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Primary Microgliopathy Presenting as Degenerative Dementias: A Case Series of Novel Gene Mutations from India

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Abstract
Introduction: Microglia exert a crucial role in homeostasis of white matter integrity, and several studies highlight the role of microglial dysfunctions in neurodegeneration. Primary microgliopathy is a disorder where the pathogenic abnormality of the microglia causes white matter disorder and leads to a neuropsychiatric disease. Triggering receptor expressed on myeloid cells (*TREM2*), TYRO protein tyrosine kinase binding protein (*TYROBP*) and colony-stimulating factor 1 receptor (*CSF1R*) are genes implicated in primary microgliopathy. The clinical manifestations of primary microgliopathy are myriad ranging from neuropsychiatric syndrome, motor disability, gait dysfunction, ataxia, pure dementia, frontotemporal dementia (FTD), Alzheimer's dementia (AD), and so on. It becomes imperative to establish the diagnosis of microgliopathy masquerading as degenerative dementia, especially with promising therapies on horizon for the same. We aimed to describe a case series of subjects with dementia harbouring novel genes of primary microgliopathy, along with their clinical, neuropsychological, cognitive profile and radiological patterns. ***Methods:*** The prospective study was conducted in a university referral hospital in South India, as a part of an ongoing clinico-genetic research on dementia subjects, and was approved by the Institutional Ethics Committee. All patients underwent detailed assessment including sociodemographic profile, clinical and cognitive assessment, pedigree analysis and comprehensive neurological examination. Subjects consenting for blood sampling underwent genetic testing by whole-exome sequencing (WES). ***Results:*** A total of 100 patients with dementia underwent genetic analysis using WES and three pathogenic variants, one each of *TREM2*, *TYROBP*, and *CSF1R* and two variants of uncertain significance in *CSF1R* were identified as cause of primary microgliopathy. *TREM2* and *TYROBP* presented as frontotemporal syndrome whereas CSF1R presented as frontotemporal syndrome and as AD. ***Conclusion:*** WES has

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widened the spectrum of underlying neuropathology of degenerative dementias, and diagnosing primary microglial dysfunction with emerging therapeutic options is of paramount importance. The cases of primary microgliopathy due to novel mutations in *TREM2*, *TYROBP*, and *CSF1R* with the phenotype of degenerative dementia are being first time reported from Indian cohort. Our study enriches the spectrum of genetic variants implicated in degenerative dementia and provides the basis for exploring complex molecular mechanisms like microglial dysfunction, as underlying cause for neurodegeneration.

Introduction

Microglia exert a crucial role in the homeostasis of white matter integrity, and emerging evidence suggests that microglial dysfunction plays a significant role in leukodystrophy and neurodegeneration [1–4]. Primary microgliopathies refer to adult leukodystrophies linked to mutations in genes expressed in microglial cells and include adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), Nasu-Hakola disease (NHD), and leukodystrophies related to variants in the negative regulator of reactive oxygen species or pseudo-TORCH syndrome [5–11]. These are considered as type-I microgliopathies [7].

Microglia are associated with a set of pattern recognition receptors at the cell surface [12, 13]. Mutations in these microbial sensome genes also lead to primary microgliopathies by promoting neurodegenerative diseases such as AD and fronotemporal dementia (FTD) in type-II microgliopathies [7, 14]. Details are shown as flow chart in Figure 1.

Mutations in microglial colony-stimulating factor 1 receptor (*CSF1R*), triggering receptor expressed on myeloid cells (*TREM2*), and TYRO protein tyrosine kinase binding protein (*TYROBP*) genes can cause leukodystrophy and FTD-like clinical syndromes, whereas mutations due to *TREM2* can be a risk factor for FTD, FTD-like syndromes, and Alzheimer's dementia (AD) [7, 15]. The clinical phenotypes of primary microgliopathy are myriad, ranging from neuropsychiatric syndrome, motor disability, gait dysfunction, ataxia, pure dementia, FTD, AD, and so on [9–11, 15, 16].

NHD (or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy [PLOSL]) and adult-onset leukoencephalopathy with ALSP exemplify how intrinsic microglial dysfunction could cause neurological or psychiatric diseases [5, 6]. NHD is caused by the mutations of genes *TYROBP* or *TREM2* (PLOSL1 and PLOSL2, respectively). The characteristic symptoms include multiple bone cysts and fractures, and frontal lobe dementia [5–8].

Several cases of early-onset FTD-like syndromes involving white matter loss but lacking overt bone phenotypes have also been associated with homozygous variants as well as rare heterozygous variants in TREM2 although the mechanism remains unclear [14, 17]. Single-nucleotide variations in *TREM2* have been linked to both late-onset Alzheimer's disease and behavioural variant FTD (bvFTD), pure dementia without bony changes, and NHD [18]. In AD, TREM2 is a risk factor that is highly associated with disease progression in amyloid-β pathology [19].

ALSP is caused by *CSF1R* mutations and is characterized by several neuropsychiatric symptoms such as cognitive decline, anxiety, depression, irritability, and behavioural FTD-like symptoms and AD. The motor symptoms include Parkinsonian symptoms, pyramidal, bulbar signs, and ataxia and are often misdiagnosed [20–22].

In this report, we describe a case series of patients clinically presenting with dementia confirmed on whole-exome sequencing (WES) to have genetic defects linked to primary microgliopathy. The range of clinical, cognitive profiles, radiological patterns, and underlying novel genetic mutations linked to primary microgliopathy is reported for the first time in the Indian context.

Patients and Methodology

A total of 100 subjects with dementia, diagnosed with FTD (n = 85) based on the standard international consensus criteria for bvFTD, progressive primary aphasia [68, 69], and AD (n = 15) based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for AD, were recruited from the Cognitive Disorders Clinic (CDC) in a university referral hospital in South India. These patients being part of an ongoing clinic-genetic study underwent WES with informed consent.

The age at enrolment of the 100 patients ranged between 32 and 83 years with an average of 58.8 years. This group consisted of 55% males, and disease duration varied from 6 months to 10 years, with an average of 2.6-years. The pedigree analysis showed significant family history in more than one-third of the patients (36.3%). Among these 100 patients, five subjects harbouring genes for primary microgliopathy form the cohort of this manuscript and are being described.

Among the 100 patients that underwent WES, patients diagnosed with dementia using the standard diagnostic criteria and

