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Brief communication

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

John Beck [a], Alan Pittman [b], Gary Adamson [a], Tracy Campbell [a], Joanna Kenny [c], Henry Houlden [b], Jon D. Rohrer [d], Rohan de Silva [e], Maryam Shoai [b], James Uphill [a], Mark Poulter [a], John Hardy [b], Catherine J. Mummery [d], Jason D. Warren [d], Jonathan M. Schott [d], Nick C. Fox [d], Martin N. Rossor [d], John Collinge [a,c], Simon Mead [a,c,*]

[a] MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
[b] Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK
[c] National Prion Clinic, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK
[d] Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
[e] Reta Lila Weston Institute, UCL Institute of Neurology, University College London, Queen Square, London, UK

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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.

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).

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

* Corresponding author at: MRC Prion Unit, Department of Neurodegenerative Disease, Queen Square, London WC1N3BG, UK Tel.: +44 (0)207 676 2164; fax: +44 (0)207 837 8047.
E-mail address: s.mead@prion.ucl.ac.uk (S. Mead).

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Table 1
Validation 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	Repeat expansion[b]	Number on validation panel	Base pairs sequenced in gene region	Exons covered	Average read depth	Standard deviation (SD) of read depth	Low coverage areas (average read depth less than 100 times)
PRNP	+++ 11	+ 1	+++ 6							18	782	1	1031	464	None
APP	+++ 8				+ 1					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						++	++ 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
CHMP2B	+	+						+++ 1		1	239	2	1478	21	None
FUS	+++			+						0	1065	8	1007	444	None
CSF1R	+++ 2									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	+								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.