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Brief communication

Targeted exome sequencing reveals homozygous *TREM2* R47C mutation presenting with behavioral variant frontotemporal dementia without bone involvement

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ABSTRACT

To identify genes associated with frontotemporal dementia (FTD) in South-East Asia, targeted exome sequencing and *C9orf72* genotyping was performed in 198 subjects (52 patients with FTD and 146 healthy controls) who were screened for mutations in 12 FTD-associated genes. We detected a homozygous *TREM2* R47C mutation in a patient with behavioral variant FTD without bone cysts or bone-associated phenotype. Two novel nonsense *GRN* mutations in 3 FTD patients from the Philippines were detected, but no known pathogenic mutations in other FTD-associated genes were found. In 45 subjects screened for *C9orf72* repeat expansions, no pathogenic expansion (≥30 repeats) was identified, but there was a higher proportion of intermediate length (≥10–29 repeats) alleles in patients compared with controls (8/90 alleles, 8.9% vs. 9/164 alleles, 5.5%). Overall, we detected a mutation rate of 7.7% (4/52 patients) in our cohort. Given recent findings of enrichment of rare *TREM2* variants (including R47C) in Alzheimer's disease, it is notable that we detected a homozygous *TREM2* R47C carrier presenting with an FTD rather than an Alzheimer's disease phenotype.

1. Introduction

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by behavioral and/or language impairment (Rascovsky et al., 2011). Genes associated with familial FTD (fFTD) include microtubule-associated protein tau (*MAPT*), progranulin (*GRN*), valosin-containing protein (*VCP*), coiled-coil-helix-coiled-coil-helix domain containing 10 (*CHCHD10*), TAR DNA-binding protein (*TARDBP*), fused-in sarcoma (*FUS*), optineurin (*OPTN*), TANK binding-kinase 1 (*TBK1*), sequestosome-1 (*SQSTM1*), charged multivesicular body protein 2B (*CHMP2B*), and chromosome 9 open-reading frame 72 (*C9orf72*). Of these, *MAPT* and *GRN* mutations and *C9orf72* hexanucleotide repeat expansions are the most common and account for about nearly half of fFTD cases (Seelaar et al., 2011). These genes were included in a targeted exome-sequencing panel of 200 neurodegenerative disease-related genes to identify disease mutations in a South-East Asian cohort of patients with FTD spectrum disorders.

2. Methods

2.1. Patient recruitment

Between January 2015 and November 2016, patients were prospectively recruited from the behavioral neurology clinics at the National Neuroscience Institute, Singapore. Eight patients were recruited from the Institute of Neurological Sciences, St Luke's

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Medical Centre in the Philippines. All patients enrolled met research criteria for bvFTD (behavioral variant frontotemporal dementia) (Rascovsky et al., 2011), semantic variant primary progressive aphasia (PPA) (Gorno-Tempini et al., 2011), nonfluent variant PPA (Gorno-Tempini et al., 2011), progressive supranuclear palsy (Litvan et al., 1996), and/or corticobasal syndrome (Armstrong et al., 2013). One hundred and forty-six age- and race-matched controls without neurological disease were included in the targeted exome sequencing analysis. Ninety-nine of these controls had participated in an earlier study (Foo et al., 2014). Control *C9orf72* data were obtained separately from 82 healthy control samples that have been previously described (Theuns et al., 2014). The institutional review boards at all participating institutions approved this study, and informed consent was obtained from all patients and caregivers.

2.2. Targeted exome sequencing

Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Maxi Kit (Qiagen, Germany). Each sample was barcoded and prepared for next-generation sequencing with the NEBNext Ultra II Library Prep kit (New England Biolabs). The FTD-associated genes were sequenced as part of a panel of 200 neurodegenerative disease-related genes (full list of genes available in Supplementary Table A.1). Exonic sequences of these 200 genes (1.2 Mb) were captured with the NimbleGen SeqCap EZ choice <7 Mb (Roche) following the manufacturer's protocol. We performed multiplexed capture in batches of 12 and pooled a total of 288 samples for 1 lane of 151 bp paired-end sequencing with HiSeq4000 (Illumina). Sequence reads were aligned to the Hg19 (Build 37) reference genome using Burrows-Wheeler aligner, and variants were called using Genome Analysis ToolKit Unified Genotyper. An average of 97.9% of the target sequences were covered with at least 15 reads across all samples, with mean coverage of 110.6x across all targets per sample. Histograms providing coverage distribution across each of the exons of the 12 FTD-related genes of interest are provided in the supplementary material (Supplementary Figs. A.1a and A.1b). *TREM2* and *GRN* mutations were further confirmed by Sanger sequencing (Sanger and C9orf72 genotyping methods and primers used are described in Supplementary Table A.2).

