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Primary microgliopathy presenting as degenerative dementias: A case series of novel gene mutations from India

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Running title -Primary microgliopathy and dementia

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Structured 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 are disorders 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*), Colony-stimulating factor 1 receptor (*CSF1R*) are genes implicated in primary microgliopathy. Clinical manifestations of primary microgliopathy are myriad ranging from neuropsychiatric syndrome, motor disability, gait dysfunction, ataxia, pure dementia, frontotemporal dementia, Alzheimer dementia and so on. It becomes imperative to establish diagnosis of microgliopathy masquerading as degenerative dementia, especially with promising therapies on horizon for the same. We aim to describe a case series of subjects with dementia harbouring novel genes of primary microgliopathy, along with their clinical, neuropsychological, and 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 clinic-genetic research on Dementia subjects and was approved by the institutional ethics committee. All patients underwent detailed assessment including socio demographic 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: 100 patients of dementia underwent genetic analysis using whole exome sequencing 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. TREM 2 and *TYROBP* presented as frontotemporal syndrome whereas *CSF1R* presented as frontotemporal syndrome and Alzheimer dementia.

Conclusion: WES has widened the spectrum of underlying neuropathology of degenerative dementias and diagnosing primary microglial dysfunction with emerging therapeutic options is of paramount importance Cases of primary microgliopathy due to Novel mutations in *TREM2*, *TYROBP* and *CSF1R* with 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 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, and leukodystrophies related to variants in the Negative regulator of reactive oxygen species (NRROS) or Pseudo TORCH syndrome. [5-11]. These are considered as Type-I microgliopathies. [7]

Microglia are associated with a set of patterns recognizing receptors (PPRs) at the cell surface [12,13]. Mutations in these microbial sensome genes also lead to primary microgliopathies by promoting neurodegenerative diseases such as Alzheimer's disease and Frontotemporal dementia in type-II microgliopathies. [7,14] Details are shown as flowchart in Fig.1.

Mutations in microglial *CSF1R*, *TREM2*, *TYROBP* genes can cause leukodystrophy and frontotemporal dementia like clinical syndromes, whereas those due to *TREM2* can be a risk factors for Frontotemporal dementia (FTD), FTD like syndromes and Alzheimer 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. [9,10,11,15,16].

Nasu-Hakola disease (NHD) or (polycystic Lipomembranous osteodysplasia with sclerosing leukoencephalopathy; PLOSL) and adult-onset leukoencephalopathy with axonal spheroids (ALSP) exemplify how intrinsic microglial dysfunction could cause neurological or psychiatric diseases. [5,6]. Nasu–Hakola disease, is caused by mutations of genes *TYROBP* or *TREM2* (PLOSL1 and PLOSL2, respectively). The characteristic symptoms include multiple bone cysts and fractures, and frontal lobe dementia [5,6,7,8].

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 rarely heterozygous variants in TREM-2 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 frontotemporal dementia (FTD), pure dementia without bony changes and in Nasu-Hakola disease. [18] In AD, TREM-2 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 behavioral frontotemporal dementia-like symptoms and AD. The motor symptoms include parkinsonian symptoms, pyramidal, bulbar signs and ataxia and is often misdiagnosed [20-22].

In this report, we describe a case series of patients clinically presenting with dementia confirmed on whole exome sequencing 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, are reported for the first time in the Indian context.

Patients and Methodology

A total of 100 subjects with dementia, diagnosed with Frontotemporal Dementia (FTD) (n=85) based on standard International consensus criteria for behavioural variant FTD (bvFTD), Progressive Primary Aphasia (PPA) (Rascovsky et al., 2011; Gorno-Tempini et al., 2011) and as Alzheimer's Dementia (n=15) based on National Institute of Neurological and Communicative disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria for Alzheimer's dementia, were recruited from the Cognitive Disorders Clinic (CDC) in a university referral hospital in South India. These patients being part of an ongoing clinico-genetic study, underwent Whole Exome Sequencing (WES) with Informed consent.

The age at enrolment of the 100 patients ranged between 32-83 years with an average of 58.8 years. This group consisted of 55% males and disease duration varied between 6 months to 10 years, with an average of 2.6 years duration. 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 standard diagnostic criteria and having additional MRI features like white matter hyperintensity, basal ganglia calcification and thinning of corpus callosum, along with presence of genetic variants causing primary microgliopathy forms the current study cohorts. All other FTD and AD cases with classical MRI features and harbouring other genetic variants were excluded from the study.

All subjects enrolled for the study underwent detailed assessment including socio-demographic details, family history using 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 (NPI) scores and

severity using Clinical Dementia Rating (CDR) Scale score were performed. [24,25,26] Participants also underwent structural imaging using a 3 Tesla magnetic resonance imaging (MRI). Testing for secondary causes of dementia including thyroid functions, vitamin B12 levels, Human immune deficiency virus (HIV), Venereal disease research lab test (VDRL), autoimmune profile, cerebrospinal fluid analysis (CSF) was carried out in all patients, to exclude other causes.