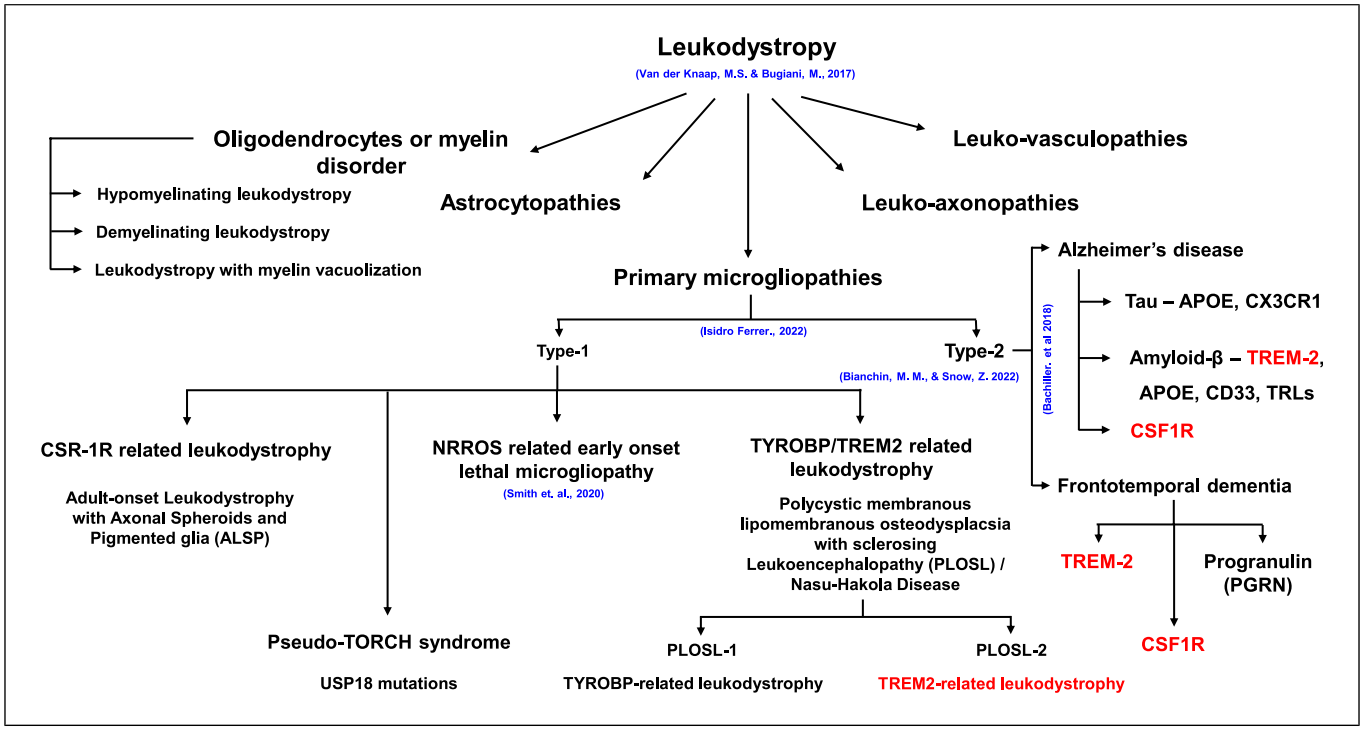

Fig. 1. Types of microgliopathies.

having additional magnetic resonance imaging (MRI) features like white matter hyperintensity, basal ganglia calcification, and thinning of the corpus callosum, along with the presence of genetic variants causing primary microgliopathy, form the current study cohort. All other FTD and AD cases with classical MRI features and harbouring other genetic variants were excluded from the study.

All subjects enrolled in the study underwent detailed assessment including sociodemographic details, family history using the modified Goldman score [23], cognitive and neuropsychological profile, and neurological examination. A comprehensive cognitive assessment using Addenbrooke's Cognitive Examination-Revised (ACE-R) or Hindi Mini-Mental Score (HMSE), frontal assessment battery, neuropsychiatry inventory scores and severity using Clinical Dementia Rating (CDR) Scale score was performed [24–26]. Participants also underwent structural imaging using a 3 Tesla MRI. Testing for secondary causes of dementia including thyroid functions, vitamin B12 levels, human immunodeficiency virus, venereal disease research laboratory test, autoimmune profile, cerebrospinal fluid analysis was carried out in all patients, to exclude other causes.

Genetics

Subjects with dementia consenting to blood sampling underwent genetic testing by WES. Genomic DNA was extracted from a peripheral blood sample using Qiagen kit (QIAamp DNA Kit). The DNA quantity and quality were assessed by NanoDrop spectrophotometer and by agarose gel electrophoresis. The quality-passed DNA samples (criteria DNA yield: 20 ng/μL; A260/280: 1.8–1.9; A260/230: 2–2.5) were further quantified by Qubit Fluorometric method (Thermo Fisher Scientific). WES libraries were prepared using 37 Mb capture probe sets from *Twist Bioscience, Inc.*, which includes protein coding genes and the mitochondrial genome as per the manufacturer's protocol. Libraries were sequenced on the Illumina NextSeq 550 platform using 2 × 150 bp read chemistry according to the manufacturer's instructions. Reads from the sequence output were aligned to the human reference genome (GRCh38) using the Burrows-Wheeler Aligner. The variants to the reference were called using the Genomic Analysis Tool Kit. The variants were annotated and filtered using the Golden Helix VarSeq analysis workflow implementing the American College of Medical Genetics (ACMG) guidelines for interpretation of sequence variants. This includes comparison against the gnomAD population catalog of variants in 730,947 exomes and 76,215 genomes and 1000 Genomes Project Consortium of 2,500 genomes, the NCBI ClinVar database and multiple lines of computational evidence on conservation and functional impact.

All variants deemed pathogenic or likely pathogenic were validated by Sanger sequencing. The pathogenicity of the variants was assessed based on the 2015 American College of Medical Genetics (ACMG) guidelines. The pathogenicity of the clinically relevant variants was further confirmed by the genotype-phenotype correlation and by a literature review of disease association studies in PubMed, HGMD, and ClinVar databases. All variants deemed pathogenic/likely pathogenic were validated by Sanger sequencing. DNA was extracted from peripheral blood

using the QIAamp DNA Minikit. Specific primers were designed using primer 3 and were checked for primer dimer and self-dimers using a primer analyser (Thermo Fisher Scientific Inc) followed by in silico PCR in UCSC genome browser. PCR-amplified products were verified on 1–1.5% agarose gel electrophoresis. The post-PCR clean-up was performed to remove unutilized primers, unused dNTPs using JetSeq Clean beads (Bioline). The purified amplicons were then subjected to bidirectional Sanger sequencing on the SeqStudio Genetic Analyzer (Thermo Fisher Scientific) using BigDye Terminator v3.1 Kit as per manufacturer's instructions (Thermo Fisher Scientific). Sanger sequencing was performed on SeqStudio Genetic Analyzer. The variant at the targeted locus was ascertained by a visual inspection of electropherogram, and also by comparing with the reference sequence and confirming the location of the mutation.

Results

A total of 100 patients diagnosed with dementia underwent genetic analysis using WES during the study period. Data from each sample had a mean depth ranging from ×70 to ×90. A total number of targets was 214,702. On-target bases were covered at least ×1 ranging between 98 and 99%; ×20 ranging between 93 and 96%; and ×100 ranging between 24 and 27%. The coverage of the coding regions of the genes of interest was 99–100%. On an average, the number of variants per sample at a depth of ×20 or more and with GQ (Phred quality scores) of 20 or more was 60,000–70,000. The variants were further filtered as described in the Methodology section. Only variants of sufficient depth and Phred quality scores of more than 20 were considered for further evaluation. Of the 100 subjects, five pathogenic variants in gene causing primary microgliopathy were identified and included three pathogenic variants of *TREM2*, *TYROBP*, and *CSF1R*, one likely pathogenic variant in *CSF1R* and one variant of uncertain significance (VUS) in *CSF1R*. Pathogenic, likely pathogenic, and a VUS in primary microgliopathy-related genes are depicted in Table 1.

Case 1

A 45-year-old female patient presented with cognitive decline, personality change, and behavioural disturbances in the form of apathy, disinhibition, overfamiliarity, a bizarre eating pattern, hyper-orality, and preference to sweets for 5 years. She had reduced attention and recent memory disturbances. She exhibited poor personal hygiene, frequent wandering, inattentiveness, loss of empathy, and problems with planning and judgement. There was a gradual decline in speech output and verbal perseverations. Subsequently, she developed navigational difficulty and difficulty in dressing and in recognizing currency. Her cognition gradually worsened and over the next 3 years, she became incontinent. There were no delusions, hallucinations, pathological bone fractures, bone pain, or swelling of ankles or wrist. There was a history of similar behavioural disturbances in the elder sister who had a premature death as shown in Figure 2.