3. Results

3.1. Genetic analysis

A total of 52 patients with FTD spectrum disorders (mean age 62.0 ± 7.7 years) were included. Their demographics are summarized in Supplementary Table A.3. A total of 12 FTD-related genes were screened: *MAPT*, *GRN*, *C9orf72*, *FUS*, *VCP*, *SQSTM1*, *TBK1*, *OPTN*, *TARDBP*, *CHCHD10, CHMP2B,* and *TREM2.* Sequencing of *MAPT*, *FUS*, *VCP*, *SQSTM1*, *TBK1*, *OPTN*, *TARDBP*, *CHCHD10*, and *CHMP2B* genes identified no known pathogenic variant. Five nonsynonymous variants of unknown significance detected in *MAPT*, *GRN, CHMP2B, SQSTM1*, and *TREM2* are listed in Supplementary Table A.4. Apart from *MAPT* p.Gln230Arg that was found in 3 heterozygous controls, the remaining 4 variants were absent in all 146 controls. None of the samples carried the *TREM2* R47H variant associated with higher risk for Alzheimer's disease (AD) (Guerreiro et al., 2013b). Three patients from the Philippines had novel nonsense *GRN* mutations: 1 was heterozygous for a *GRN* p.Cys92* (c.276 C>A) mutation and 2 were heterozygous for a *GRN* p.Ser301* (c.902 C>A) mutation. Both patients carrying the *GRN* p.Ser301* mutation were unrelated but from the same region in the Philippines (Bicolano). Importantly, we identified a homozygous *TREM2* p.Arg47Cys (c.139 C>T) mutation predicted in silico to be damaging [SIFT (Kumar et al., 2009) score 0.01, PolyPhen-2 (Adzhubei et al., 2010) score 0.998], with recent

Table 1
Variant and clinical details of 4 FTD patients with mutations in *TREM2* and *GRN*

Variant and clinical details	*TREM2* p.Arg47Cys	*GRN* p.Cys92*	*GRN* p.Ser301* Patient 1	*GRN* p.Ser301* Patient 2
Position (hg19)	Chr 6: 41129253, exon 2	Chr 17: 42427046, exon 4	Chr 17: 42428797, exon 9	Chr 17: 42428797, exon 9
cDNA position	c.139, NM_018965	c.276, NM_002087	c.902, NM_002087	c.902, NM_002087
Mutation	Homozygous	Heterozygous	Heterozygous	Heterozygous
Codon	CGC>TGC	TGC>TGA	TCG>TAG	TCG>TAG
Amino acid change	p.Arg47Cys	p.Cys92X	p.Ser301X	p.Ser301X
SIFT score	0.01	N/A	N/A	N/A
SIFT prediction	Damaging	Damaging	Damaging	Damaging
PolyPhen-2 score	0.998	–	–	–
PolyPhen-2 prediction	Probably damaging	–	–	–
ExAC freq (East Asians)	0	absent	absent	absent
ExAC freq (South Asians)	0.0001218	absent	absent	absent
ExAC freq (Non-Finnish Europeans)	1.509e-05	absent	absent	absent
gnomAD freq (East Asian)	0.0001160	absent	absent	absent
gnomAD freq (South Asians)	0.00006499	absent	absent	absent
gnomAD freq (Non-Finnish Europeans)	0.00001816	absent	absent	absent
Patient clinical data and demographics				
Gender (M/F)	F	M	M	F
Race	South Asian	Filipino	Filipino	Filipino
Age at onset	58	58	66	60
Phenotype	bvFTD	bvFTD	nfvPPA	nfvPPA
Duration of symptoms (y)	5.0	5.0	2.0	1.0
Neuroimaging findings	Bilateral symmetrical frontotemporal and biparietal atrophy	Bilateral moderate frontotemporal atrophy	Left temporal and parietal lobe atrophy	Left anterior temporal lobe atrophy
Progranulin levels (ng/mL)	N/A	88.8 (<112.0)	51.2 (<112.0)	Not performed

Key: bvFTD, behavioral variant frontotemporal dementia; ExAC, exome aggregation consortium; gnomAD, genome aggregation database; *GRN*, progranulin; PolyPhen-2, polymorphism phenotyping 2; N/A, not applicable; nfvPPA, nonfluent variant primary progressive aphasia; SIFT, sorting intolerant from tolerant; SNP, single-nucleotide polymorphism; *TREM2*, triggering receptor expressed on myeloid Cells 2.