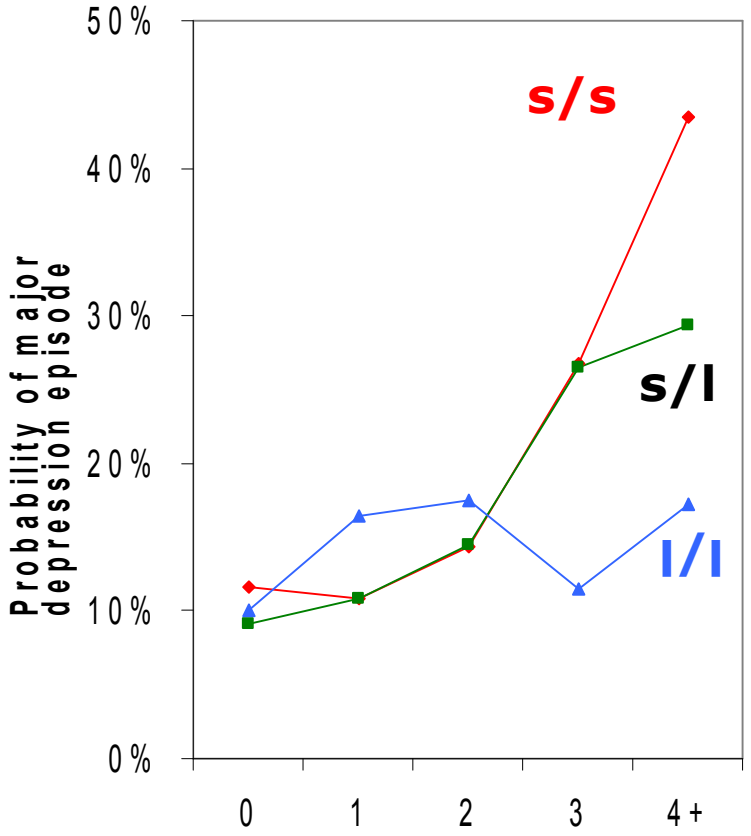
Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene

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] and Richie Poulton[3]

Science 18 July 2003:
Vol. 301 no. 5631 pp. 386-389

Fig. 1. Results of multiple regression analyses estimating the association between number of stressful life events (between ages 21 and 26 years) and depression outcomes at age 26 as a function of 5-HTT genotype. Among the 146 s/s homozygotes, 43 (29%), 37 (25%), 28 (19%), 15





c/o Will Coventry & David Duffy

Does it matter? Probably not

The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis Revisited

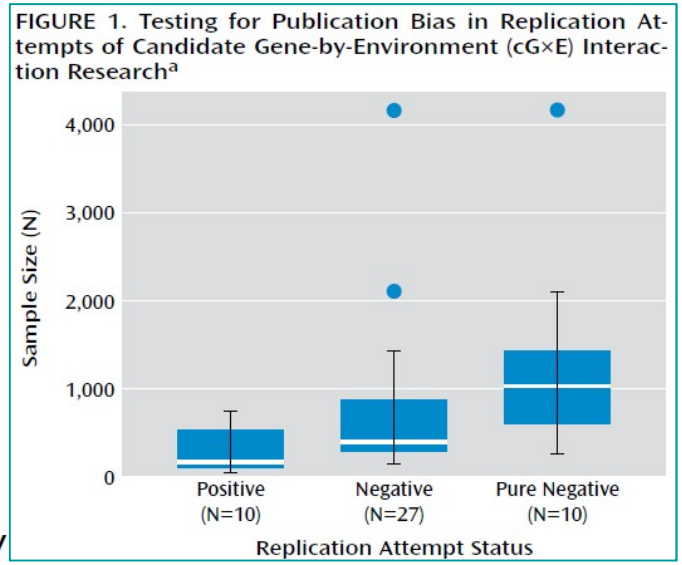
Arch Gen Psychiatry. 2011;68(5):444-454.

