Next generation sequencing approaches to diagnosis of early onset dementia

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NHS National Prion Clinic
MRC Prion Unit
Early onset dementia

- Between common and very rare
- Often cause by a single mistake (mutation) in DNA
- Often runs in families
- Major developments in gene discovery in recent years
  - “next generation” sequencing technology
- Opportunities for families for genetic diagnosis
- Probably under recognised/ opportunities not taken because of lack of awareness
Some issues re genetic testing in the memory clinic...

• What is a dementia gene and mutation?
• How well does a dementia mutation link with symptoms?
• Who should be referred for testing?
• What are the risks/benefits of testing?
• What is the best/what are the new technologies for testing?
Causes of genetic neurodegenerative disorders

Not simple, many possibilities

Some discrete diagnoses (Huntington’s disease, HTT; Alzheimer’s disease, prion disease/CJD),
   ...more usually a continuum (FTLD-ALS)

Level of clinical syndrome, tissue pathology, molecular cause?
   ...increasing hierarchy

Most conveniently for the clinician based on gross differential eg.
   • Dementias
   • Movement disorders
   • Ataxias

Traditional neurogenetics done on the basis of “cherry-picking”
genes based on detailed clinical assessment
DNA, Proteins and Mutations

DNA sequence (762 bases) for prion protein gene:

ATGGCGAACCCTTGGCTGATGCTGTTCTATTGTATCGGACATGGAGGCTGGCTCTGCAAGA
AGCGCCGGAAGCGTTGAGAAGAAGCTGGGGAAGCCGATACCCGGGGCAAGGGGACGCCTGGAGGCAA
CGGCTACACCACCTAGGGGCTGGTGGCTGGGGGGACGCTATGGTCGGTGCGGGGACGCCCAGCATG
GGTTGGCTGGGCGACGCCCATGCTGGTGCTGGCTGGGAGACACGCTATGATGGTGCGGGGTGCTCAAGGAGGTG
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GAGCAGATTGTGATTCACCAGTACAGAGAGGAAATCCAGCCTATTACCAGAGGATCCAGCATGTGCTC
TCTTCTCCTCCTCCACCTGTATCCCTCTGGATCTTCTCCTCCTCTCCTCTCCTGATAGTGCGGATGA

Translation of the code to amino acids (253):

MANLGCWMLVLFLVFATWSDLGLCKKRPKPGWGGNTGGSRY
PGQGSPGGNRYPQQQGGGGGQGPHGGGWGPQPHG
GGWGQPHGGGGGGQGPHGGGWQGGGGSQCNKNK P/L
SKPKTNMKGAGAAAAGAVVGGGLGGYLGSAMSRIIH
FGSRYEDRYYRENMRNPQRNVYYRPMDEYSNQNNFVHD
CVNIKIQHTVTNTTKGNTFTEDVKMMERVV
EQMCITQYERESQAYYQRGSMVLFSPPPVPILLISFLFLIVG
A typical inherited dementia family tree
Inheritance of prion disease

Parents

Affected father

Unaffected mother

Children

50%

affected father

unaffected mother

50%

50%
At risk over a life-time
Dementia genes

- **APP**
- **APP dup**
- **PSEN1**
- **PSEN2**
- **APOE, TREM2**

\{ 
  \textit{Alzheimer’s disease syndromes}
\}

- **GRN**
- **MAPT**
- **C9orf72**

\{ 
  \textit{Frontotemporal dementia syndromes}
\}

- **PRNP**

\{ 
  \textit{Rapid dementia, or dementia + ataxia/myoclonus. AD/FTLD mimic}
\}

- **FUS, TARDBP, CHMP2B, VCP, SCAs, HTT, ATP7B, NPC1/2, NOTCH3, BRI2, TYROBP, TREM2**

\{ 
  \textit{mitochondrial, metabolic}
\}

- **Rare causes, and prompts if white matter lesions, ataxia, movement disorder, chorea, dystonia etc.**
Complexity of variants in a single gene