Genetics:

Subjects with dementia consenting for blood sampling, underwent genetic testing by whole exome sequencing (WES). Genomic DNA was extracted from peripheral blood sample using Qiagen kit (QIAamp DNA Kit). The DNA quantity and quality was assessed by Nanodrop spectrophotometer and by Agarose gel electrophoresis. The quality passed DNA samples (criteria DNA yield: 20ng/ µl; A260/280: 1.8-1.9; A260/230: 2-2.5) were additionally quantified by Qubit Fluorometric method (Thermo Fisher scientific). Whole exome sequencing 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 Illumina NextSeq 550 platform using 2x150 bp read chemistry according to manufacturer's instructions. Reads from the sequence output were aligned to the human reference genome (GRCh38) using the Burrows-Wheeler Aligner (BWA). Variants to the reference were called using the Genomic Analysis Tool Kit (GATK). The variants were annotated and filtered using the Golden Helix VarSeq analysis workflow implementing the ACMG guidelines for interpretation of sequence variants. This included comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes.

Project Consortium's publication 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 2015 American College of Medical Genetics (ACMG) guidelines. Pathogenicity of the clinically relevant variants was further confirmed by genotype-phenotype correlation and by 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 QIAamp DNA Minikit. Specific primers were designed using primer 3, checked for primer dimer and self-dimers using primer analyzer (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. 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 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 visual inspection of electropherogram, as well as comparing with the reference sequence and confirming the location of the mutation.

Results:

100 patients diagnosed to have dementia underwent genetic analysis using whole exome sequencing during the study period. Data from each sample had a mean depth ranging from 70x to 90x.Total number of targets was 214,702. On target bases were covered at least 1x ranging between 98-99%; 20x ranging between 93-96% and ranging 24-27% at 100x. 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 20x or more and with GQ (Phred quality scores) of 20 or more was 60,000-70,000. Variants were further filtered as described in methodology. 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 variant of uncertain significance in Primary microgliopathy related genes are depicted in Table1.

Case 1: A 45-year-old lady, presented with cognitive decline, personality change and behavioural disturbances in the form of apathy, disinhibition, overfamiliarity, a bizarre eating pattern, hyper-orality and sweet preference for five years. She had reduced attention and recent memory disturbances. She had poor personal hygiene, frequent wandering, inattentiveness, loss of empathy and problems with planning and judgement. There was gradual decline of speech output and verbal perseverations. Subsequently, she developed navigational difficulty, difficulty in dressing and in recognizing currency notes and coins. She gradually worsened and over the next three years, 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 Fig.2.

On neurological exam, the lady was disoriented and inattentive. She had non-fluent aphasia, frontal release signs, along with utilization behaviour and environmental dependency. ACE-R score was three and Clinical Dementia Rating score was three. Investigations for young onset dementia were negative.

A clinical diagnosis of behavioural variant FTD 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. There were T2/FLAIR hypo intensity in the bilateral putamen, globus pallidus, subcortical FLAIR intensities in the frontal lobe along with a striking thinning of corpus callosum as shown in Fig.2. Whole exome sequencing revealed a pathogenic splice donor variant NM_018965.4:c.40+1G>A in *TREM2* gene (NC_000006.12:g.41163042C>T; rs766712618) in 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 gnomAD South Asian background in gnomAD in only heterozygous state and 2 other individuals with similar phenotype in homozygous state in our inhouse 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 Fig.2. On telephonic follow up, her family reported that she had expired 1.5 years after diagnosis.

Case 2: A 43-year-old lady, presented with four years' history of insidious onset progressive behavioral symptoms in the form of compulsive behavior 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, 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 Fig. 3

On examination, she was found to have 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 were negative. MRI brain showed fronto-parietal 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 Fig.3

Whole exome sequencing revealed a ClinVar reported pathogenic (Accession: VCV001935007.2) stop gained NM_003332.4: c.82C>T; NP_003323.1: p. Gln28Ter variant in *TYROBP* gene (NC_000019.10: g.35907742G>A) in 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 Fig 3.

Case 3: A 54-year-old lady, presented with rapidly progressive cognitive decline, with predominantly language difficulties associated with behavioural disturbances characterised by aggressiveness, anger outbursts and poor

self-care of 1.5 years' duration. She developed slowness of gait, incontinence and became dependent on all activities of daily living over the next three months. There was no family history of 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. Clinical Dementia Rating score was 3 and Neuropsychiatry Inventory score was 6. She had prominent language disturbances and the Aphasia Quotient was 50.1 and a provisional clinical diagnosis of primary progressive aphasia was made. 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, DWI hyper intense signal changes in white matter periventricular region, centrum semiovale, corona radiata and corpus callosal atrophy with diffusion restriction in splenium along with diffuse atrophy as shown in Fig 4. Whole exome sequencing 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 heterozygous state in the proband. The c.1969+1G>A variant is novel (not in any individuals) in 1kG All, gnomAD as well as in our inhouse 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 *CSF1R* gene. For these reasons, this variant has been classified as Pathogenic (PM2 PVS1 PP5). Sanger validation confirmed the genetic variation as shown in Fig.4.