Evidence of Genetic Moderation

Katja Karg, BSc; Margit Burmeister, PhD; Kerby Shedden, PhD; Srijan Sen, MD, PhD

We included 54 studies and found strong evidence that 5-HTTLPR moderates the relationship between stress and depression, with the 5-HTTLPR s allele associated with an increased risk of developing depression under stress (P = .00002). When stratifying our analysis by the type of stressor studied, we found strong evidence for an association between the s allele and increased stress sensitivity in the childhood maltreatment (P = .00007) and the specific medical condition (P = .0004) groups of studies but only marginal evidence for an association in the stressful life events group (P = .03). When

Our initial search identified 148 publications. Of these studies, we identified 54 studies that included 40 749 subjects meeting criteria for inclusion (**Table 1**).



Laramie E. Duncan, Ph.D.

Matthew C. Keller, Ph.D.

A Critical Review of the First 10 Years of Candidate Gene-by-Environment Interaction Research in Psychiatry

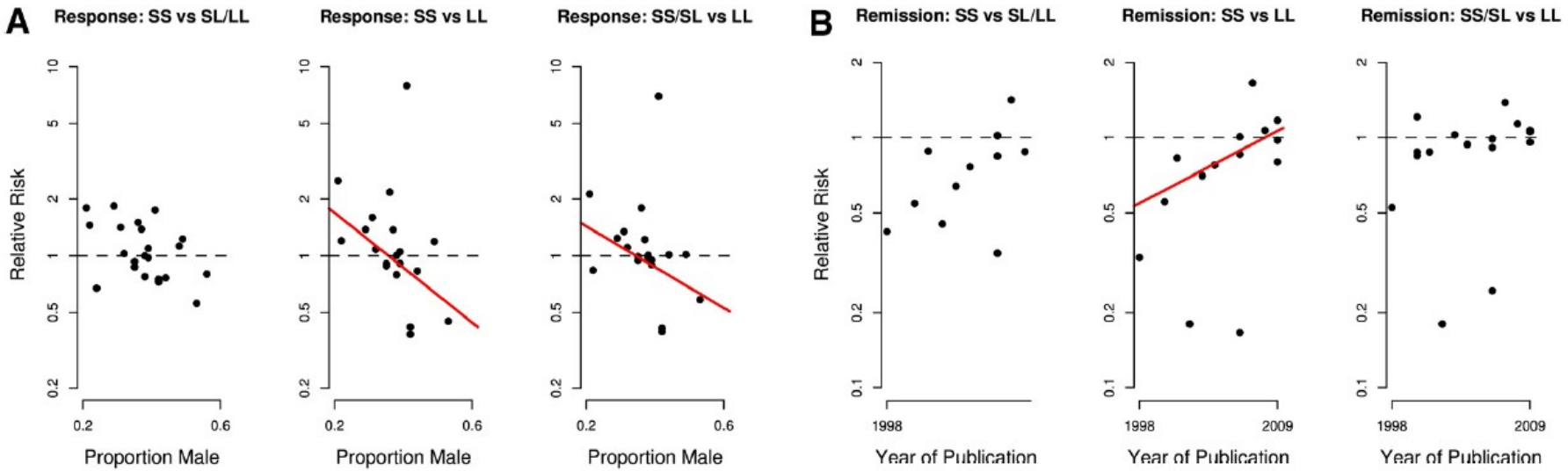
(Am J Psychiatry 2011; 168:1041–1049)

Antidepressant Response and the Serotonin Transporter Gene-Linked Polymorphic Region