Some cause disease, some increase risk, some are protective, others make no difference, some unknown.
More complex look at variants in *PRNP*

- E219K
- G127V
- Partially protective variant
- 129MV
- 1-OPRD
- Benign (neutral) variant
- 129MM/VV
- M232R
- Weak risk factor
- V180I
- Strong risk factor
- E200K
- 4-OPRI
- Partially penetrant disease causing mutation
- P102L
- Highly penetrant disease causing mutation
- A117V
Therefore…. Problems with the traditional approach

Far too many genes now, new ones discovered monthly

Phenotypic and allelic heterogeneity, range of effect sizes particularly in the early stages

Perception that findings are rare and only useful if a family history

Sequential testing therefore slow

Lack of confidence in a negative many remain unexplained after exhaustive study

Accessibility of tests UK-Genetic testing network website

Expense

Sense of futility – why bother
# Sequential gene testing

What is available through the Genetic Testing Network?

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Number of patients with FTD in UK >3,500 (Ratnavalli et al.)

Mutation frequencies (~20% total)
MAPT (2-11%),
GRN (5-11%),
C9ORF72 (2-19%)

~700 symptomatic patients with inherited FTD with mutation in a known gene
>3000 individuals at-risk
Annual incidence new cases ~100

Likely to be significantly low ascertainment

Comparison with National Prion Clinic experience new cases per year around 12 (4-17), similar number predictive tests
Advantages of Genetic Diagnosis in Dementia

- Patients and families want definitive and early diagnosis
- Ends need for further diagnostic tests
- Gives prognostic information
- Access to therapeutics/ trials
- Predictive testing may give sense of control over family illness
- Prenatal testing/ Pre-implantation genetic diagnosis
Next generation sequencing approaches to these problems

Want to have,

Cost effective,
accurate,
way to identify all genetic variants of clinical relevance without having to pick the right gene

We have been looking at next generation sequencing as an approach to these problems
MRC Dementia Gene Panel

• Next-generation sequencing technology (Ion Torrent)
• Looks at a panel of related genes simultaneously
• Runs multiple barcoded samples in parallel (32 per chip)
• Quick, cost effective (£60 per sample)
Ion PGM sequencing chemistry

Polymerase integrates a nucleotide.

Hydrogen and pyrophosphate are released.

The nucleotide does not compliment the template - no release of hydrogen.

The nucleotide compliments the template - hydrogen is released.

The nucleotide compliments several bases in a row - multiple hydrogen ions are released.

Sequential flood of dNTP
Strengths and Weaknesses

- Quicker and cheaper than sequential Sanger sequencing
- Not dependent on correct diagnosis
- 10x cheaper than exome
- Greater depth and uniformity than exome
- Reduced number of variants of unknown significance and artefacts compared with exome
Example of Coverage- VCP
# Geneticist Assistant software

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Samples to analyse

- Sporadic CJD- 200
- Early-onset Alzheimer’s disease- 700
- Late-onset Alzheimer’s disease- 500
- Fronto-temporal dementia- 600
- HD-like (mutation negative)- 250
- Other neurodegeneration- 400

- Total- 2650
Classification of Mutations

- Class 5- Pathogenic
- Class 4- Probably/possibly pathogenic
- Class 3- Uncertain
- Class 2- Likely benign
- Class 1- Not pathogenic
# Mutations Found (first 1500)

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<th>POSSIBLE</th>
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<td><strong>16</strong></td>
<td><strong>67</strong></td>
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## Mutations Found