Case 4: A 56-year-old lady, presented with four years' history of episodic and recent memory loss, attention deficits, misplacing objects and repeated questioning, navigational difficulty of one and half years' duration, followed by a one-year history of difficulty in recognizing and using common objects, suggestive of apperceptive agnosia. Her Hindi mental state score was 18 and she had attention 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 Alzheimer Dementia (AD) was considered. PET MR showed hypometabolism in temporoparietal and posterior cingulate region as shown in Fig. 5. MR images also showed bifrontal asymmetric T2-weighted/FLAIR hyperintensities in the sub cortical deep and periventricular white matter with corresponding T1 hypointensities. Whole exome sequencing revealed a novel Likely pathogenic missense variant NM_005211.4:c.2768A>G (NP_005202.2:p.Tyr923Cys) in *CSF1R* gene (NC_000005.10:g.150054220T>C) in heterozygous state in the proband. The NP_005202.2:p.Tyr923Cys variant is novel (not in any individuals) in 1kG All, in gnomAD as well as in our inhouse 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 be damaging by both SIFT and PolyPhen2. The gene *CSF1R* has a low rate of benign missense variation as indicated by a high missense variants 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 *CSF1R* gene. For these reasons, this variant has been classified as Likely Pathogenic (PM2 PP2 PP3 PP4_Moderate) with ClinVar 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 man presented with memory disturbances, disinhibitory behaviour, way finding difficulty and decreased interaction of three years' duration, followed by slowness of activities over two and half years. The symptoms rapidly worsened and he became dependant for his activities of daily living within the next year. There was history of a traumatic head injury 13 years ago, requiring surgery, after which he recovered without significant deficits. On examination, HMSE was 13. Cognitive examination demonstrated inattention, impaired new learning ability, visuospatial and executive dysfunction. 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 fronto-parietal region as shown in Fig.6. Whole exome sequencing revealed a ClinVar reported (Accession: VCV000870766.16) missense variant of uncertain significance NM_005211.4: c.658G>A (NP_005202.2:p.Ala220Thr) in *CSF1R* gene (NC_000005.10: g.150078183C>T; rs757109045) in 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 heterozygous state of unrelated phenotype in our inhouse database. There is a small physicochemical difference between alanine and threonine, 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 variants 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 *CSF1R* gene. For these reasons, this variant has been classified as Uncertain Significance (PP2 PP4_Moderate). Sanger sequencing electropherogram confirmed 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 adult leukodystrophies linked to mutations in genes expressed in microglial cells, we have highlighted the spectrum of phenotypes associated with primary microgliopathies.

Our study highlights five cases of dementia with four presenting as frontotemporal dementia and one with features of atypical Alzheimer disease from a large Indian cohort of cognitive disorders registry of subjects with dementia who subsequently, had evidence of primary microgliopathy and white matter disease, as evidenced by WES and clinical imaging .All the three pathogenic variants of *TREM2*,*TYROBP* and *CSF1R* reported are novel and *TREM2* (without osseous changes)and *TYROBP* in dementia are first time described from a single center in Indian subcontinent. There are few case reports of *CSF1R* reported in literature from India and there is no published literature where *CSF1R* is associated with atypical AD phenotype so far. [28-32]

TREM2 is an immune receptor found on myeloid lineage cells and forms a receptor-signaling 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 Nasu- Hakola disease. [33-35]. There are also reports of behavioral variant and language variants of FTD associated with *TREM2* mutation. [36] Rare variants like p.R47H, T66M, T96K, p. T96K, p. L211P, Q33X, S116C mutations represent candidates for FTD risk. [36-39,40-42]

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 frontotemporal dementia without the PLOSL bone phenotypes from Turkey, Lebanon, Columbia, Malaysia and Singapore.[43-47].The case we report is the first case of *TREM2* homozygous mutation masquerading as behavioral variant FTD (without bony changes) , from India and South Asia, and second case of homozygous mutation of *TREM2* from India and emphasizes that genetic screening should be performed in FTD with atypical phenotypes, characterized by a very young age at onset, early parietal and hippocampal deficits, the presence of seizures and parkinsonism, extensive white matter lesions and corpus callosum thinning.[47,48]

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. Her navigational difficulties and parietal atrophy are well described in *TREM2* mutation and the associated clinical-genetic features provide insight into the pathogenic role of *TREM2* in neurodegenerative disorders and its varied phenotypes. c.377T>G mutation (*TREM2* gene of Exon 2) in the homozygous state presenting behavioural variant FTD and bony cysts have been previously reported as second case of Nasu Hakola from India. [48]

TYRO protein tyrosine kinase binding protein (*TYROBP*, also known as DAP12) is a transmembrane signaling protein. [49,50] Recessive mutations in *TYROBP* have been described as causative of Nasu-Hakola disease. (NHD). [51,52]. *TYROBP* also regulates macrophage proliferation through *CSF1R*, and can further explain the phenotypes observed in both in NHD and ALSP. [53] *TYROBP* may also be involved in Aβ turnover and in the differentiation and function of osteoclasts. In NHD, no changes in amyloid plaques have been observed with loss-of-function mutation of *TYROBP*. [54]

Nasu-Hakola disease cases without apparent skeletal symptoms occur in *TREM2* mutations, but not *TYROBP*. [8] Initial cases of *TYROBP* due to deletion or non- functional mutations have been reported mainly from Japan, Finland, UK etc. [55,56]. In a previous case report of first case of Nasu Hakola disease from India, homozygous nonsense variation in exon 2 of the *TYROBP* gene (PGln28Ter) was detected in a younger brother of a patient with NHD. [57].