On neurological examination, the patient had disorientation and inattentiveness. She had non-fluent aphasia and frontal release signs, along with utilization behaviour and environmental dependency. The ACE-R score was three, and the CDR score was three. Investigations for young onset dementia were negative.

A clinical diagnosis of bvFTD (behavioural variant) was considered. Her brain imaging showed symmetrical atrophy of frontal, temporal lobes, and superior parietal lobules with relative preservation of occipital lobe and inferior parietal lobe. T2/FLAIR hypointensity was observed in the bilateral putamen, globus pallidus, and subcortical FLAIR intensities in the frontal lobe along with a striking thinning of the corpus callosum as shown in Figure 2. WES revealed a pathogenic splice donor variant NM_018965.4:c.40 + 1G>A in *TREM2* gene (NC_000006.12:g.41163042C>T; rs766712618) in the homozygous state in the proband. The c.40 + 1G>A variant is novel (not in any individuals) in 1kG All. The c.40 + 1G>A variant is observed in 3/30,780 (0.0097%) alleles from individuals of a gnomAD South Asian background in gnomAD in only a heterozygous state and 2 other individuals with a similar phenotype in the homozygous state in our in-house database. This variant mutates a splice donor sequence, potentially resulting in the retention of large segments of intronic DNA by the mRNA and nonfunctional proteins. This variant results in the loss of a donor splice site for the clinically relevant transcript. This variant disrupts the donor splice site for an exon upstream from the penultimate exon junction and is therefore predicted to cause nonsense-mediated decay. The c.40 + 1G>A variant is a loss-of-function variant in the gene *TREM2*, which is intolerant of loss-of-function variants, as indicated by the presence of existing pathogenic loss-of-function variant NP_061838.1:p.E14* and 4 others. In addition, the phenotype of the proband matches with that of the disorder caused by pathogenic variants in *TREM2* gene. For these reasons, this variant has been classified as Pathogenic (PM2 PVS1 PP4_Moderate PS4_Moderate) (submission ID to ClinVar: SUB13901156). This variant was validated on Sanger sequencing as shown in Figure 2. On a telephonic follow-up, the patient's family reported that she had expired 1.5 years after diagnosis.

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Table 1. Pathogenic, likely pathogenic, and VUS in primary microgliopathy-related genes

Gene symbol (transcript) location	Variant (HGVS nomenclature)	Genomic coordinate of the variant	Zygosity and metrics (depth and Phred quality score)	Effect	ACMG classification	ClinVar accession ID	Allele frequency gnomAD 4.0[a]	1kG All[b]	In-house database	In silico predictions MSA-SIFT	PolyPhen2	CADD score*
TREM2 (NM_018965.4) Intron 1	NM_018965.4: c.40+1G>A	chr6: g.41163042C>T	Homozygous 86 × 251.00	LoF (splice donor variant)	Pathogenic: PM2 PVS1 PP5 PP4 PS4	VCV002583155.1	5 in heterozygous state AF = 0.0000034203	N	2 similar phenotypes in homozygous state	D	D	4.81
TYROBP (NM_003332.4) Exon 2	NM_003332.4: c.82C>T; NP_003323.1: p.Gln28Ter	chr19: g.35907742G>A		Stop gained	Pathogenic: PM2 PVS1 PP5 PP4	VCV001935007.2	2 in heterozygous state AF = 0.00000318088	N	N	D	D	9.00
CSF1R (NM_005211.4) Intron 14	NM_005211.4: c.1969+1G>A	chr5: g.150060861C>T	Heterozygous 18x/52 × 42.00	LoF (splice donor variant)	Pathogenic: PM2 PVS1 PP5 PP4	VCV000978469.4	N	N	N	D	D	5.01
CSF1R (NM_005211.4) Exon 22	NM_005211.4: c.2768A>G; NP_005202.2: p.Tyr923Cys	chr5: g.150054220 T>C	Heterozygous 35x/60 × 47.00	Missense	Likely pathogenic: PM2 PP2 PP3 PS1 PP4	VCV002583156.1	N	N	N	D	PD	4.0
CSF1R (NM_005211.4) Exon 5	NM_005211.4: c.658G>A; NP_005202.2: p.Ala220Thr	chr5: g.150078183C>T		Missense	VUS: PP2 PP4_Moderate	VCV000870766.17	13 in heterozygous state AF = 0.00000889273	N	9 of unrelated phenotype in heterozygous state	D	D	3.27

PD, probably damaging; D, deleterious; CADD, combined annotation-dependent depletion; N, novel; ACMG, American College of Medical Genetics. [a]gnomAD, gnome Aggregation Database version 4 (https://gnomad.broadinstitute.org/). [b]1kG All, the 1000 Genomes Project Consortium's publication of 2,500 genomes (https://www.genome.gov/27528684/1000-genomes-project). *In-house* database (of ~2000 healthy controls and non-FTD cases at NIMHANS). *CADD score (https://cadd.gs.washington.edu/developed by the University of Washington and precomputed on every substitution in the human genome as well as for the insertion/deletions (InDels) in the 1000 Genomes dataset. For novel InDels, the maximum value of the overlapping or adjacent bases is provided). Multiple lines of computational evidence support a D effect on the gene or gene product. Examples include in silico protein function predictions, conservation, splicing impact, etc. The variants are classified according to the guideline of ACMG.