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<tr>
<th>Gene</th>
<th>Pathogenic</th>
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<th>Total</th>
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<tr>
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<tr>
<td>TOTAL</td>
<td>148</td>
<td>16</td>
<td>67</td>
<td>231</td>
</tr>
</tbody>
</table>
## Mutations in genes not associated with diagnosis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Patient Diagnosis</th>
<th>Usual Diagnosis</th>
<th>Diagnosis Additional Note</th>
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</thead>
<tbody>
<tr>
<td><strong>GRN</strong></td>
<td>p.Ala303Profs</td>
<td>HD-like</td>
<td>FTD</td>
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<tr>
<td><strong>MAPT</strong></td>
<td>p.Pro301Leu</td>
<td>Familial AD</td>
<td>FTD</td>
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<tr>
<td><strong>TARDBP</strong></td>
<td>p.Ile383Val</td>
<td>Familial AD</td>
<td>FTD</td>
<td>Patient with FTD has same mutation</td>
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<tr>
<td><strong>APP</strong></td>
<td>p.Ala701Thr</td>
<td>FTD</td>
<td>AD</td>
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</tbody>
</table>
Double mutations

- 2 patients (both FTD cohort) with \textit{C9ORF72} expansion and pathogenic \textit{GRN} mutation
- 1 patient (FTD cohort) with \textit{C9ORF72} expansion and probably pathogenic \textit{PSEN2} mutation
- 1 patient (EOAD cohort) with pathogenic \textit{APP} and \textit{GRN} mutations
Known PRNP mutations

P102L
- Patient in EOAD Cohort
- Age of onset 47
- No known family history

A117V
- FTD patient from Cambridge cohort
- No clinical details known

5OPRI
- FTD patient from Cambridge cohort
- No clinical details known
Novel *PRNP* variants

**R156C**
- Predicted pathogenic
- Not previously reported
- Patient diagnosed with semantic dementia
- Age of onset 51, Goldman score 3.5
Implications

Single gene/variant testing will remain valid in many circumstances
- known disorder in the family
- clear genotype – phentype relationship (eg. HTT, prion disease)

But for the majority of circumstances custom panels or medical exome sequencing will inevitably become dominant, and will happen quickly

Major issues remain to annotate all the variants discovered. NHS is potentially an excellent organisation to do this, but this is not an issue for the referring neurologist

Practical aspects remain the same 10-20mls EDTA blood, delay up to 3-5 days acceptable, ambient temperature
Written consent, clear labelling
Implications

What is the point?

Definite diagnosis, avoid other tests – easy to underestimate importance of this for families

Preimplantation genetic diagnosis

Postnatal genetic testing

Participation in clinical trials/research

Opportunities for sub-speciality clinics, effective concentration of expertise for rare disorders

Comradeship, community and support networks - empowering

Patients usually know of genetic risk from internet research, opportunity is to free up from this risk (and for children)

Downsides – start-up costs, enthusiasm required and complexity
   - psychological reactions if support not available
Implications

What does this mean for practice?

Given that the disorders are common this should not be the domain of subspecialists only – less need for this as not dependent on details of the phenotype

Familiarity with genetic counselling, partnership with Clinical Geneticists, counsellors. Best practice – written consent

Incidental findings (decide about feedback before testing)

Long term follow up and support

Direct access by patients on the high street/ internet

How to do it – speak to local Clinical Genetics lab
Papers

Diagnostic exome sequencing: a new paradigm in neurology.
Delanty N, Goldstein DB.
Accessible 3 page review of implications of genomics in neurology

Validation of next-generation sequencing technologies in genetic diagnosis of dementia.
Development of a custom gene panel in dementia here at MRC Unit, UCL

The inherited ataxias: genetic heterogeneity, mutation databases, and future directions in research and clinical diagnostics.
Hersheson J, Haworth A, Houlden H.
Hum Mutat. 2012 Sep;33(9):1324-32.

Genetics of dementia.
Loy CT, Schofield PR, Turner AM, Kwok JB.
Lancet. 2014 Mar 1;383(9919):828-40

Genetics of dystonia: what's known? What's new? What's next?
Lohmann K, Klein C.
Mov Disord. 2013 Jun 15;28(7):899-905
Three recent reviews which list genes relevant to neurodegenerative disease genetics
Thank you

• Human Genetics group especially Gary Adamson
• Teams at UCLH Dementia Research Centre and external sites centres for samples and clinical information
• Janna Kenny
• Professor Collinge