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

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

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

Brain parenchymal calcifications mainly in frontal and periventricular areas in CT (75%), T2 and FLAIR hyperintense lesions in the periventricular, deep, and subcortical bifrontal or bifronto-parietal white matter with central atrophy in MRI are the classical findings [58,61] In addition, thinning of the corpus callosum and diffusion-restricted lesions in the white matter are hallmarks, as was demonstrated in the 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 have shown to reduce disease progression in mouse models of neurodegenerative diseases, such as Alzheimer's disease [10]. Several studies have shown microglia-mediated regulation of plaque deposition and/or p-tau propagation by *CSF1R* inhibitor. [63]

With recent advances in role of allogenic hematopoietic stem cell transplant in microgliopathy and narrow therapeutic window due to rapid progression, diagnosing these primary microgliopathies early becomes very crucial. Hematopoietic stem cell transplantation. (HSCT) in “microglial leukoencephalopathies have been used in a few clinical trials. Beneficial effects of immunosuppressive therapy have also been reported. [63-67].

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 underlying cause for 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

Study approval statement

This study protocol was reviewed and approved by the Institutional Ethical Committee No./NIMHANS/ (BS & NS Division)/24th meeting /2020 dated 11.6.2020

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, Dr Gautham A were involved in Conception and design of the study. Dr Arun, Dr Susan Bosco, Dr Gautham Dr Karthik K, Dr Subasree, Dr Faheem, Dr Suvarna Alladi were involved in Acquisition of data, Subasree R, Subhash Chandra

Bose, Hariharakrishnan Chidambaram, Faheem, Karthik K, Gautham A, Suvarna Alladi were involved in drafting the work, revision, and approval of the paper

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Description of Figures:

Fig.1: Types of Microgliopathies

Fig. 2:

a: Roman numerals (I, II, III, IV) indicate the generations, 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: 48y); 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,d,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.

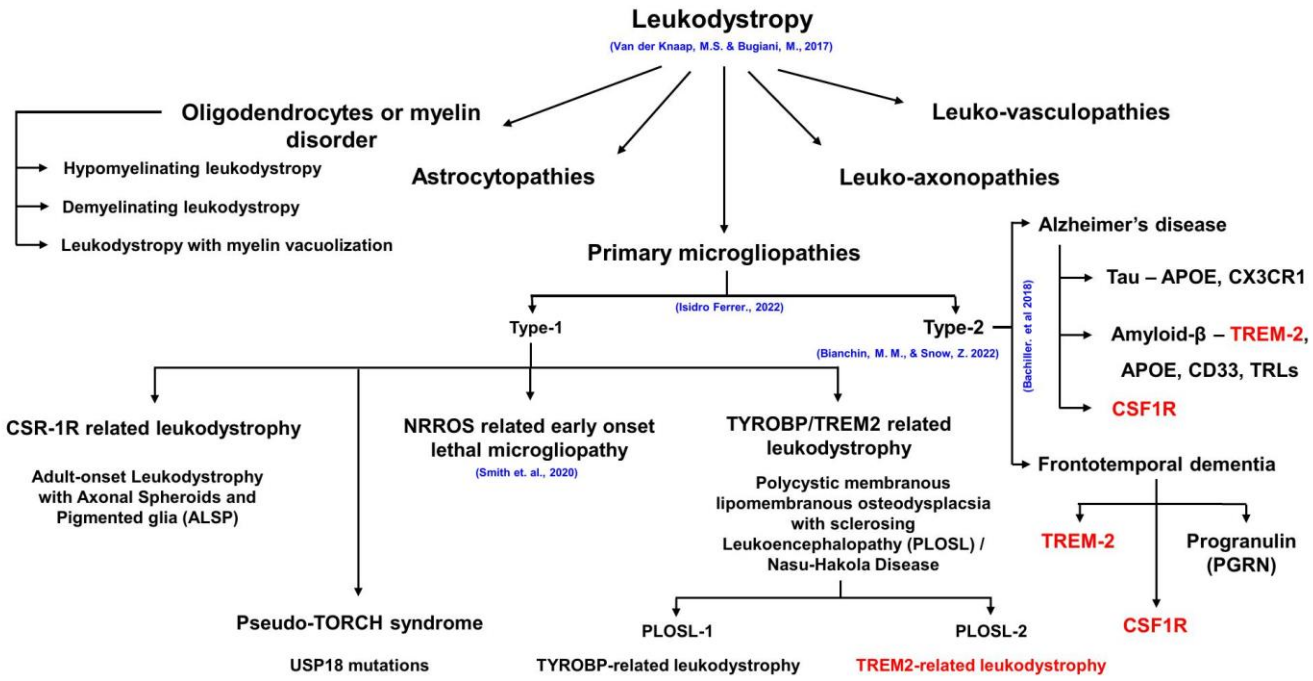
Fig. 3:
a: family tree: Roman numerals (I, II, III, IV) indicate the generations, the numbers (1-17) indicate individuals in each generation. The proband is indicated by the arrow; black filled symbols represent subjects affected by: (III.7: psychiatric illness: Age at onset: 43 y); diagonal lines indicate 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 gain 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 images 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: Last image is 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.