Matthew J. Taylor, Srijan Sen, and Zubin Bhagwagar

BIOL PSYCHIATRY 2010;68:536–543

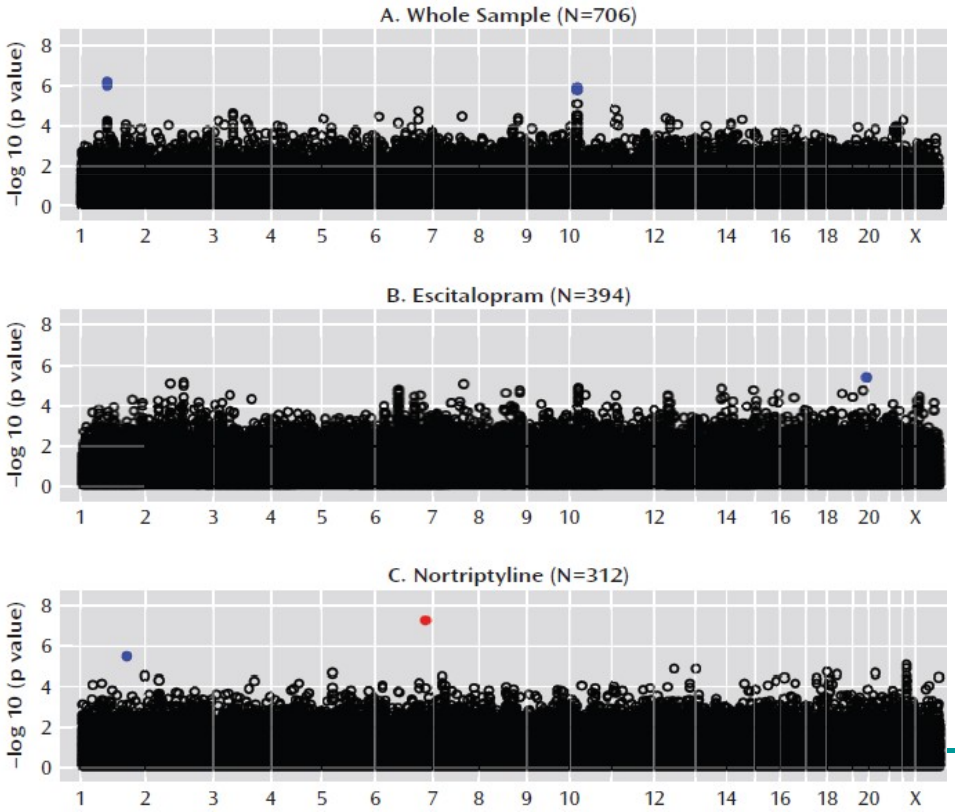
Results: There was no statistically significant effect on antidepressant response. Compared with L carriers, there was an apparent effect of the SS genotype on remission rate (relative risk: .88; 95% confidence interval: .79–.98; $p = .02$). However, after trim and fill correction for missing data, the effect disappeared (relative risk: .92; 95% confidence interval: .81–1.05; $p = .23$), indicating that the initial significant effect was likely the result of publication bias. No significant effect on remission rate was seen for SS versus LL and SS/SL versus LL. Substantial unexplained heterogeneity of effect sizes was observed between studies, pointing to additional interacting factors contributing to an association in some cases.



Am J Psychiatry 2010; 167:555-564
(published online April 1, 2010; doi: 10.1176/appi.ajp.2009.09070932)

Genome-Wide Pharmacogenetics of Antidepressant Response in the GENDEP Project

Rudolf Uher, M.D., Ph.D., M.R.C.Psych., Nader Perroud, M.D., Mandy Y.M. Ng, Ph.D., Joanna Hauser, Ph.D., Neven Henigsberg, M.D., Wolfgang Maier, M.D., Ole Mors, Ph.D., Anna Placentino, Psy.D., Marcella Rietschel, M.D., Daniel Souery, Ph.D., Tina Žagar, Ph.D., Piotr M. Czerski, Ph.D., Borut Jerman, B.Sc., Erik Roj Larsen, M.D., Ph.D., Thomas G. Schulze, M.D., Astrid Zobel, M.D., Sarah Cohen-Woods, Ph.D., Katrina Pirlo, B.Sc., Amy W. Butler, Ph.D., Pierandrea Muglia, M.D., Michael R. Barnes, Ph.D., Mark Lathrop<, Ph.D., Anne Farmer, M.D., F.R.C.Psych., Gerome Breen, Ph.D., Katherine J. Aitchison, M.D., M.R.C.Psych., Ph.D., Ian Craig, Ph.D., Cathryn M. Lewis, Ph.D., and Peter McGuffin, F.R.C.P., F.R.C.Psych., Ph.D.



