Bildgebung und Genetik

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Zentralinstitut für Seelische Gesundheit, Mannheim
Complex path from gene to behavior

Systems-level intermediate phenotype: imaging genetics

<table>
<thead>
<tr>
<th>Study name</th>
<th>Std diff in means</th>
<th>Standard error</th>
<th>Variance</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-Value</th>
<th>p-Value</th>
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<td>Bertolino; 2006a</td>
<td>0.922952</td>
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</tbody>
</table>

Mier et al, Mol Psych 2009

Effect size = 0.73
Imaging genetics

- Common genetic variants
  - Need around 80 subjects for functional variants with reasonable MAF (Meir et al, Munafo et al.
  - „packing your lunchbox and then looking what‘s inside“
  - Make sense if querying a given system
- Genome-wide significant variants
  - Better evidence for being linked to psychiatric illness
  - Same considerations with regard to sample size
- Rare variants – CNVs
- Epigenetics
- Forward genetics approaches
5-HT Transporter Promoter Variant (5-HTTLPR)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
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<tr>
<td>I/I</td>
<td>32%</td>
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<tr>
<td>I/s</td>
<td>49%</td>
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<tr>
<td>s/s</td>
<td>19%</td>
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</table>
t test (5-HTTLPR)

LL > S

SPM\text{mip}^{-1, 2, 1, 1}

SPM\{T, 108\}

SPM\text{results: 14
corr lvs-carriers}
Height threshold T = 1.66
Extent threshold k = 0 voxels

Published in Pezawas*, Meyer-Lindenberg* et al. *Nat Neurosci* 2005
SNPs

- 2 alleles, usually coded 1 and 2 (frequent and rare)
- For autosomal genes, three genotypes: 11, 12, 22
- ANOVA with three groups – can look at heterozygote effects, must be careful to specify the correct contrasts if looking for a codominant effect
- Regression: genotypes coded as 1 (11), 2 (12), 3 (22), mean centered or not doesn’t matter if a constant is included in the model, codominant effect is “hardwired” in the model but heterozygote effects could be missed
The COMT val^{158/108}met polymorphism

"high-activity" thermo-stable ancestral allele

...CGTG... ..AGVKD...

“low-activity” thermo-labile human allele

...CATG... ..AGMKD...

SOURCE: NCBI, GEN-BANK, ACCESSION # Z26491
ANOVA (COMT val158met)

Honea*, Verchinski* et al. Neuroimage 2009
Simple regression

PRODH1311 neg

SPM\{T_{50}\}

Fitted responses effects of interest

Kempf et al.

PLoS Genetics 2008
X-linked variants

- Men are hemizygotes, women can be homozygotes
- Gene by gender interaction must be included in the model
- ANOVA: 5 groups m1, m2, f11, f12, f22
- Can also be done by multiple regression with gender interaction term (see next example)
MAO-A uVNTR:

Caspi et al. Science 2002

Sabol et al. Hum Genetics 1999
ANOVA: Genotype by gender

Published in Meyer-Lindenberg et al. *PNAS* 2006
Genotype x gender interaction (MAO-A)

Published in Meyer-Lindenberg et al. *PNAS* 2006
Can do ANOVAs with as many groups as there are cells: 11-11, 11-12, 11-22, 12-11 etc.

- Gets to be a lot of cells and contrasts to run
- Regression approach is preferred: code both genes as regressors, mean-center (?), and put an interaction regressor for each interaction you want to do together with the main effect regressors
- The procedure is exactly the same for continuous predictors
- To test for interaction, just run +1 and -1 contrast over the interaction regressor column
- Easily generalizes to more than one interaction term: for a three-way interaction, multiply the three main effects together, etc.: can use createcovs script
- Not easy to see what a significant interaction reflects: best to pull out values and plot
Interaktion PRODH and COMT

Human chromosome 22

3-Mb TDR (>85%)
1.5-Mb deletion (~8%)

wt      kd
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10

1449183_at  2.002E-5  COMT catechol-O-methyltransferase
1429906_at  6.228E-4  NRXN3 neurexin III
1454988_x_at  0.011203  TRIM9 tripartite motif protein 9
1444973_at  0.001546  KCNMA1 potassium large conductance calcium-activated channel, subfamily M, a
1452423_at  0.00338  PCLO piccolo (presynaptic cytomatrix protein)
1451850_at  0.003486  SPNB2 spectrin beta 2
1445537_at  0.007765  SRGAP2 SLIT-ROBO Rho GTPase activating protein 2
1432415_at  0.009047  RAB3C RAB3C, member RAS oncogene family
1421136_at  2.211E-4  EDN3 endothelin 3
1433894_at  7.436E-4  SYT1 synaptotagmin 1
1431812_a_at  7.518E-4  SLC6A9 solute carrier family 6 (neurotransmitter transporter, glycine), member 9
1434582_at  0.001122  D14ERD171E DNA segment, Chr 14, ERATO Doi 171, expressed -1.51948043
1455766_at  0.001551  GABRA1 gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 1
1442707_at  0.00155  CAMK2A calcium/calmodulin-dependent protein kinase II alpha
1448907_at  0.002005  HRH3 histamine receptor H3
1417746_at  0.002071  CPLX1 complexin 1
1418496_at  0.004798  STX7 syntaxin 7
1417702_a_at  0.004904  HNMT histamine N-methyltransferase
1415766_at  0.005717  SEC22L1 SEC22 vesicle trafficking protein-like 1 (S. cerevisiae)
1447863_s_at  0.005933  NR4A2 nuclear receptor subfamily 4, group A, member 2
1440843_at  0.006112  SLC5A10 solute carrier family 5 (sodium/glucose cotransporter), member 10
1420418_at  0.006817  SYT2 synaptotagmin 2
1444455_at  0.007049  CAACNA1A calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
1416561_at  0.007419  GAD1 glutamic acid decarboxylase 1
1456406_at  0.007637  NRXN2 neurexin II
1419246_s_at  0.008669  RAB14 RAB14, member RAS oncogene family

