Case notes | Dementia genetics

**Genetic investigation in dementia: new interpretive challenges**

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Genetic testing is increasingly used in the assessment and investigation of individuals with dementia and cognitive impairment. Next generation sequencing dementia panels allow simultaneous parallel examination of multiple genes. But with this increased diagnostic power comes also the possibility of incidental findings, of identifying sequence variants which are benign rather than pathogenic. Dr Larner and colleagues discuss two such cases.

The capacity to define the genetic causes of dementia disorders has proliferated over the past two decades as deterministic mutations have been defined. This knowledge has informed clinical practice as well as advancing understanding of disease pathogenesis, although has not as yet afforded disease-modifying therapies. The increasing availability of genetic testing and its falling cost has increased usage. Moreover, whereas previously a sequential or stratified, gene-by-gene, approach to testing was necessarily adopted, the advent of next generation sequencing (NGS) dementia panels allows simultaneous, massively parallel, examination of multiple genes. NGS panels for dementia include genes that are deterministic for Alzheimer’s disease (APP, PSEN1, PSEN2), frontotemporal dementia (e.g. MAPT, GRN, VCP, TREM2, FUS, TYROBP, CHMP2B), and conditions in which dementia or cognitive impairment are associated with movement disorder (e.g. DCTN1). These panels do not, however, encompass repeat expansions, a type of mutation in which short, 3-6 nucleotide, repeats in certain genes or introns exceed the normal, stable threshold resulting in abnormal gene function (e.g. C9orf72 GGGGCC hexanucleotide repeat expansion). These may need to be tested separately.

A corollary of this advanced diagnostic power is the discovery of sequence variants within genes that are of uncertain clinical significance. Perhaps such sequence changes may be novel pathogenic changes, but equally they may be benign or inconsequential variants. Such polymorphisms have been previously encountered in dementia disorders (the non-pathogenicity of the PSEN1 p.Glu318Gly sequence variant, originally described as a ‘mutation’, is a case in point). Classification systems for sequence variants of undetermined significance have been developed by the American College of Medical Genetics (ACMG), the most recent publication suggesting criteria for classifying sequence variants as pathogenic or benign.

We present two cases recently encountered in a dedicated cognitive disorders clinic to illustrate these issues.

**Case 1**
A 58-year-old lady attended the cognitive clinic with her daughter, who provided the history. The patient had suffered cognitive decline from her late 40s/early 50s, beginning with memory problems. Seen in this clinic approximately 10 years after the onset of illness, she was dependent on others for activities of daily living, including help with self-care, asked repetitive questions, and called family members by the wrong names. Previous cognitive screening three years earlier had shown impairments in all domains of the ACE-III (59/100) and formal neuropsychological assessment using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) found performance below the 5th percentile for immediate and delayed memory, visuospatial function, semantic fluency, and assessments of executive function. Magnetic resonance (MR) brain imaging showed some volume loss for age with slight parietal predominance and interval progression over three years.

The patient’s mother and aunt were also reported to have had dementia, also with age at onset in their late 40s/early 50s – both were now dead. Unaffected family members were concerned about the possibility of an inherited form of dementia. Diagnostic genetic testing in the patient was therefore undertaken with appropriate consent, via the local regional NHS genetic testing centre.

Next generation sequencing dementia panel was entirely negative with the exception of a sequence change found in exon 6 of the amyloid precursor protein (APP) gene (c.682G>A, predicted protein change p.Val228Ile)
located on chromosome 21q21.3, a sequence change not previously described in the literature. The clinical significance of this variant was uncertain, mandating further consideration. No other affected family members were available to examine for possible co-segregation of the sequence change and clinical dementia.