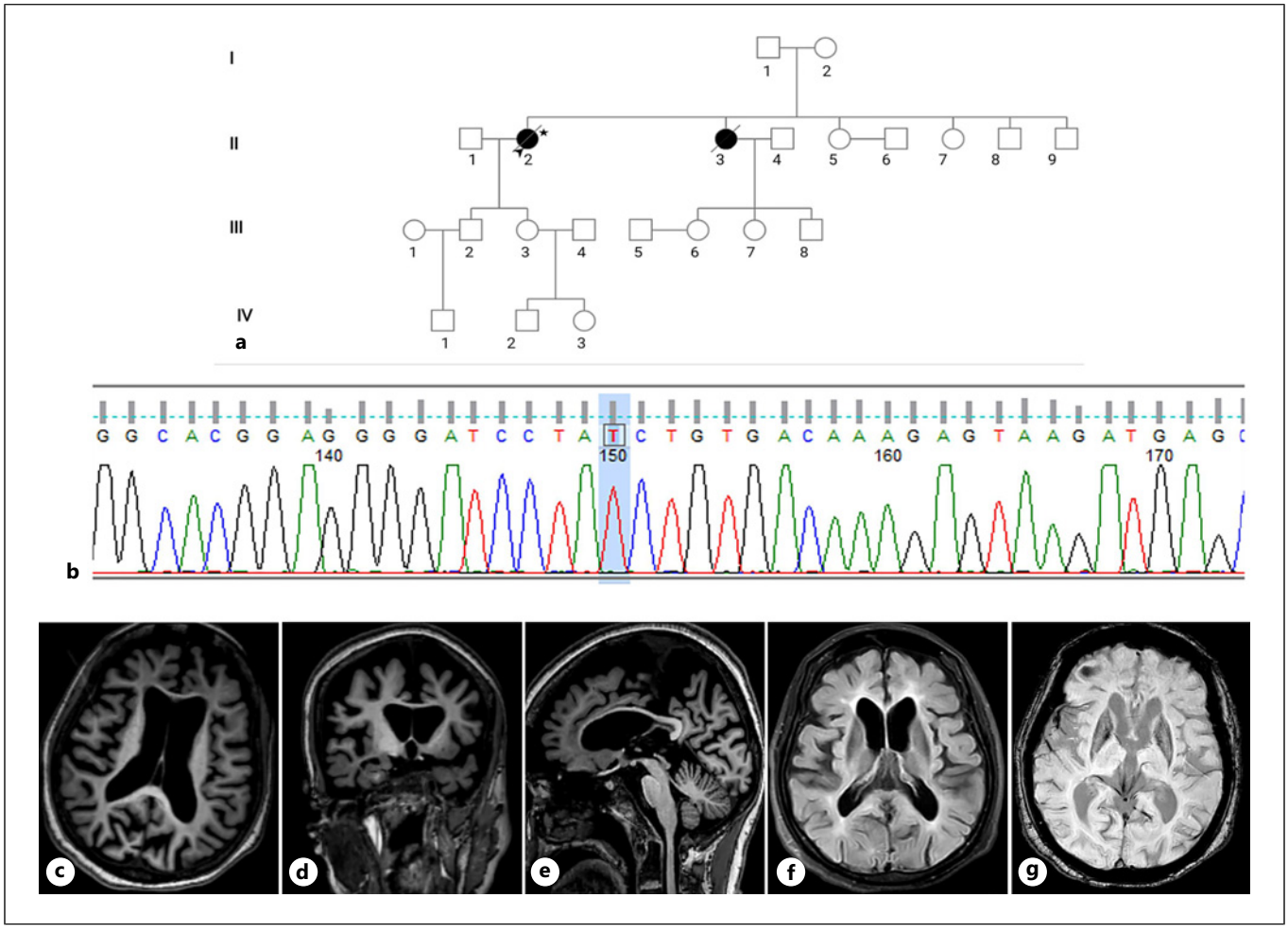

Fig. 2. a Roman numerals (I, II, III, IV) indicate the generations, and the numbers (1–9) indicate the individuals in each generation. The proband is indicated by the arrow; black-filled symbols represent subjects affected by II.2 by FTD; age at onset: 40 y, age at death: 48 y); II.3: dementing illness similar to the proband, age at onset: 40; age at death: 46 years. Diagonal lines indicate the deceased and asterisk in whom the variant has been demonstrated. The proband is II.2 (filled, arrow, and asterisk), a 45-year-old female, carrying the (c.40 + 1G>A) splice donor variant TREM2. **b** Chromatogram of the proband highlighted at intron 1 of TREM2 (between exons 1 and 2 of 5) (transcript ID: NM_018965.4). The proband has been identified with homozygous targeted mutation in intron 1 of TREM2 gene (highlighted in blue). **c**–**e** T1-weighted multiplanar imaging shows frontoparietal predominant atrophy and ventricular horn prominence. **f** Axial FLAIR image showing frontoparietal periventricular and deep white matter hyperintense signal changes and volume loss. **g** SWI demonstrates blooming in the bilateral lentiform nucleus.

Case 2

A 43-year-old female patient presented with 4 years history of insidious onset progressive behavioural symptoms in the form of compulsive behaviour such as buying things, spending money excessively, aggressiveness, and abusive nature which required antipsychotics. Over the next one and a half years, she developed overfamiliarity with strangers, attention and recent memory problems. She was also noted to have slurring of speech and reduced fluency and was speaking only in single words or phrases. Caregivers reported a loss of self-hygiene, social disinhibition, lack of empathy, and sweet preference. Subsequently, few months later, she developed progressive difficulty in jaw opening, chewing, and swallowing that required Ryle's tube feeding and difficulty in speaking that progressed to mutism. There was sleep talking and periodic complex limb movements. There was history of frequent pathological fractures. Family history of psychiatric illness with onset at 43 years was present in her first cousin as shown in Figure 3.