true association. Drug-specific analyses revealed a genome-wide significant association between marker rs2500535 in the uronyl 2-sulphotransferase gene and response to nortriptyline. Response to escitalopram was best predicted by a marker in the interleukin-11 (*IL11*) gene. A set of 72 a priori-selected candidate genes did not show pharmacogenetic associations above a chance level, but an association with response to escitalopram was detected in the interleukin-6 gene, which is a close homologue of *IL11*.

← SSRI

← Tricyclic

Would you see the 5-HTTLPR effects via GWAS?

Identification of tag haplotypes for 5HTTLPR for different genome-wide SNP platforms

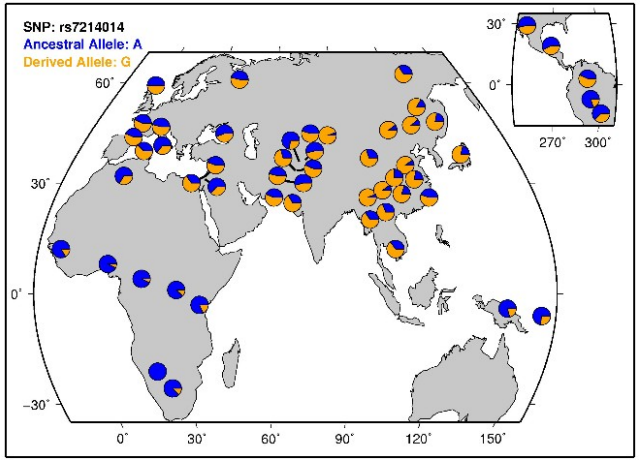
Molecular Psychiatry advance online publication, 14 June 2011; doi:10.1038/mp.2011.68

AAE Vinkhuyzen[1], T Dumenil[1], L Ryan[1], SD Gordon[1], AK Henders[1], PAF Madden[2], AC Heath[2], GW Montgomery[1], NG Martin[1] and NR Wray[1]

5HTTLPR/rs2129785/rs11867581

L/T/G: 0.422
S/T/A: 0.412
L/C/A: 0.106
L/T/A: 0.039
S/T/G: 0.018
$r^2 = 0.775$

tively). The highest r^2 between any single SNP and 5HTTLPR was $r^2 = 0.50$, for both rs7214014 genotyped on Illumina HumanHap610 quad and its proxy ($r^2 = 1$) rs8072345 genotyped on Affymetrix 6.0. For Illumina



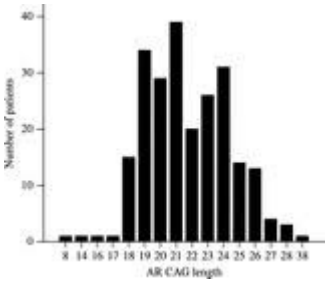
Will you see all the usual suspects via GWAS?

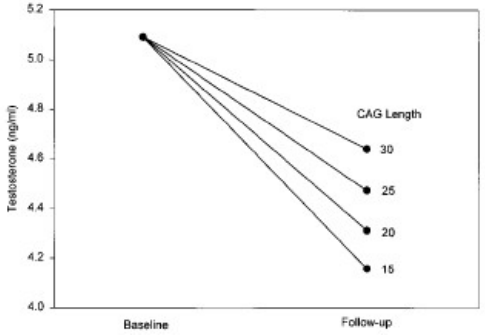
- Not always
- XAR

Opposite effects of androgen receptor CAG repeat length on increased risk of left-handedness in males and females.
Medland SE, Duffy DL, Spurdle AB, Wright MJ, Geffen GM, Montgomery GW, Martin NG.
Behav Genet. 2005 Nov;35(6):735-44.
PMID: 16273319 [PubMed - indexed for MEDLINE]
Related citations