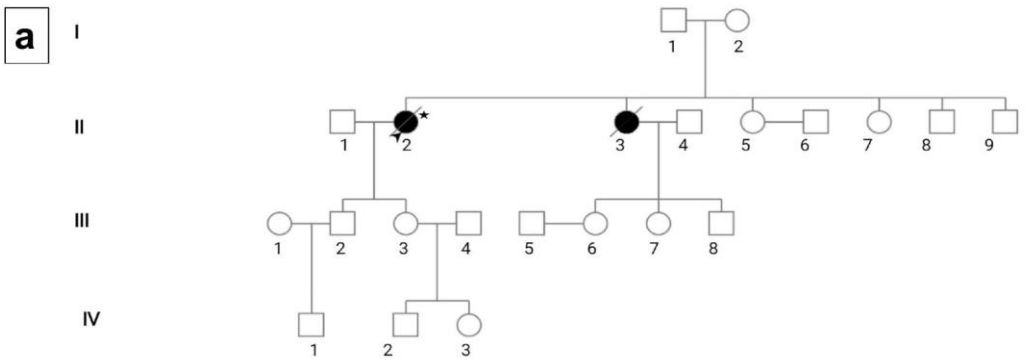
Fig. 4:
a: Chromatogram of the proband highlighted at Intron 14 of CSF1R (between Exons 14 and 15 of 22) ;(Transcript ID: NM_05211.4).
The proband has been identified with heterozygous targeted mutation in intron 14 of CSF1R gene (highlighted in blue).
b: T1 weighted multiplanar imaging (J-K) shows frontoparietal predominant atrophy and ventricular horn prominence.
c: Axial FLAIR images (E, F) 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.

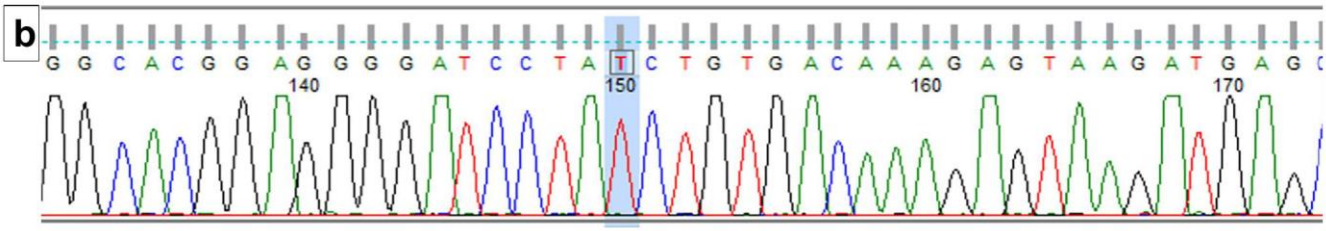
Fig. 5:
a: Sagittal T2 weighted image reveals moderate cerebral atrophy.
b, c, d: Axial T2 weighted images show moderate frontal and parietal atrophy with mild temporal atrophy and relative occipital sparing.
e, f: Axial FLAIR images reveal frontoparietal periventricular and deep white matter hyperintense signal changes.

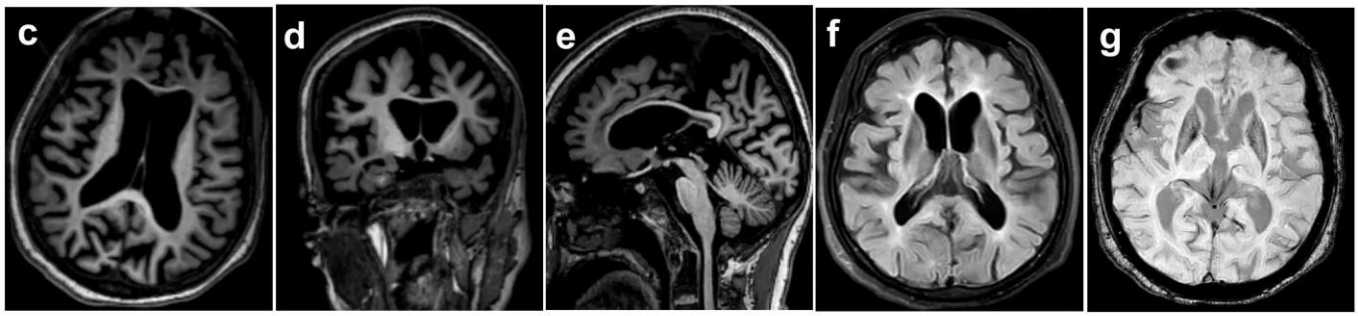
g: SWI demonstrates no blooming.
h, i: DWI has no evidence of diffusion restriction.
j: T1 weighted multiplanar imaging shows parietal predominant atrophy.
k, l: PET imaging shows hypometabolism in the bilateral temporoparietal and lateral occipital regions
m.Sanger sequencing electropherogram showing heterozygous variant at c.2768A>G position in CSF1R mutation of the proband's sample.

