Are there foreseeable applications of genomic medicine for the management of neuropsychiatric conditions?

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“Are there foreseeable applications of genomic medicine for the management of neuropsychiatric conditions?”

Discuss!
Plan

• Personal perspective on ‘genetics and psychiatry’
• Making a genetic diagnosis
• Making use of a diagnosis
  – syndromic diagnosis or a unique diagnosis
• Rett syndrome as an example
A perspective on the history

- Recording family histories
- Attempts to measure heritability
- Genetic linkage studies in large families
- Mini-linkage studies: allele sharing and transmission studies in ‘familial clusters’
A perspective on the history

Association studies in sporadic cases

de novo mutations in sporadic cases
Where is this leading?

- Diagnosis => explanation, prognosis, clarity about reproduction
- Informed health care, health surveillance and epidemiology
- Opportunities to understand pathology and (perhaps) develop rational treatments
Family History

- Documenting specific inherited causes of neuropsychiatric and neurodevelopmental disorders
- Mode of inheritance
- Description of phenotypes, especially syndromic, indicating possible mechanisms
Heritability

• $H^2 = G^2/P^2$

• Heritability is not a fixed, biological entity but depends upon (varies with) the environment

• Concept has been (is being) abused

• Consider ‘intelligence’

• How ‘fine-grained’ is the environment?
Heritability

• Measures of heritability - usually from twin studies - may be ‘correct’ but misleading, with values of $H^2 \sim 0.5-0.8$

• Identifying polygenes and accounting for heritability in Drosophila (Trudy McKay)

• To what extent is the high level of polymorphism maintained actively by selection?

• The flawed notion of single best allele
Linkage studies in Mendelian disorders

• Tracking inherited disease by using the best available markers (sites of genetic variation)
• Good at identifying genes ‘for’ Mendelian disorders
• Tuberous sclerosis (TSC1, TSC2), fragile X (FRAXA), ...
• Insights into disease mechanisms ...
Chromosome Studies

- Trisomies/Monosomies
- Deletions, duplications, etc ...
- Convergence with molecular studies => imprinting (e.g. Angelman vs Prader-Willi)
- FISH to identify specific sites at higher resolution than light microscopy - Williams syndrome, 22q11 deletion, del 1p36, ......
- array Comparative Genomic Hybridisation (aCGH) as successor technology
Loose family clusters

• Genes or gene regions shared by affected or transmitting members of a family
Genome Wide Association Studies (GWAS)

• Allele-sharing between sporadic ('unrelated') cases
• Common Disease, Common Variant (CDCV) model of the common, complex disorders
• Some pointers to schizophrenia, autism, etc ...

Common = Ancient

- Necessarily ancient variation
- Necessarily weak effects
- Accounts for only a small proportion of the genetic contribution to most disorders
- Heritability may be 0.5 or more but these studies only account for ~10-15% of $H^2$
- => part of the cause of the ‘missing heritability’
Or modern and (individually) rare?

• Neurodevelopmental disorders and psychiatric disease often arise from *de novo* genetic change
  – aCGH - Copy Number Variants
  – high throughput (genomic) sequencing
    (DGCR2 - within the 22q11 region)

• These *de novo* mutations are of high penetrance, so (usually) concordant in MZ twins and discordant in DZ
• Nature Genetics September 2012

• **Increased exonic de novo mutation rate in individuals with schizophrenia**
  • pp860 - 863; Simon L Girard et al
  • and:
  • Exome sequencing supports a *de novo* mutational paradigm for schizophrenia
  • pp864 - 868; Bin Xu et al
Keep an open mind

- $H^2$ will appear to be high - BUT - this should **not be taken to** support the CDCV model

- Missing heritability’: partly GxG and GxE interactions

- plus **epigenetic** influences (including Predictive Adaptive Responses)

- No ‘single best allele’ - always provisional and contextual
Achieving a diagnosis

• When will patient management be modified by specific, molecular diagnosis?
  – Down; Noonan; 22q11; 1p36;

• UVs - variants of unknown significance on aCGH or NGS

• Incidental findings from sequencing?
Value of the Findings

• Some shared factors predispose both to bipolar disease and schizophrenia

• => implications for disease taxonomy (contra Kraepelin...)