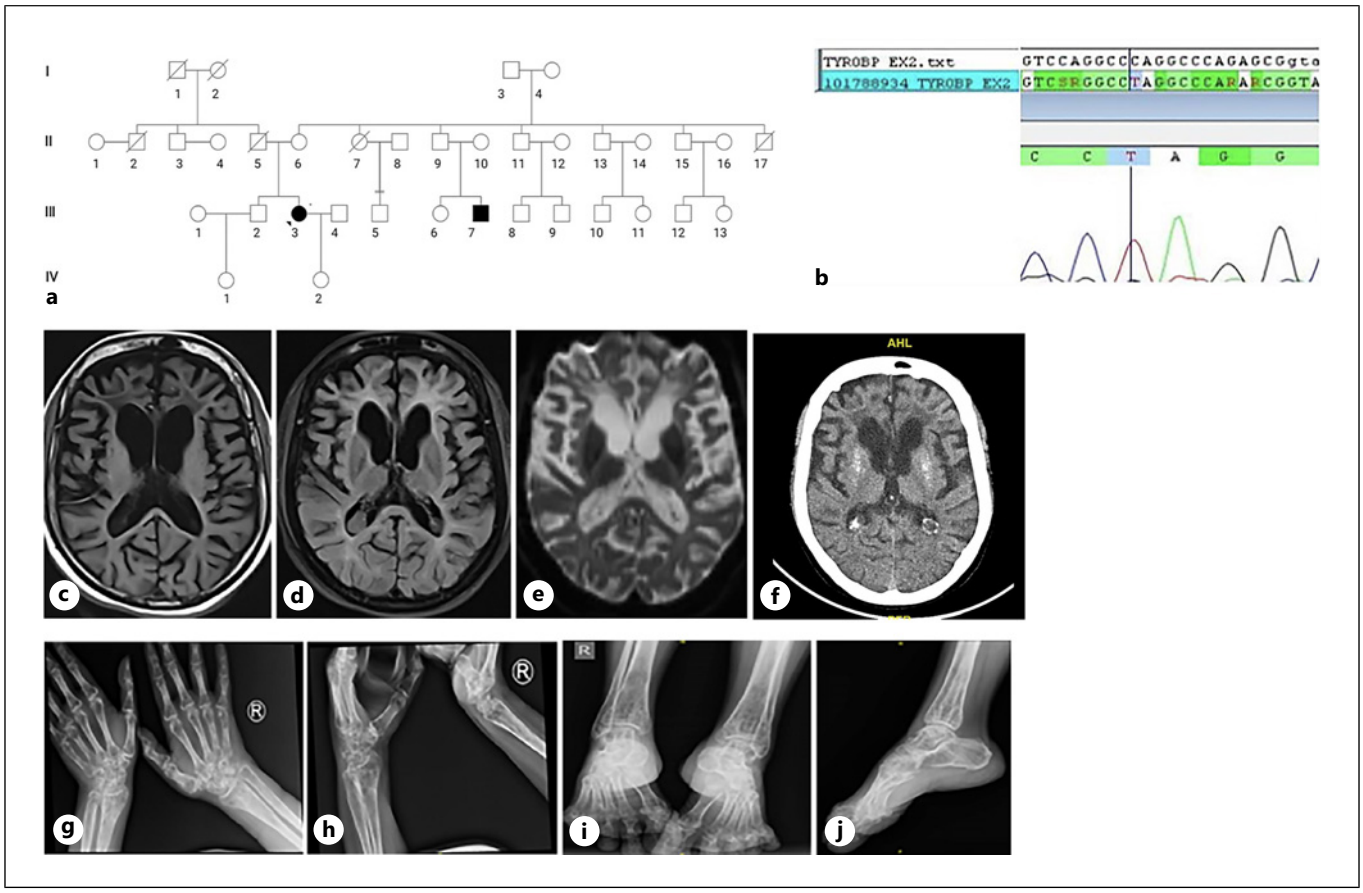

Fig. 3. a Family tree: Roman numerals (I, II, III, IV) indicate the generations, and the numbers (1–17) indicate individuals in each generation. The proband is indicated by the arrow; black-filled symbols represent subjects affected by III.7: psychiatric illness; age at onset: 43 y; diagonal lines indicate the deceased and asterisk in whom the variant has been demonstrated. The proband is III.3 (filled, arrow, and asterisk), a 43-year-old female carrying the (p.Gln28Ter*) stop gained variant TYROBP. **b** Chromatogram showing homozygous stop gained variant in exon 2 of 5 in TYROBP mutation NM_00332.4(TYROBP): C.82C>T(p.Q28*); transcript ID: NM_00332.4. **c** First image from left is T1-weighted axial images showing frontoparietal atrophy with hypointense periventricular white matter changes. **d** Second image is T2-weighted axial image showing periventricular white matter hyperintensity. **e** SWI axial view image showing lenticular nucleus blooming. **f** NCCT shows ill-defined calcification in bilateral lentiform nuclei. **g**, **h** AP and lateral X-ray of the bilateral hands and wrists show multiple variable size ill-defined trabecular lucencies, a few of which have a cystic morphology in a periarticular and metaphyseal distribution. **i**, **j** AP and lateral X-ray of the bilateral legs and ankle show multiple variable size ill-defined trabecular lucencies, a few of which have a cystic morphology in a periarticular and metaphyseal distribution.

On examination, the patient exhibited apathy, social disinhibition, executive dysfunction, parkinsonism, mutism, and pyramidal signs. She had spasticity with brisk tendon reflexes and release reflexes. She was evaluated for secondary causes of early-onset dementia and the results were negative. MRI brain scan showed frontoparietal T2/FLAIR hyperintensities with severe frontal predominant atrophy. X-rays of hands, wrists, legs, and ankles showed multiple variable size ill-defined trabecular lucencies, with a few demonstrating cystic morphology in a periarticular and metaphyseal distribution as shown in Figure 3.

WES revealed a ClinVar-reported pathogenic (Accession: VCV001935007.2) stop gained NM_003332.4:c.82C>T and NP_003323.1:p. Gln28Ter variant in *TYROBP* gene (NC_000019.10:g.35907742G>A) in the homozygous state in the proband. The p. Gln28Ter variant is novel (not in any individuals) in 1kG All and nomad as well as in our in-house database. This variant is predicted to cause loss of normal protein function through protein truncation. This variant is a stop gained variant which occurs in an exon of *TYROBP* upstream of where nonsense-mediated decay is predicted to occur. This

variant has been previously classified as pathogenic, indicating that the region is critical to protein function. There is another pathogenic loss-of-function variant 60 residues downstream of this variant, indicating that the region is critical to protein function. The p. Gln28Ter variant is a loss-of-function variant in the gene *TYROBP*, which is intolerant of loss-of-function variants, as indicated by the presence of existing pathogenic loss-of-function variant NP_003323.1:p. Q28*. In addition, the phenotype of the proband matches with that of the disorder caused by pathogenic variants in *TYROBP* gene. For these reasons, this variant has been classified as Pathogenic (PM2 PVS1 PP5). This variant has been Sanger validated as shown in Figure 3.

Case 3

A 54-year-old female patient presented with rapidly progressive cognitive decline, with predominantly language difficulties associated with behavioural disturbances characterized by aggressiveness, anger outbursts, and poor self-care for 1.5 years. Over the next 3 months, the patients developed slowness of gait and incontinence and became dependent for all activities of daily living. There was no family history of a similar illness. She was inattentive and understood only simple commands and gestures. ACE-R was 5, while ACE-R of 44 was documented a year ago indicating a rapid decline. The CDR score was 3, and the Neuropsychiatry Inventory score was 6. She had prominent language disturbances, the aphasia quotient was 50.1, and a provisional clinical diagnosis of primary progressive aphasia was performed. She also had impairment on frontal lobe assessment battery, Luria test, trail making test, verbal perseveration, verbal fluency, etc., tests along with language, and visuospatial dysfunction. All secondary causes for young onset dementia were negative [27].

Imaging revealed T1, T2, FLAIR, and DWI hyperintense signal changes in the white matter periventricular region, centrum semiovale, corona radiata, and corpus callosal atrophy with diffusion restriction in the splenium along with diffuse atrophy as shown in Figure 4. WES revealed a ClinVar-reported (accession: VCV000978469.4) pathogenic splice donor variant NM_005211.4:c.1969 + 1G>A in *CSF1R* gene (NC_000005.10:g.150060861C>T; rs1757478199) in the heterozygous state in the proband. The c.1969 + 1G>A variant is novel (not in any individuals) in 1kG All and gnomAD as well as in the in-house database. This variant mutates a splice donor sequence, potentially resulting in the retention of large segments of intronic DNA by the mRNA and nonfunctional proteins. This variant results in the loss of a donor splice site for the clinically relevant transcript. This variant disrupts the donor splice site for an exon upstream from the penultimate exon junction and is therefore predicted to cause nonsense-mediated decay. The c.1969 + 1G>A variant is a loss-of-function variant in the gene *CSF1R*, which is intolerant of loss-of-function variants, as indicated by the presence of existing pathogenic loss-of-function variant NP_005202.2:p. K185Rfs*2 and 5 others. In addition, the clinical phenotype of the proband matches completely with that of the disorder caused by pathogenic variants in the *CSF1R* gene. Hence, this variant has been classified as Pathogenic (PM2 PVS1 PP5). Sanger validation confirmed the genetic variation as shown in Figure 4.

Case 4

A 56-year-old female patient presented with 4 years history of episodic and recent memory loss, attention deficits, misplacing objects and repeated questioning, and navigational difficulty for 1.5 years, followed by a 1-year history of difficulty in recognizing and using common objects, suggestive of apperceptive agnosia. The patient's Hindi Mental State Examination (HMSE) score was 18, and exhibited attentional errors, difficulty in recent memory and recall, visuospatial disorientation, clock drawing errors, simultagnosia, and dressing apraxia with apperceptive agnosia. Hence, a clinical diagnosis of posterior cortical variant of AD was considered. PET MR showed hypometabolism in the temporoparietal and posterior cingulate region as shown in Figure 5. MR images also showed bifrontal asymmetric T2-weighted/FLAIR hyperintensities in the subcortical deep and periventricular white matter with corresponding T1 hypointensities. WES revealed a novel Likely Pathogenic missense variant NM_005211.4:c.2768A>G (NP_005202.2:p.Tyr923Cys) in the *CSF1R* gene (NC_000005.10:g.150054220T>C) in the heterozygous state in the proband. The NP_005202.2:p.Tyr923Cys variant is novel (not in any individuals) in 1kG All, gnomAD and in the in-house database. There is a large physicochemical difference between tyrosine and cysteine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size, and/or other properties. The p.Tyr923Cys missense variant is predicted to cause damaging effect by both SIFT and PolyPhen2. The gene *CSF1R* has a low rate of benign missense variation as indicated by a high missense variant Z-Score of 1.57. The gene *CSF1R* contains 28 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. In addition, the clinical phenotype of the proband especially the MRI matches completely with that of the disorder caused by pathogenic variants in the *CSF1R* gene. As a result, this variant has been classified as Likely Pathogenic (PM2 PP2 PP3 PP4_Moderate) with ClinVar