Relevant to this case, APP mutations were the first to be described (1991) in early-onset autosomal dominant Alzheimer’s disease (AD). The AD & FTD Mutation Database registers more than 50 mutations (duplications, point mutations) in APP, as well as non-pathogenic sequence changes. Hence the finding of a novel APP sequence variant might be deemed a biologically plausible explanation of this patient’s (and family’s) dementia. However, because all the pathogenic APP point mutations reported hitherto are located in exons 16–17 of the gene, in and around the transmembrane domain of the protein, and because the current sequence variant represented a conservative substitution in a non-functional domain (exon 6) of APP, it was thought more likely that this was a benign variant sequence. In silico evolutionary conservation analysis predicted the variant to be benign. Using the ACMG criteria, there was judged to be ‘supporting evidence of benign impact’ (criterion BP1: multiple lines of computational evidence suggest no impact on gene or gene product) but in the absence of any other criteria the final classification was ‘variant of uncertain significance’.

Case 2
A man in his fifties became increasingly withdrawn, expressed delusional ideas, and a psychiatric diagnosis of depression was made. He progressively developed limb tremors, parkinsonism, falls, and loss of spontaneous verbal output. Various neurodegenerative disorders were considered and excluded after extensive investigation, with a residual working diagnosis of corticobasal syndrome, possibly a tauopathy.

Although there was no family history of similar disorder, blood was sent for the dementia/movement disorder NGS panel with appropriate consent. This was negative aside from a sequence change in exon 5 of the integral membrane protein 2B (ITM2B) gene (c.682A>G, predicted protein change p.Thr228Ala) located on chromosome 13q14.2, not previously described. It was not apparent whether this represented a pathogenic mutation or an incidental benign sequence variant.

ITM2B gene mutations, relevant to this case, are found in familial British and Danish dementias (FBD, FDD), conditions characterised as cerebral amyloid angiopathies, but sometimes categorised with the frontotemporal dementias. Parkinsonism is not a recognised clinical feature. Moreover, these conditions result from point mutations in the stop codon of the ITM2B gene generating a longer open reading frame, hence a larger precursor protein from which an amyloidogenic subunit is released. Missense mutations (point mutations in which a single nucleotide change results in a codon that codes for a different amino acid) have not previously been described to our knowledge. The variant in our patient was predicted to result in a conservative substitution in a highly conserved residue, albeit within a functional domain of the protein. Hence the biological credibility for pathogenicity of this ITM2B sequence change in our patient was uncertain. Subsequent amyloid (18F florbetapir) positron emission tomography (PET) imaging was negative. A previous report of amyloid PET imaging in one patient carrying the FBD ITM2B mutation reported high cerebellar uptake, consistent with the prominence of ataxia in this condition.

Using the ACMG criteria, there was judged to be ‘supporting evidence of benign impact’ for the ITM2B sequence variant found in our patient, based on criterion BP1 (missense variant in a gene for which primarily truncating variants are known to cause disease) and BP4, suggesting a final classification of ‘likely benign’ based on two items of supporting evidence of benign impact.

Discussion
The hope of having discovered a new disease-causing (pathogenic) mutation when NGS results show a novel sequence variant must be tempered by the possibility that such findings are incidental (benign).

While NGS panels certainly enhance the potential to define pathogenic mutations in neurodegenerative (and other neurological) disorders, they may also reveal incidental sequence variants of uncertain significance. Functional studies or familial segregation studies may be unavailable, or may give indefinite answers, meaning that the interpretation of NGS results can be challenging. In this context the importance of liaison with colleagues in clinical genetics services cannot be overemphasised, along with attention to published guidelines for interpretation of sequence variants (often encompassed in the report produced by the genetics laboratory). Counselling of both patients and potentially affected family members prior to NGS
testing concerning the possibility of non-specific findings, irrelevant to disease state, may be need to become part of routine clinical practice.

Using technologies such as magnetic resonance imaging (MRI), clinicians have become familiar with the possibility of findings which are incidental to disease pathogenesis. Clinicians may advise patients of this possibility before scanning, and routinely liaise with colleagues in neuroradiology for interpretation of imaging results. These generic learning points need to be applied to each diagnostic technology when newly introduced.

Declaration of interests
No conflicts of interest were declared.

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References