Gender diagnosticity and androgen receptor gene CAG repeat sequence.
Loehlin JC, Jönsson EG, Gustavsson JP, Schalling M, Medland SE, Montgomery GW, Martin NG.
Twin Res. 2004 Oct;7(5):456-61.
PMID: 15527661 [PubMed - indexed for MEDLINE]
Related citations

- CAG_n
- Continuous ~linear effect of number of repeats on T levels





Journal of Endocrinology (1999) **162**, 137–142
K KRITHIVAS and others

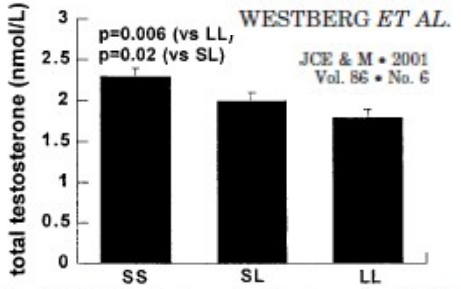


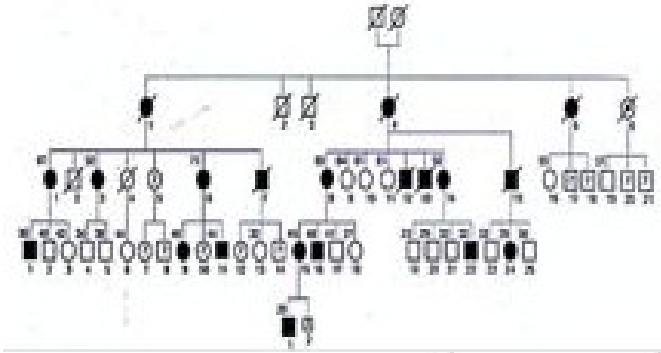
FIG. 2. Levels of total testosterone in subjects carrying the SS, SL, and LL variants of the AR gene, respectively.

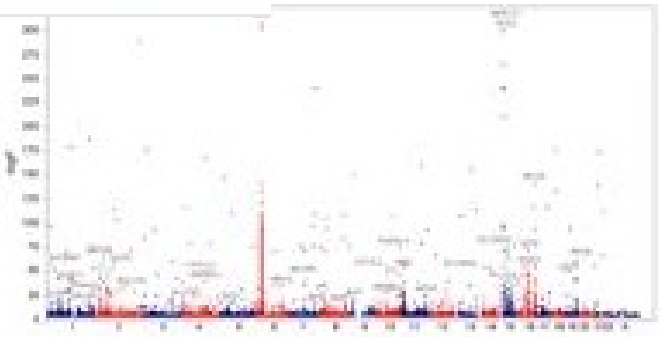
Take home message 1

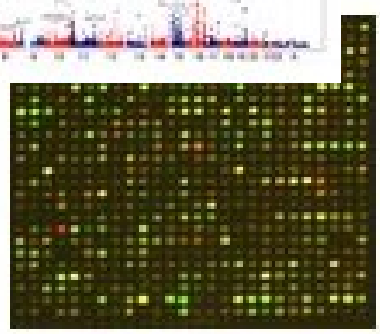
- The usual candidates *maybe* important but *NOT* sufficient
- By definition 20 studies *funded* to study candidate genes will find *less novel* effects than 20 studies that adopt an exploratory approach
- Do you want to find novel variants?
- Do you want to dig deeper into previous findings?