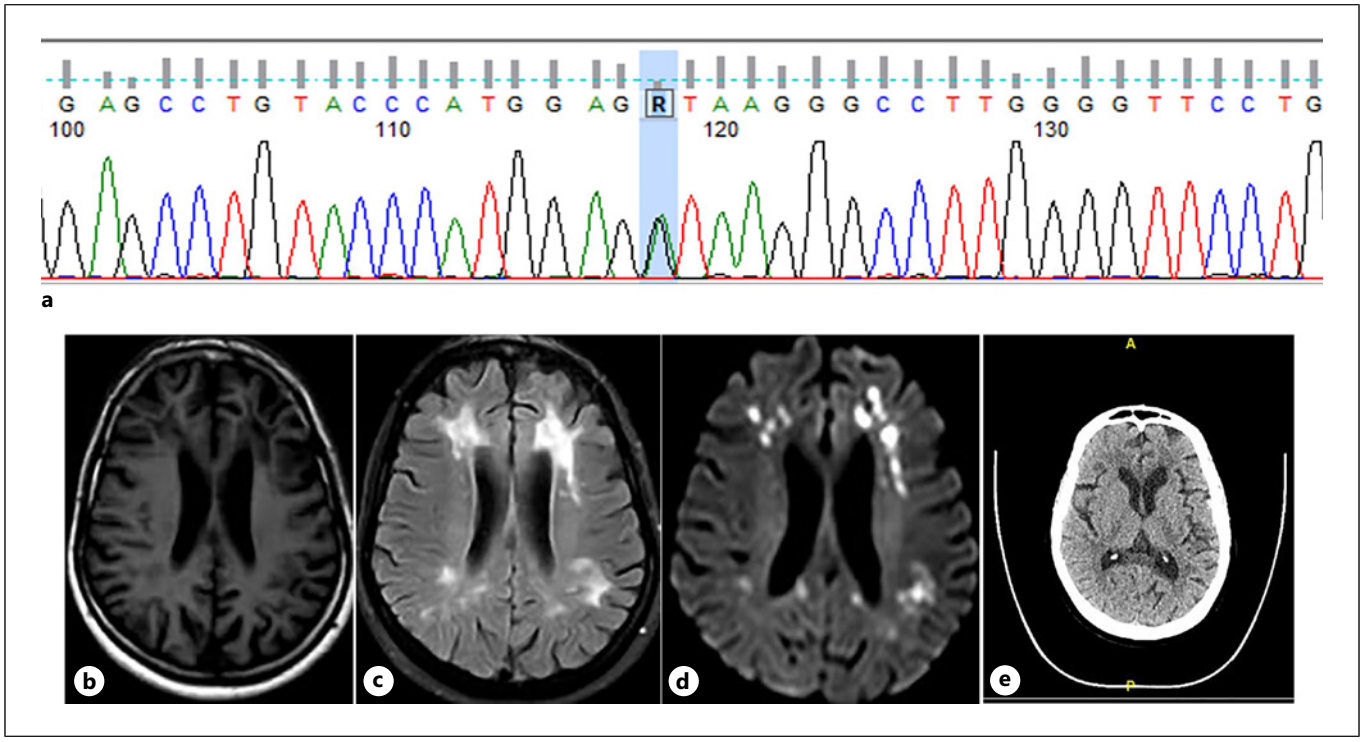

Fig. 4. a Chromatogram of the proband highlighted at intron 14 of CSF1R (between exons 14 and 15 of 22) (transcript ID: NM_05211.4). The proband has been identified with heterozygous targeted mutation in intron 14 of CSF1R gene (highlighted in blue). **b** T1-weighted multiplanar imaging shows frontoparietal predominant atrophy and ventricular horn prominence. **c** Axial FLAIR images reveal frontoparietal periventricular and deep white matter hyperintense signal changes and volume loss. **d** DWI has evidence of multifocal scattered areas of diffusion restriction in the bilateral frontal and parietal deep white matter. **e** NCCT scan brain shows periventricular hypodensity.

submission ID: SUB13901225. Sanger sequencing electropherogram revealed heterozygous variant at c.2768A>G position in the proband's sample as depicted in Figure 5.

Case 5

A 65-year-old male patient presented with memory disturbances, disinhibitory behaviour, way-finding difficulty, and decreased interaction since 3 years, followed by slowness of activities over 2.5 years. The symptoms rapidly worsened, and resulted in patient becoming dependant for his activities of daily living within the next year. There was a history of a traumatic head injury 13 years ago, requiring surgery, after which he recovered without significant deficits. On examination, the HMSE score was 13. Cognitive examination demonstrated inattention, impaired new learning ability, visuospatial and executive dysfunction. The examination revealed impaired anti-saccades, dystonia, rigidity, and asymmetric bradykinesia. Investigations for reversible causes of dementia were negative. Serial neuroimaging revealed progressive white matter hyperintensities in bilateral frontoparietal region as shown in Figure 6. WES revealed a ClinVar-reported (accession: VCV000870766.16) missense VUS NM_005211.4:c.658G>A (NP_005202.2:p.Ala220Thr) in the *CSF1R* gene (NC_000005.10:g.150078183C>T; rs757109045) in the heterozygous state in the proband. The NP_005202.2:p.Ala220Thr variant is novel (not in any individuals) in 1kG All. The p.Ala220Thr variant is observed in 7/30,782 (0.0227%) alleles from individuals of gnomAD South Asian background in gnomAD and 9 individuals in the heterozygous state of an unrelated phenotype in the in-house database. A small physicochemical difference between alanine and threonine is observed, which is not likely to impact secondary protein structure as these residues share similar properties. The gene *CSF1R* has a low rate of benign missense variation as indicated by a high missense variant Z-Score of 1.57. The gene *CSF1R* contains 28 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. In addition, the clinical phenotype of the proband matches completely with that of the disorder caused by pathogenic variants in the *CSF1R* gene.