• Predictive/Susceptibility Testing?: knowledge of one’s (genetic) risk could in itself be an (environmental) risk and likely to increase anxiety / ‘expressed emotion’
Rett Syndrome

• Lessons for genomic neuropsychiatry
Career of a Diagnosis

- Recognition of a clinical entity
- Narrow diagnostic criteria =>
- Progress in recognising (+/- understanding) the underlying pathology
- Recognition of wider range of phenotypes associated with same pathology
- Appropriate clinical indications for further investigation
Career of Rett Syndrome

• Recognition of “cerebral atrophy with hyperammonaemia” by Andreas Rett 1966
• English language publication 1983 by Hagberg et al
Career of Rett Syndrome

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• English language publication 1983 by Hagberg et al
• Diagnostic criteria formalised and then progressively revised (latest 2010)
Initial Diagnostic Criteria

- Normal development to 6 months
- Developmental stagnation
- Regression - social contact, hand use
- Hand stereotypies
- Recovery of social contact
- Persisting profound cognitive impairment
- Gait and truncal ataxia
- Absence of other neurodevelopmental problem
- (Only girls)
Other associated features

- Muscle tone, including spasticity in legs
- Ventilatory rhythm
- Vasomotor disturbances including cool, atrophic feet
- Seizures
- Scoliosis
- Impaired growth
Rett syndrome is primarily a **clinical** diagnosis

with a highly characteristic timecourse and evolution,

although most cases associated with mutations in *MECP2* gene at Xq28
Degrees / Variants of Rett Syndrome

- Recognition of clinical features before 6 months in (otherwise) classic cases

- Variant / Atypical forms
  - Forme fruste (late stagnation, no regression)
  - Preserved speech (Zapella)
  - Congenital onset (no regression)
  - Early onset of seizures (Hagberg)
  - Angelman-like
  - Male cases (some with 47,XXY; some 46,XY)
Search for genetic basis

• X-linked dominant, male-lethal disorder would account for unusual pattern of occurrence
  – Sporadic
  – Occurring only in females
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- Gender bias in (high) mutation rates
  \[\Rightarrow\] a better explanation
Search for genetic basis

• X-linked dominant, male-lethal disorder would account for unusual pattern of occurrence
  – Sporadic
  – Occurring only in females
  – Variability between MZ twins

• Gender bias in (high) mutation rates
  => a better explanation

• Wide clinical variability of same mutation from X inactivation
FAMILIAL RETT SYNDROME: SISTER - SISTER PAIRS
Search for genetic basis: mainly red herrings

• Cytogenetic “clues” … t(Xp;A) x 2
• Uncertain significance of common ancestry in Swedish genealogy cases
• Linkage analysis difficult
  – Few families
  – Familial cases perhaps atypical
    (criteria relaxed ??)
• Xq28 a likely region
  – Amir et al 1999 => MECP2 gene
MeCP2 Protein: Adrian Bird 1992

• Already implicated in repression of transcription via methylated CpG groups
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• Already implicated in repression of transcription via methylated CpG groups

• **Rapid** progress in the molecular biology

• **Steady** progress in diagnostic utility

• **Slower** progress in understanding the pathogenesis

  – or moving to effective treatments
Rett syndrome is (usually) caused by mutations in \textit{MECP2}

- Methyl-CpG-binding protein 2
- Global transcription repressor
- Locus at Xq28

\cite{Amir1999}
A loose correlation between the mutation and the disorder

- truncating vs missense mutations
- early truncating vs late truncating mutations
- some common mutations associated statistically with greater or lesser severity
  - R133C and C-terminal deletions milder
  - R270X more severe
Large deletions in MECP2

Exon 1 & 2 (n=3)
Exon 3 & 4.1 (n=3)
Exon 3 & 4 (n=3)
Exon 3 – 4.3 (n=5)
Exon 4.1 - 4.3 (n=1)
Exon 4 (n=1)
Exon 4.2 (n=1)
Exon 4.2 - 4.3 (n=1)
Exon 4.2 - 4.4 (n=1)
Exon 4.3 (n=1)
Exon 4.3 – IRAK1 (n=1)
Severe Rett syndrome

• TRD-NLS mutations more severe
  – Floppy
  – Immobile
  – Often no hand use
  – Microcephaly
  – Severe scoliosis
  – Poor health
  – Increased mortality
Mild Rett syndrome

- Walk
- Swim
- Ride a bike
- Talk
- Use hands – self-feed, write
- Better growth
- Greater survival
- But significant learning disability
How do the mutations cause the disease?