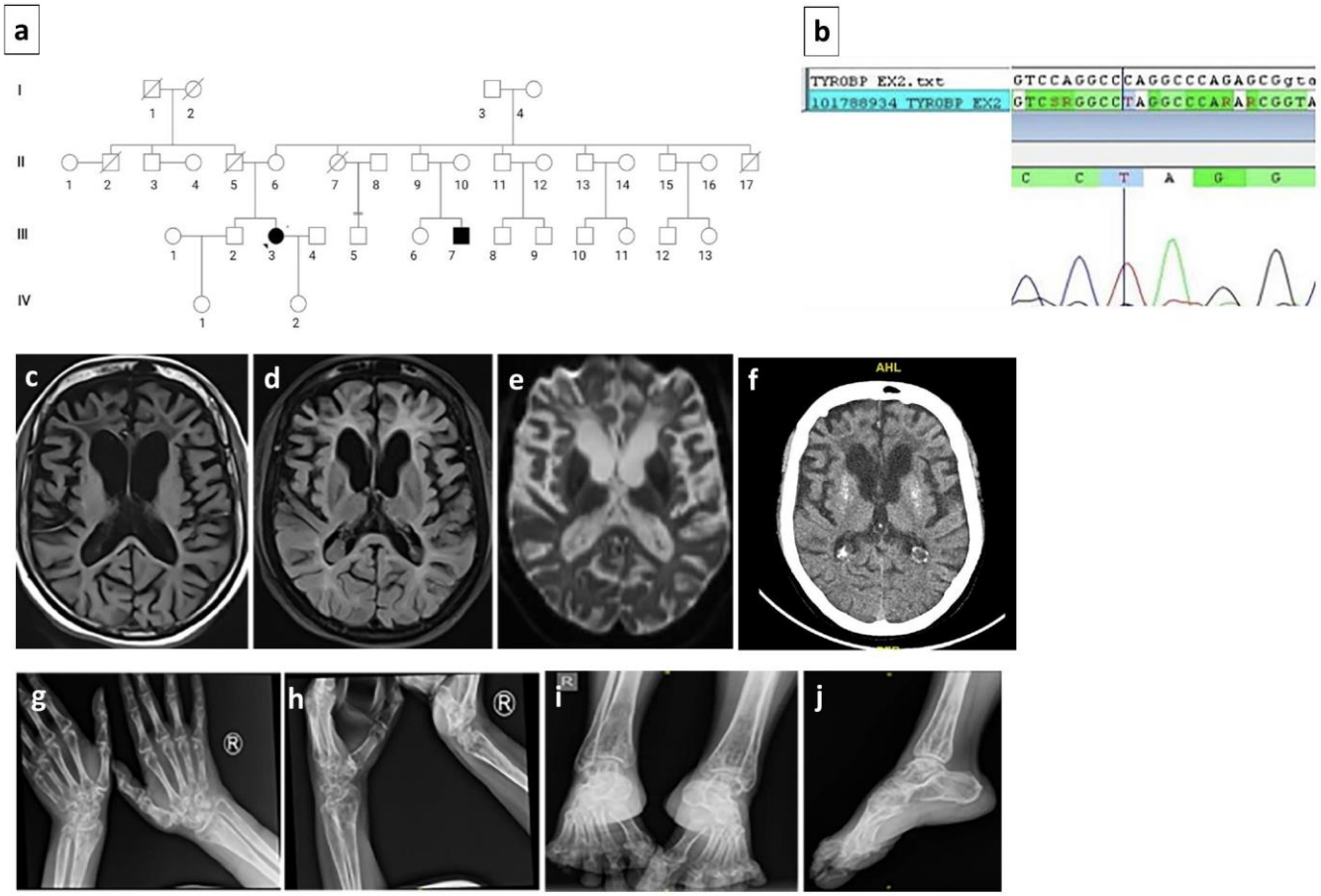
Fig. 6:
a,b,c: Axial T2 weighted images show gliosis at bilateral temporal poles, basifrontal regions, left superior frontal and right superior parietal lobule region with frontal depressed fracture on the left side.
d: Hemorrhagic residue is seen in these locations on SWI.
e,f: Gliosis is demonstrated on FLAIR images in these locations.
g: NO diffusion abnormality is seen.
h: T1 weighted image shows mild diffuse cerebral atrophy.
i, j, k, 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)