Exploratory approaches

- Linkage
- GWAS
  - Multivariate
- Sequencing
- Pathways
- Copy Number Variants
- Expression
- Methylation







FTO

(the GWAS poster child)

- GWAS for T2D discovered a significant hit in an unexpected region on chr 16
- Subsequently discovered to be associated with obesity and BMI not diabetes

Science 11 May 2007:
Vol. 316. no. 5826, pp. 889 - 894
DOI: 10.1126/science.1141634

REPORTS

A Common Variant in the *FTO* Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity

Timothy M. Frayling,[1,2*] Nicholas J. Timpson,[3,4*] Michael N. Weedon,[1,2*] Eleftheria Zeggini,[3,5*] Rachel M. Freathy,[1,2] Cecilia M. Lindgren,[3,5] John R. B. Perry,[1,2] Katherine S. Elliott,[3] Hana Lango,[1,2] Nigel W. Rayner,[3,5] Beverley Shields,[2] Lorna W. Harries,[2] Jeffrey C. Barrett,[3] Sian Ellard,[2,6] Christopher J. Groves,[5] Bridget Knight,[2] Ann-Marie Patch,[2,6] Andrew R. Ness,[7] Shah Ebrahim,[8] Debbie A. Lawlor,[9] Susan M. Ring,[9] Yoav Ben-Shlomo,[9] Marjo-Riitta Jarvelin,[10,11] Ulla Sovio,[10,11] Amanda J. Bennett,[5] David Melzer,[1,12] Luigi Ferrucci,[13] Ruth J. F. Loos,[14] Inês Barroso,[15] Nicholas J. Wareham,[14] Fredrik Karpe,[5] Katharine R. Owen,[5] Lon R. Cardon,[3] Mark Walker,[16] Graham A. Hitman,[17] Colin N. A. Palmer,[18] Alex S. F. Doney,[19] Andrew D. Morris,[19] George Davey Smith,[4] The Wellcome Trust Case Control Consortium[†] Andrew T. Hattersley,[1,2‡§] Mark I. McCarthy[3,5‡]

Nature Genetics **39**, 724 - 726 (2007)
Published online: 13 May 2007 | Corrected online: 26 September 2007 |
doi:10.1038/ng2048

There is a Corrigendum (October 2007) associated with this Brief Communication.

Variation in *FTO* contributes to childhood obesity and severe adult obesity

Christian Dina[1], David Meyre[1], Sophie Gallina[1], Emmanuelle Durand[1], Antje Körner[2], Peter Jacobson[3], Lena M S Carlsson[3], Wieland Kiess[2], Vincent Vatin[1], Cecile Lecoeur[1], Jérome Delplanque[1], Emmanuel Vaillant[1], François Pattou[4], Juan Ruiz[5], Jacques Weill[6], Claire Levy-Marchal[7], Fritz Horber[8], Natascha Potoczna[8], Serge Hercberg[9], Catherine Le Stunff[10], Pierre Bougnères[10], Peter Kovacs[11], Michel Marre[12], Beverley Balkau[13,14], Stéphane Cauchi[1], Jean-Claude Chèvre[1] & Philippe Froguel[1,15]

FTO

(the GWAS poster child)

per by Frayling *et al.*, "*FTO* is a gene of unknown function in an unknown pathway" (p. 893).[8]

- Huge effect
  - Adults who are homozygous for the risk allele weigh about 3 kg more than those without risk alleles
  - Obese individuals are 1.5 times more likely to have inherited risk genotypes than lean controls
- Replicates in many european samples
- But it never would have been a candidate gene
  - (even though it was called the *Fatso* gene)

Mamm Genome. 1999 Oct;10(10):983-6.

Cloning of Fatso (Fto), a novel gene deleted by the Fused toes (Ft) mouse mutation.

Peters T, Ausmeier K, Rüther U.

Institut für Molekularbiologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany.

- Since renamed… '*fat mass and obesity associated gene*'

The way forward?

- Innovation costs money
  - Sequencing is hugely expensive
    - Requires extensive analytical and biological knowledge
  - Deep phenotyping is also expensive
- We have barely scratched the surface of common variation
- Little functional work has been done
- Replication is essential
- Ethical burden of communication to the public should not be underestimated!