Paterlini et al. Nat Neurosci 2005
Gene-gene interaction: multiple regression approach

Kempf et al. under review
So it turns out 5-HTTLPR is actually triallelic …

Serotonin Transporter Promoter Gain-of-Function Genotypes Are Linked to Obsessive-Compulsive Disorder

Xian-Zhang Hu,¹ Robert H. Lipsky,¹ Guanshan Zhu,¹ Longina A. Akhtar,¹ Julie Taubman,¹ Benjamin D. Greenberg²,³ Ke Xu,¹ Paul D. Arnold,⁴ Margaret A. Richter,⁴ James L. Kennedy,⁴ Dennis L. Murphy,⁵ and David Goldman¹

¹Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD; ²Butler Hospital and ³Department of Psychiatry, Brown University School of Medicine, Providence; ⁴Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto; and ⁵Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD

A functional serotonin transporter promoter polymorphism, HTTLPR, alters the risk of disease as well as brain morphometry and function. Here, we show that HTTLPR is functionally triallelic. The L_C allele, which is the L allele with a common G substitution, creates a functional AP2 transcription-factor binding site. Expression assays in 62 lymphoblastoid cell lines representing the six genotypes and in transfected raphe-derived cells showed co-dominant allele action and low, nearly equivalent expression for the S and L_C alleles, accounting for more variation in HTT expression than previously recognized. The gain-of-function L_A L_A genotype was approximately twice as common in 169 whites with obsessive-compulsive disorder (OCD) than in 253 ethnically matched controls. We performed a replication study in 175 trios consisting of probands with OCD and their parents. The L_A allele was twofold overtransmitted to the patients with OCD. The HTTLPR L_A L_A genotype exerts a moderate (1.8-fold) effect on risk of OCD, which crystallizes the evidence that the HTT gene has a role in OCD.
Modeling individual alleles /haplotypes

- We could do an anova with all possible combinations 11, 12, 13, 22, 23, 33
- Or a regression where we code these 1-6
- We can save df and increase power if we assume codominance, because then we can model individual alleles
- Set up as multiple regression with one regressor for each the three alleles: LA, LG, and S which contains the number of alleles for that person (0, 1, or 2)
Triallelic system: 5-HTTLPR: assuming codominance

Published in Pezawas*, Meyer-Lindenberg* et al. Mol Psychiatry 2008
Triallelic system: 5-HTTLPR: F test strategy

Published in Pezawas*, Meyer-Lindenberg* et al. *Mol Psychiatry* 2008
Multiallelic variant: RS3 microsatellites in AVPR1A

(GATA)$_{14}$

RS1

(CT)$_4$-TT-(CT)$_8$-(GT)$_{24}$

RS3

Meyer-Lindenberg et al. *Mol Psychiatry* 2009
PR1A expression and social behavior in voles

Hammock and Young, Science 2005
Multiallelic system – AVPR1A

Meyer-Lindenberg et al. Mol Psychiatry 2009
Genetic variation in AVPR1A (RS 3) predicts activation of amygdala

p < 0.001, uncorrected, p = 0.040, corrected in ROI (left)
Meyer-Lindenberg et al. *Mol Psychiatry* 2009
Haplotypes

Ancestral haplotype: ATXGTA

10,000 bp

Contemporary haplotypes:
1. GAATXGTA
2. TAGATXGTA
3. TAGATXGTA
4. CGATXGTA

DNA sequence:

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<th>Individuals</th>
<th>SNP1</th>
<th>SNP2</th>
<th>Indel</th>
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<td>G</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>G</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
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</tr>
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<td>A</td>
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<tr>
<td>6</td>
<td>G</td>
<td>T</td>
<td>A</td>
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</table>
Haplotype estimation problem

- Current genotyping methods do not resolve phase
- The number of haplotypes compatible with a given genotype rises exponentially with the number of SNPs
- Haplotypes can be estimated from pedigrees and population data but are inherently probabilistic
Assessing effects of probabilistic haplotypes in neuroimaging

- Estimation of haplotype (-pairs, diplotypes) and associated probabilities using a Bayesian approach: PHASE 2.1
- Multivariate regression to estimate BOLD response associated with each haplotype
- Inference and multiple comparison correction using Gaussian random fields
Impact of P2 promoter SNP

Chen et al. AJHG 2004
Characterization of a risk haplotype through neuroimaging

Published in Meyer-Lindenberg et al. Mol Psychiatry 2006
CNV imaging project

500 subjects with CNVs, controls structure completed; function starts 3/

Steffansson*, Brammer*, Morgen* et al. in progress, Abbott Nature 2010
CNV imaging project

Gray matter, duplication > controls > deletion

White matter, duplication > controls > deletion

Steffansson*, Brammer*, Morgen* et al. in progress

p<0.05, FWE corrected in mask (GM), whole brain (WM)
Cellular mechanisms of GxE

- Histone methylation (repressive)
- DNA methylation (repressive)
- Histone acetylation (permissive)

Chronic defeat stress

Krishnan and Nestler *Nature* 2008

Altar, *TIPS* 1999
neural glucocorticoid receptor (NR3C1)

McGowan et al., Nat Neurosci 2009

Tyrka et al., PLoS One 2012
Hypermethylation of NR3C1 and cingulate-amygdala connectivity

Tost*, Walter*, Nieratschker* et al., in progress