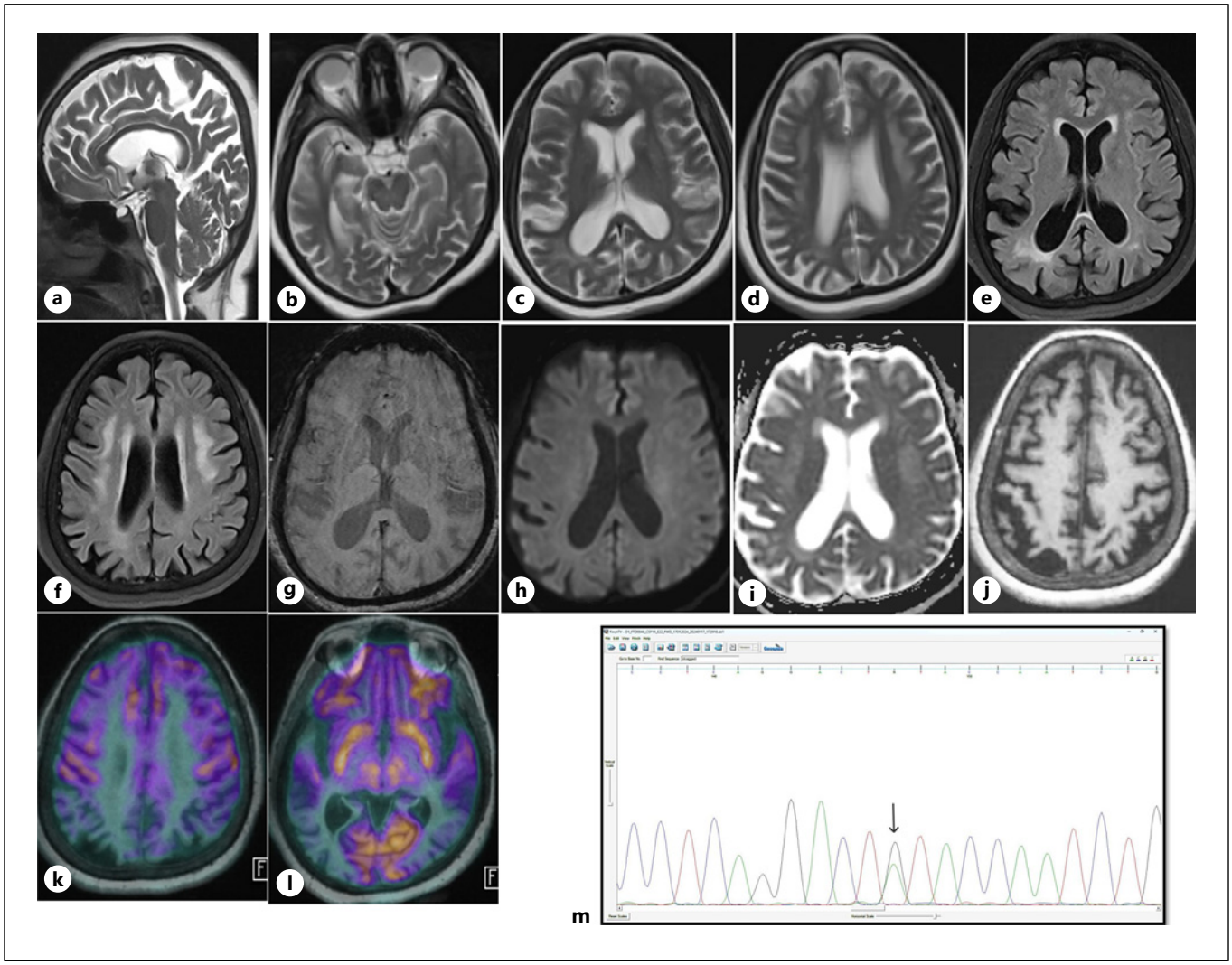

Fig. 5. a Sagittal T2-weighted image reveals moderate cerebral atrophy. **b–d** Axial T2-weighted images show moderate frontal and parietal atrophy with mild temporal atrophy and relative occipital sparing. **e**, **f** Axial FLAIR images reveal frontoparietal periventricular and deep white matter hyperintense signal changes. **g** SWI demonstrates no blooming. **h**, **i** DWI has no evidence of diffusion restriction. **j** T1-weighted multiplanar imaging shows parietal predominant atrophy. **k**, **l** PET imaging shows hypometabolism in the bilateral temporoparietal and lateral occipital regions. **m** Sanger sequencing electropherogram showing heterozygous variant at c.2768A>G position in CSF1R mutation of the proband's sample.

Hence, this variant has been classified as Uncertain Significance (PP2 PP4_Moderate). Sanger sequencing electropherogram confirmed the heterozygous variant at c.658G>A (p. Ala220Thr) in the proband's sample (Fig. 6).

Discussion

The clinical and genetic spectrum of white matter diseases due to primary microgliopathies is expanding. In this clinical case series of degenerative dementias linked to mutation in genes expressed in microglial cells, we have highlighted the spectrum of phenotypes associated with primary microgliopathies.

Our study highlights 5 cases of dementia with 4 patients presenting as FTD and one with features of atypical Alzheimer's disease from a large Indian cohort of cognitive disorder registry of subjects with dementia who subsequently had evidence of primary microgliopathy and white matter disease, as evidenced by WES and clinical imaging. All three pathogenic variants of *TREM2*, *TYROBP*, and *CSF1R* reported are novel, and *TREM2*

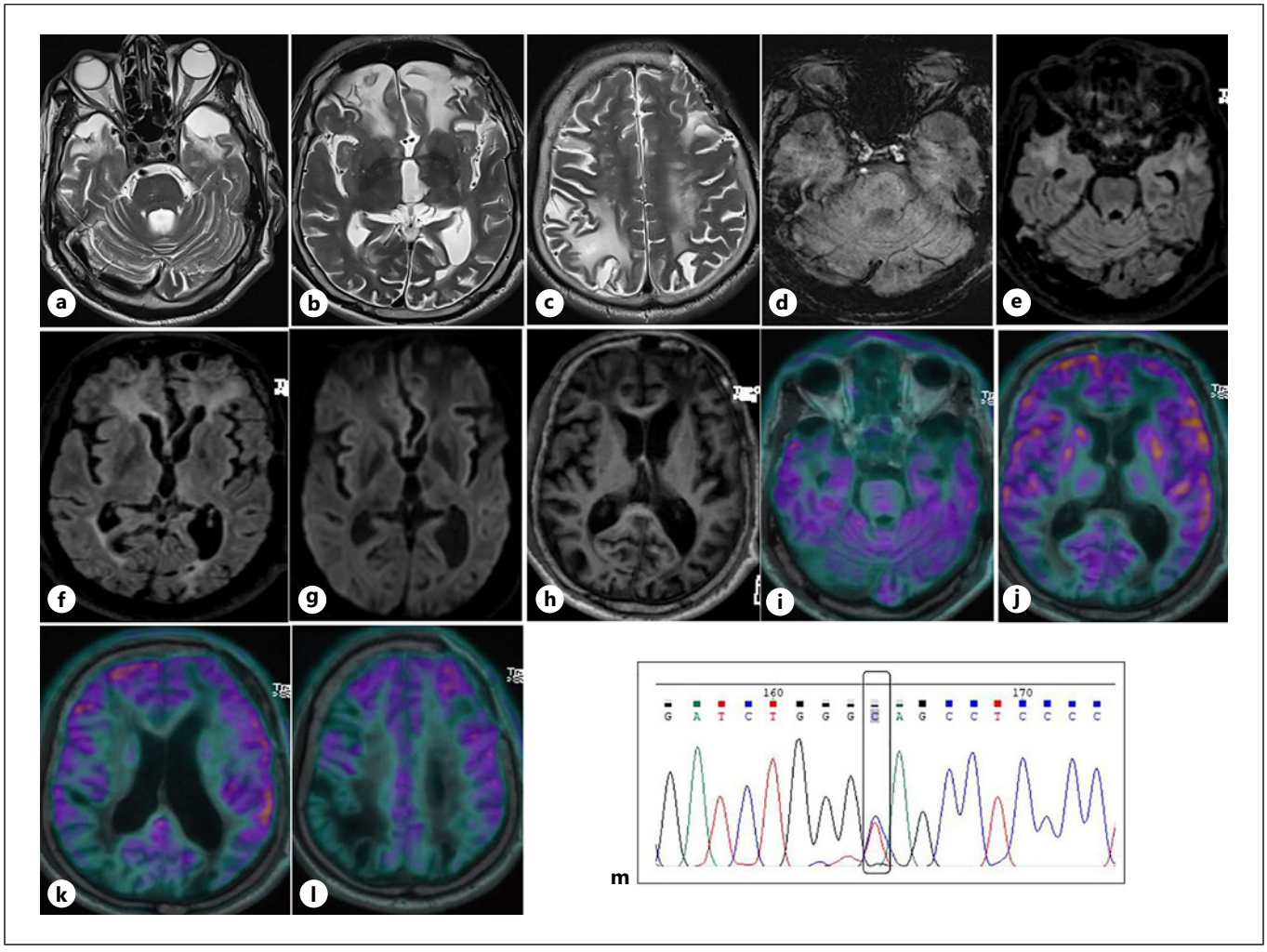

Fig. 6. a–c Axial T2-weighted images show gliosis at bilateral temporal poles, basifrontal regions, left superior frontal and right superior parietal lobule region with frontal depressed fracture on the left side. **d** Haemorrhagic residue is seen in these locations on SWI. **e**, **f** Gliosis is demonstrated on FLAIR images in these locations. **g** No diffusion abnormality is seen. **h** T1-weighted image shows mild diffuse cerebral atrophy. **i–l** PET images show hypometabolism in the gliotic foci. **m** Sanger sequencing electropherogram confirmed heterozygous variant at c.658G>A (p. Ala220Thr) in CSF1R mutation of the proband's sample (Fig. 6).