• ?? MeCP2 deficiency => ~2 fold up-regulation of many genes (Ballestar et al 2005)

• Rett syndrome is a disease of “chromatin configuration” that “should” have global consequences
  – hard to understand how mutation leads to the very specific disease phenotype
MeCP2 target genes

- **UBE3A/GABRB3** (Samaco *et al* 2005, Makedonski *et al* 2005) related to Angelman phenotype
- **BDNF** (Chen *et al* 2003, Martinowich *et al* 2003) neuronal plasticity, eating behaviour
- **DLX5** (Horike *et al* 2005) silent chromatin loop, GABA synthesis
- **FMR1** (Harikrishnan *et al* 2005)
- **Hairy2a** (Stancheva *et al* 2003)
- Glucocorticoid response elements
But there may be no (very) specific targets

- MeCP2 binding is proportional to methylation at CpG groups
- Binding of MeCP2 is not concentrated at promoter regions or CpG islands
- Levels of illegitimate (‘nonspecific’) transcription is raised in *MECP2* mutant mice – Adrian Bird’s lab
Diagnostic Applications of *MECP2* testing

- Classical Rett Syndrome
  >90% mutations

- ‘Atypical’ Rett syndrome
  50% mutations

- Early seizure variant
  <10% mutations, none with infantile spasms
Diagnostic Applications of \textit{MECP2} testing

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- Is the mutation pathogenic? 
  – \textit{de novo} ? synonymous ? conserved ?
  – present in healthy male ?
The complexity is enormous

- Coding sequence variants in healthy newborns
- Variation in transcription: not only the two isoforms, also extension at 5’ and 3’ ends
- Conserved sequences in the 8.5 kb 3’UTR and intron 2
- Silencers, enhancers and miRNA binding
Increase in MECP2 dosage

- Cognitive impairment
- Hypotonia then spasticity
- Absent speech
- Seizures
- Susceptibility to infection

(not all features from MECP2 dosage alone)
Specific Mutations

• Some associated with non-syndromic XLMR (?)

• A140V => distinct phenotype in males
  – Problems with development and behaviour
  – Hypotonia and Seizures

• Later:
  – Parkinsonism
  – Macro-orchidism
  – Spasticity
Tissue- and Cell-type specific expression of MECP2

• Apparent from conditional mutations with a range of different promoters
• Guy and Bird: reversal of pathology in the Cre-lox Mecp2 +/- mouse
Family Consequences of Mutation Testing for RTT

• Confirmation of diagnosis
  – Reproductive confidence in face of mosaicism
  – But still an emotional kick

• “Disconfirmation” of diagnosis
  – An anomalous category
  – A different emotional kick

• “Disconfirmation of normality” when MECP2 mutation found in absence of RTT
## Diagnostic Test => 2 x 2 Table

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>Mutation Test</th>
<th>Test Positive: mutation found</th>
<th>Test Negative: mutation NOT found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical diagnosis: typical or ‘atypical’ case of RTT</td>
<td></td>
<td>expected</td>
<td>! New and anomalous</td>
</tr>
<tr>
<td>Clinical diagnosis: NOT typical of RTT</td>
<td>! New and anomalous</td>
<td></td>
<td>expected</td>
</tr>
</tbody>
</table>
No (MECP2) mutation ? …
No MECP2 mutation? …

- Look harder in MECP2 (promoter, 3’UTR, …)
- In the mouse, duplications of MECP2 in males associated with some features of RTT
- In boys, duplications of Xq28 associated with delay (and ? some features of RTT)
  - Duplications including MECP2, filamin A, …
- In girls, CDKL5 gene disrupted in girls with infantile spasms and ? RTT or autism
  - Mutations in 3/20 girls with early seizure variant of RTT, including infantile spasms (15%)
Two patients with $CDKL5$ mutations

