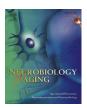
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Brief communication

Validation of next-generation sequencing technologies in genetic diagnosis of dementia

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ABSTRACT

Identification of a specific genetic cause of early onset dementia (EOD) is important but can be difficult because of pleiotropy, locus heterogeneity and accessibility of gene tests. Here we assess the use of next generation sequencing (NGS) technologies as a quick, accurate and cost effective method to determine genetic diagnosis in EOD. We developed gene panel based technologies to assess 16 genes known to harbour mutations causal of dementia and combined these with PCR based assessments of the *C9orf72* hexanucleotide repeat expansion and the octapeptide repeat region of *PRNP*. In a blinded study of 95 samples we show very high sensitivity and specificity are achievable using either Ion Torrent or MiSeq sequencing platforms. Modifications to the gene panel permit accurate detection of structural variation in *APP*. In 2/10 samples which had been selected because they possess a variant of uncertain significance the new technology discovered a causal mutation in genes not previously sequenced. A large proportion (23/85) of samples showed genetic variants of uncertain significance in addition to known mutations. The MRC Dementia Gene Panel and similar technologies are likely to be transformational in EOD diagnosis with a significant impact on the proportion of patients in whom a genetic cause is identified.

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1. Introduction

Genetic diagnosis of the inherited dementias currently relies on sequential Sanger sequencing of genes selected on a clinical basis. This process is costly, time-consuming, and gene tests are variably available, contributing to the limited ascertainment of inherited dementia in the population (Stevens et al., 2011). Here we show that next-generation sequencing (NGS) technology offers a 1-step, accurate, and cost-effective method of screening many causal genes simultaneously, and is likely to be a transformational technology in early-onset dementia diagnosis (Rehm, 2013).

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2. Methods

We used Life Technology's Ion Torrent PGM sequencer with a polymerase chain reaction (PCR) amplicon-based library preparation (AmpliSeq) and Illumina's MiSeq with a PCR amplicon-based (TrueSeq custom amplicon) target enrichment to screen for variants across 16 dementia disease genes (PRNP, PSEN1, PSEN2, APP, GRN, MAPT, TREM2, CHMP2B, CSF1R, FUS, ITM2B, NOTCH3, SERPINI1, TARDBP, TYROBP, and VCP) (Bettens et al., 2013; Rademakers et al., 2012). These sequencing-based technologies were coupled with repeat-primed PCR assessment for C9orf72 hexanucleotide expansions and DNA size fractionation to detect alterations of the octapeptide repeat motif within PRNP because we predicted that the NGS technology would fail to ascertain these variants (DeJesus-Hernandez et al., 2011). To validate this approach we assessed a blinded 95-sample panel which included 85 samples with causal mutations (n = 75) or

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Table 1Validation panel details and Ion Torrent performance metrics

| Gene | Missense | Termination | Large insert/ deletion ^a | Small insert/ deletion | Gene duplication | Gene deletion | Intronic variant | Splice site mutation | | Number on validation panel | | Exons covered | Average read depth | Standard deviation (SD) of read depth | Low coverage areas (average read depth less than 100 times) |
|----------|----------------|-------------|---|------------------------------|---------------------|------------------|---------------------|-------------------------|-----|----------------------------------|--------------------------------------|------------------|--------------------------------------|--|---|
| PRNP | +++ | + | +++ | | | | | | | 18 | 782 | 1 | 1031 | 464 | None |
| APP | 11 +++ 8 | 1 | 6 | | + | | | | | 9 | 268 | 2 ^c | 1092 | 377 | None |
| PSEN1 | +++ 29 | | | + 1 | • | | | + 2 | | 32 | 1504 | 10 | 1442 | 312 | None |
| PSEN2 | +++ 4 | | | | | | | | | 4 | 852 | 5 | 1168 | 519 | 92 bp of second exon; no known mutation; average depth 72, SD 6 |
| GRN | ++ 1 | +++ 2 | | +++ 4 | | + 1 | | + 1 | | 9 | 2304 | 12 | 1166 | 379 | None |
| MAPT | ++ 5 | 2 | | • | | • | ++ | ++ 1 | | 6 | 1730 | 7 | 775 | 301 | None |
| VCP | +++ 2 | | | | | | | | | 2 | 2981 | 17 | 1365 | 513 | 17 bp of first exon; no known mutation; average depth 22, SD 7 |
| СНМР2В | + | + | | | | | | +++ 1 | | 1 | 239 | 2 | 1478 | 21 | None |
| FUS | +++ | | | + | | | | | | 0 | 1065 | 8 | 1007 | 444 | None |
| CSF1R | +++ | | | | | | | | | 2 | 1404 | 11 | 1266 | 426 | None |
| ITM2B | _ | +++ | | | | | | | | 0 | 167 | 1 | 1152 | 657 | 5 bp of last exon; no known mutation; average depth 89, SD 23 |
| TREM2 | + 1 | ++ | | | | | | ++ | | 1 | 842 | 5 | 1365 | 321 | None |
| TYROBP | ++ | | | ++ | | | | | | 0 | 474 | 5 | 1081 | 262 | None |
| TARDBP | +++ | + | | | | | | | | 1 | 1308 | 5 | 1326 | 404 | None |
| NOTCH3 | +++ | | | | | | | | | 0 | 502 | 2 | 1076 | 369 | None |
| SERPINI1 | +++ | | | | | | | | | 0 | 1313 | 7 | 1301 | 333 | None |
| C90RF72 | | | | | | | | | +++ | 0 | NA Total bp screened 17,735 | NA | NA Average read depth, 1193 | NA Average SD, 381 | NA |

The type and frequency of mutations present in genes are shown (+++ = common, ++ = occasional, + = rare; relative to other mutation types in that gene) and the actual number assessed within the validation set. The number of exons and bp screened per gene are indicated along with the average read depth achieved across 95 samples. Areas of lower coverage are shown in the far right column.

Key: bp, base pair; CNV, copy number variation; PCR, polymerase chain reaction.

^a Determined using size fractionation of PCR products of repeat regions.

^b Expansion detected using repeat-primed PCR.

^c Additional 9 exons included in the next design for *APP* CNV detection.

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