(without osseous changes) and *TYROBP* in dementia are described for the first time from a single centre in the Indian subcontinent. There are a few case reports of *CSF1R* reported in the literature from India, but there is no published literature where *CSF1R* is associated with an atypical AD phenotype so far [28–32].

TREM2 is an immune receptor found on myeloid lineage cells, forms a receptor-signalling complex with protein tyrosine kinase binding protein, and causes phagocytosis. Heterozygous variants including R47H and R62H are risk factors for Alzheimer's disease, whereas homozygous loss of function was found in families with the rare recessive NHD [33–35]. There are also reports of behavioural variant and language variants of FTD associated with *TREM2* mutation [36]. Rare variants like p.R47H, T66M, T96K, p. T96K, p. L211P, Q33X, and S116C mutations represent candidates for FTD risk [18, 36–41].

NHD is characterized by early-onset progressive dementia, bone cysts, and pathological bone fractures [5, 6, 11]. Our case 1 illustrates an interesting presentation of *TREM2* as FTD without osseous changes. To date, biallelic *TREM2* mutations have only been described in ten families diagnosed with FTD without the PLOSL bone phenotypes from Turkey, Lebanon, Columbia, Malaysia, and Singapore [42–46]. The case we report is the first case

of *TREM2* homozygous mutation masquerading as behavioural variant FTD (without bony changes) from India and South Asia, the second case of homozygous mutation of *TREM2* from India and emphasizes that genetic screening should be performed in FTD with atypical phenotypes, characterized by early onset, early parietal and hippocampal deficits, the presence of seizures and parkinsonism, extensive white matter lesions, and corpus callosum thinning [46, 47].

The novel homozygous c.40 + 1G>A variant *TREM2* in our cohort presented with early-onset bvFTD, white matter signal changes, and thin corpus callosum. The navigational difficulties and parietal atrophy are well described in the patient with *TREM2* mutation, and the associated clinical-genetic features provide insight into the pathogenic role of *TREM2* in neurodegenerative disorders and its varied phenotypes. The c.377T>G mutation (*TREM2* gene of exon 2) in the homozygous state presenting as bvFTD and bony cysts has been previously reported as the second case of Nasu-Hakola from India [47].

TYROBP (also known as DAP12) is a transmembrane signalling protein [48, 49]. Recessive mutations in *TYROBP* have been described as causative of NHD [50, 51]. *TYROBP* also regulates macrophage proliferation through *CSF1R* and can further explain the phenotypes observed in both NHD and ALSP [52]. *TYROBP* may also be involved in Aβ turnover and the differentiation and function of osteoclasts. In NHD, no changes in amyloid plaques have been observed with loss-of-function mutation of *TYROBP* [53].

Cases of NHD without apparent skeletal symptoms occur in *TREM2* mutations, but not in *TYROBP* [8]. The initial cases of *TYROBP* due to the deletion or non-functional mutations have been reported mainly from Japan, Finland, United Kingdom, etc. [54, 55]. In an earlier case report on the first case of NHD from India, the homozygous nonsense variation in exon 2 of the *TYROBP* gene (PGln28Ter) was detected in a younger brother of a patient with NHD [56].

Our case 2 with *TYROBP* with FTD phenotype had significant extrapyramidal features, spasticity, severe dysphagia, and mutism which have not been so far reported commonly in *TYROBP*. The patient had NM_003332.4 (*TYROBP*):C.82C>T (p. Q28*), a stop gained variant in the homozygous state in exon 2 of 5. The p. Gln28Ter variant is novel in 1kG All, gnomAD and in the in-house database. Hence, case 2 in our cohort represents the first confirmed Pathogenic variant of *TYROBP* presenting as NHD, FTD and bony cysts from India.

This reported case series of 3 patients of *CSF1R*-related leukoencephalopathy or ALSP highlights the variability in the phenotypic presentation of *CSF1R* mutation: cases 3 and 5 presented with FTD and case 4 presented as atypical AD. Although a few Indian reports of dementia patients with *CSF1R* mutation are published, the genetic novelty in case 3 was pathogenic intronic 14 mutation and the clinical novelty was that aphasia was prominent in the course of illness [28, 29, 30, 31, 32, 57, 58, 59, 60]. Aphasia is described only in 19% of the series in literature [27, 57].

Presently, a total of 115 C*SF1R* mutation sites have been identified worldwide in approximately 300 cases reported [57, 58, 59, 60] and only 13 intronic pathogenic variants have been reported in the literature. Although several variants of *CSF1R* are implicated as a risk for AD, phenotypically atypical variant AD confirmed by the PET MR hypoperfusion pattern in case 4 is a rarity [16].

Brain parenchymal calcifications mainly in the frontal and periventricular areas in CT (75%), T2, and FLAIR hyperintense lesions in the periventricular, deep, and subcortical bifrontal or bifrontoparietal white matter with central atrophy in MRI are the classical findings [57, 61]. In addition, thinning of the corpus callosum and diffusion-restricted lesions in the white matter are hallmarks, as was demonstrated in cases 3 and 5. The functional trio of *CSF1R*, *TREM2*, and *TYROBP* plays a crucial role in microglial population dynamics, viability and survival [62]. The prospect of targeting microglia for the treatment of neuro-psychiatric disorders and degenerative dementias is intriguing [1].

Transient microglial depletion by clodronate liposomes or *CSF1R* inhibitors has been shown to reduce disease progression in mouse models of neurodegenerative diseases, such as Alzheimer's disease [10]. Several studies have shown the microglia-mediated regulation of plaque deposition and/or p-tau propagation by the *CSF1R* inhibitor [63].

With recent advances in the role of allogenic hematopoietic stem cell transplantation (HPSCT) in microgliopathy and the narrow therapeutic window due to rapid progression, early diagnosis of these primary microgliopathies becomes very crucial. Hematopoietic stem cell transplantation in microglial leukoencephalopathies has been used in a few clinical trials. The beneficial effects of immunosuppressive therapy have also been reported [63, 64, 65, 66, 67].

Conclusion

Our study enriches the spectrum of genetic variants implicated in dementia and provides the basis for exploring the complex molecular mechanisms like primary

microgliopathy as an underlying cause of inflammation and neurodegeneration. Our reports suggest that genetic testing should be offered to all patients who develop early-onset dementia especially with emerging therapeutic options.

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Statement of Ethics

This study protocol was reviewed and approved by the Institutional Ethical Committee No./NIMHANS/(BS & NS Division)/24th meeting/2020 dated June 11, 2020. Written informed consent was obtained from the patient/legal guardian for participation in the study. No vulnerable patients were included in this study.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

Dr. Subasree Ramakrishnan, Dr. Arun Gokul Pon, Dr. Sandeep, Keerthana BS, Sandeep, Susan Bosco, Faheem, and Dr. Gautham Arunachal were involved in the conception and design of the study. Dr. Arun, Dr. Susan Bosco, Dr. Gautham Arunachal, Dr. Karthik Kulanthaivelu, Dr. Subasree Ramakrishnan, Dr. Faheem Arshad, and Dr. Suvarna Alladi were involved in the acquisition of data. Subasree Ramakrishnan, Subhash Chandra Bose, Hariharakrishnan Chidambaram, Faheem Arshad, Karthik Kulanthaivelu, Gautham Arunachal, and Suvarna Alladi were involved in drafting the work, revision, and approval of the paper.

Data Availability Statement

Research data are not publicly available on legal or ethical grounds. All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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