- 2/13 (15%) girls with seizures in the first 6 months of life had a novel mutation in $CDKL5$
• Presence of CDKL5 results in phosphorylation of MeCP2, releasing it from the methylated CpGs – CDKL5 mutations amplify effects of MeCP2
Pathogenesis

What could plausibly count as an “explanation” of RTT pathogenesis?
Pathogenesis

• Descriptive explanations
  Pattern recognition
  = natural history

• Mechanistic / Linear explanations
  A => B => C => D; upstream and target loci
  = science

• Systemic explanations
  Complex web of interactions; neuronal plasticity
  ........ = ? despair or reality ?
Complex Causation

• Generalised dysregulation of cell function: How could this account for the illness?

• Plasticity / substitutability of CNS cell functions alters during development.

• Ability to mask dysfunction in ~50% of neurons may be impaired as plasticity declines
How was RTT not “spotted” until 1966?

- 6-12 months: “She’ll catch up”
- Acute regression
  - degenerative cerebral disease
  - undiagnosed encephalopathy
  - childhood psychosis or autism
- Dystonic, ataxic or diplegic cerebral palsy; microcephaly and epilepsy

Recognition of the temporal pattern
"Early concerns"

Systemic disease
Non-progressive intellectual impairment
Dysmorphism – dx by genetic test or opinion
Regression – Rett syndrome
  - severe autism
  - diagnosis by biochemical methods
    Leigh’s disease
  - diagnosis by MRI
    Brain tumour
Neurological problem – seizures
  - microcephaly
  - cerebral palsy
Socio-emotional problem
the later diagnostic landscape ...

cerebral palsy
post-encephalitic
microcephaly, delay, spasticity
MATURE SYNDROME
syndromic disorders
RETT SYNDROME
Angelman
severe autism
severe epilepsies
West’s and
MECP2 diagnostic landscape

"MECP2 disease"

(UBE3A)
Angelman syndrome

forme – mild – CLASSIC – severe – early - congenital
fruste RTT RTT RTT fits onset
(no regression) (with regression) (no regression)

infantile spasms
(CDKL5)
(TSC1/2)
(ARX) etc ..
The Jigsaw of Neurodevelopmental Disease

- Mutations in *MECP2* can be associated with classic or atypical RTT, non-RTT, or normal female phenotypes
- Mutations at other loci (*CDKL5*) result in related disorders
Interventions

• How to replicate the “cure” seen in the Guy/Bird mouse?

• Modify neurogenic amines: desipramine, fluoxetine

• Modifiers of γ-ergic neuronal activity

• Agents to increase BDNF

• IGF-1 action on growth of synapses

• Reverse tissue hypoxia

• Rebreathing to reduce effects of hyperventilation
Towards effective treatments ... 

• Physiological studies in mice (Abdala et al PNAS 2010) suggest 5HT1A agonist plus GABA reuptake inhibitor can correct respiratory disorganisation

• Suppressors of nonsense mutations (readthrough agents, e.g. gentamicin) *may* lead to major benefits (... or not)
Towards effective treatments ...

• Gene therapy?

• Reactivate the inactivated X??
Treatments for other conditions

- Therapeutic guidance - choice of drugs for the individual
- If deletions/duplications of whole exons, antisense oligos may correct the reading frame (as in DMD)
Lessons (1)

• Identifying genetic basis of developmental or behavioural disorder can be of value to the family
  – explanation
  – prognosis
  – genetic counselling

• Establishing genetic basis allows natural history studies to begin

• Many incidental findings: confirming a mutation as pathogenic may be difficult

• Some ‘incidental’ findings will be important
Lessons (2)

- **GxG and GxE interactions** may take decades to sort out.
- **Identifying genetic basis of developmental or behavioural disorder** can give insights into disease mechanisms:
  - animal models and treatment trials
  - opportunities for effective treatment
- **Treatments** may depend upon the mutation.
Some further reading