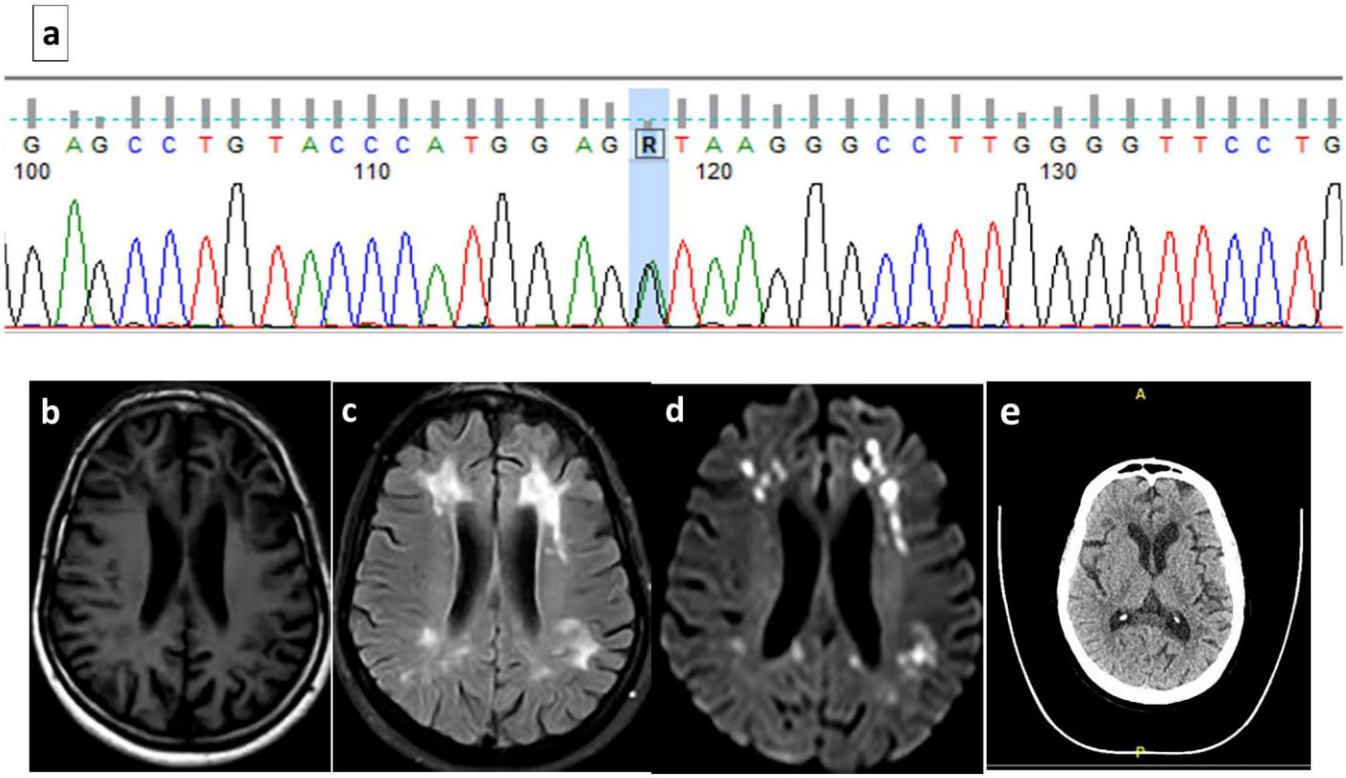


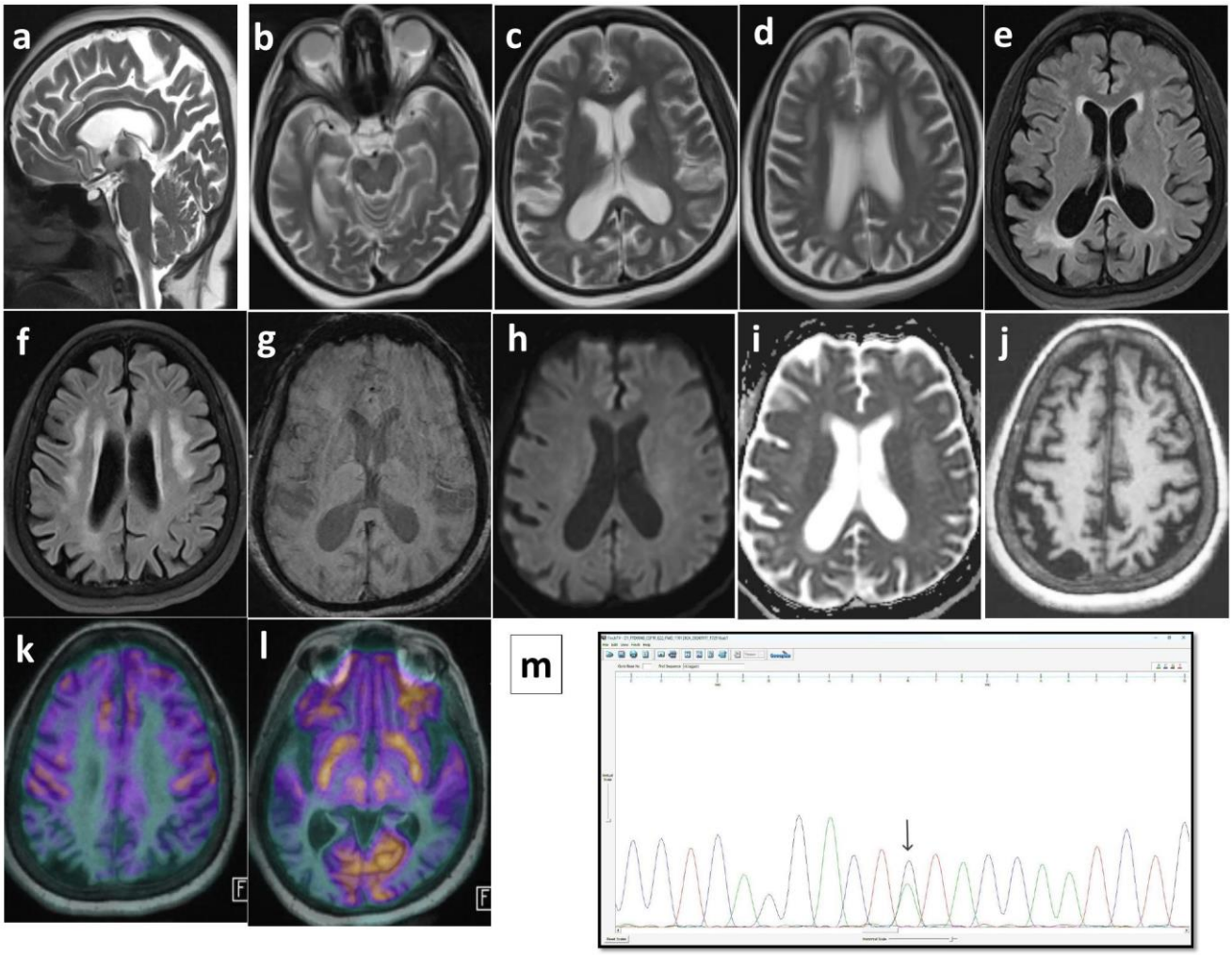












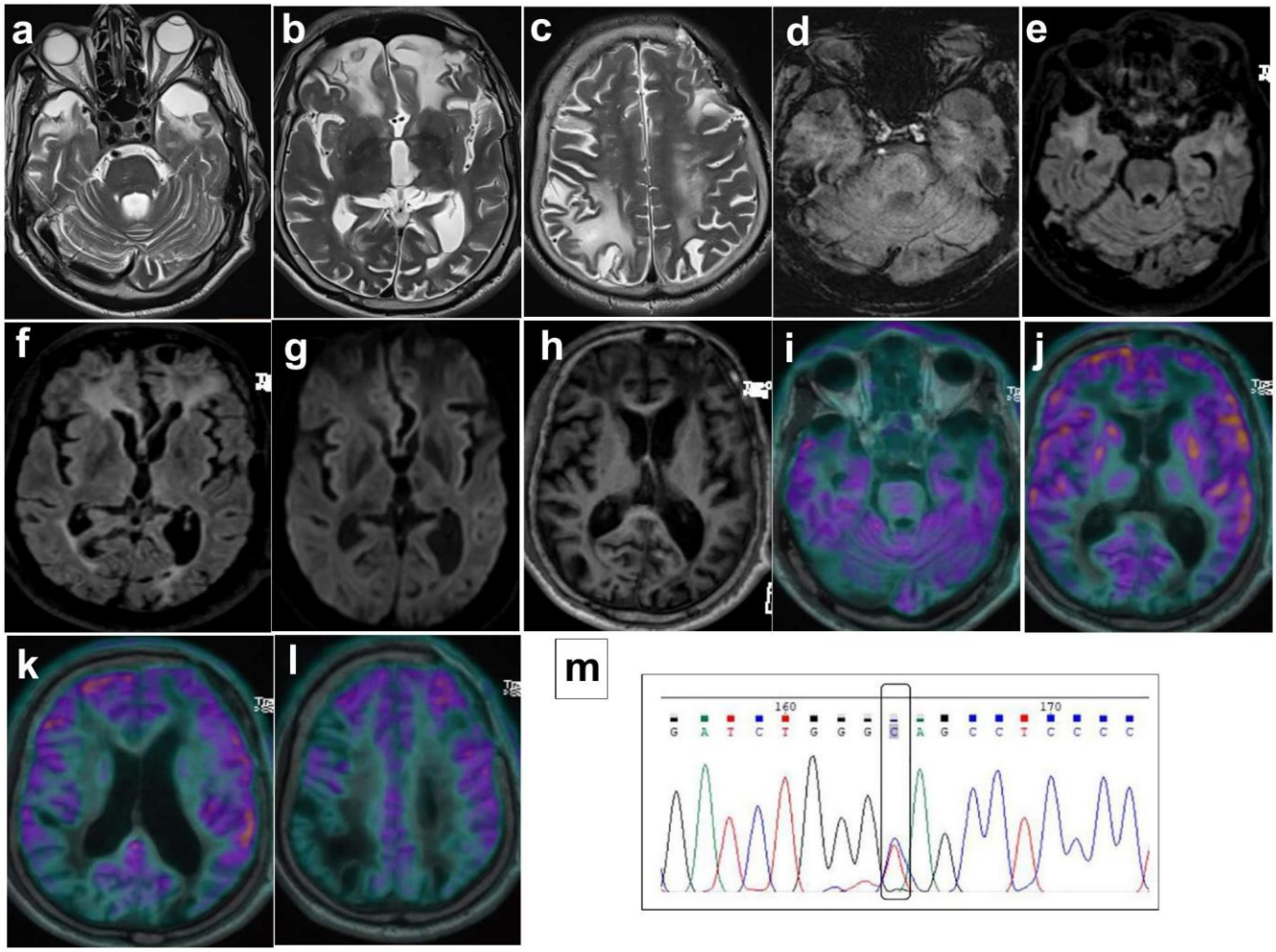


Table 1- Pathogenic, likely pathogenic and variant of uncertain significance in Primary microgliopathy related genes

Gene Symbol, Transcript & Location	**Variant (HGVS Nomenclature)**	**Genomic coordinate of the variant**	**Zygosity & Metrics (depth & Phred Quality score)**	**Effect**	**ACMG Classification**	**ClinVar Accession ID**	**Allele frequency gnomAD 4.0†**	**1KG All‡**	***In-house* database**	***In-silico* predictions MSA- SIFT**	**Poly-Phen2**	**CADD Score***
TREM2 (NM_018965.4) Intron 1	NM_018965.4:c.40+1G>A	chr6:g.41163042 C>T	Homozygous 86x 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.35907742 G>A		Stop Gained	**Pathogenic** PM2 PVS1 PP5 PP4	VCV001935007.2	2 in heterozygous state AF=0.0000318088	N	N	D	D	9.00
CSF1R (NM_005211.4) Intron 14	NM_005211.4:c.1969+1G>A	chr5:g.150060861 C>T	Heterozygous 18x/52x 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/60x 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.150078183 C>T		Missense	**VUS** PP2 PP4_Moderate	VCV000870766.17	13 in heterozygous state AF=0.0000889273	N	9 of unrelated phenotype in heterozygous state	D	D	3.27

PD, probably damaging; **D**, deleterious; **DC**, disease causing; **P**, polymorphism**; CADD**, Combined Annotation Dependent Depletion; **N**, Novel.

†**gnomAD**, gnome Aggregation Database version 4 (https://gnomad.broadinstitute.org/)

‡**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 (Combined Annotation Dependent Depletion Score:) (**https://cadd.gs.washington.edu/ developed by the University of Washington and is 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).