Original Research Article

Uncovering genetic mimics in multiple sclerosis: A single-center clinical exome sequencing study

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Abstract

Background: Multiple sclerosis (MS) shares clinical/radiological features with several monogenic diseases that can mimic MS.

Objective: We aimed to determine if exome sequencing can identify monogenic diseases in patients diagnosed with MS according to the McDonald criteria thus uncovering them as being misdiagnosed.

Methods: We performed whole exome sequencing in a cohort of 278 patients with MS, clinically or radiologically isolated syndrome without cerebrospinal fluid-specific oligoclonal bands (CSF-OCBs) (n = 228), a positive family history of MS (n = 44), or both (n = 6), thereby focusing on individuals potentially more likely to have underlying monogenic conditions mimicking MS. We prioritized 495 genes associated with monogenic diseases sharing features with MS.

Results: A disease-causing variant in *NOTCH3* was identified in one patient without CSF-OCBs, no spinal lesions, with non-response to immunotherapy, and a family history of dementia, thereby converting the diagnosis to cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Moreover, 18 patients (6.5% of total) carried variants of unclear significance.

Conclusion: Monogenic diseases being misdiagnosed as MS seem rare in patients diagnosed with MS according to the McDonald criteria, even in CSF-OCB negative cases. The detected pathogenic *NOTCH3* variant emphasizes CADASIL as a rare differential diagnosis and highlights the relevance of genetic testing in selected MS cases with atypical presentations.

Keywords: Multiple sclerosis, CADASIL, misdiagnosis, genetic mimic, genetic testing, exome sequencing

Introduction

The 2017 revision of the McDonald criteria for diagnosis of multiple sclerosis (MS) introduced the detection of cerebrospinal fluid-specific oligoclonal bands (CSF-OCBs) as a diagnostic criterion for dissemination in time.[1] Evaluation of CSF is recommended to improve diagnostic certainty and exclude differential diagnoses.[1,2] Caution is advised in cases where CSF-OCBs cannot be detected.[1] However, this does not rule out the presence of MS.[1] Indeed, a recent study in Sweden re-evaluated OCB-negative MS patients and identified that 33% had been misdiagnosed.[3] Striving for early diagnosis as aimed for by the 2017 revision of the McDonald criteria and the desire for timely initiation of disease-modifying therapy may increase the risk of misdiagnosis.[1,4] Possible differential diagnoses that resemble the clinical or radiological phenotype of MS include monogenic diseases such as hereditary spastic paraplegias or leukoencephalopathies. There have been numerous cases published describing monogenic diseases in patients initially thought to suffer from MS.[5] For example, in a cohort of 38 PPMS patients, pathogenic variants in genes causal for spastic paraplegia (*KIF5A*) or leukodystrophy (*MLC1*) were detected in two cases.[6] Several clinical, paraclinical, and radiological findings have been outlined as "red flags" that might hint towards a monogenetic differential diagnosis in patients with suspected MS. Amongst others, a positive family history of neurological symptoms or

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negative OCBs should raise suspicion for genetic differential diagnoses.[5]

Comprehensive data on how many patients diagnosed with MS instead suffer from an underlying monogenic disease that causes their MS-like phenotype and thus have been misdiagnosed with MS is lacking. The objective of this study was to investigate the prevalence of such monogenic diseases in a selected cohort of patients with the diagnosis of MS, encompassing all subtypes, including those with clinically or radiologically isolated syndrome (CIS or RIS, respectively). To this end, we selected patients who were more likely to be misdiagnosed with MS due to negative CSF-OCBs ("OCB neg."), a positive family history of MS ("Familial"), or both ("Familial OCB neg.").

Patients and methods
Written informed consent was obtained from all included patients prior to inclusion into our local biobank and subsequent whole exome sequencing (WES) analysis. The study was approved by the local ethics committee at the Technical University of Munich (project number 691/20S).

Patient cohort
In this retrospective study, patients diagnosed with early MS, CIS, and RIS treated at the Department of Neurology of the Technical University of Munich, who donated DNA to the local biobank between 2008 and 2019 were considered.

Patient data at blood sample collection date was retrospectively assessed using the contemporary diagnostic criteria at the time when analysis of the genetic data was conducted in 2020–2022. Thus, at blood sample collection date, considered patients met the diagnostic criteria for remitting relapsing MS (RRMS), primary and secondary progressive MS (PPMS, SPMS), and CIS according to the 2017 revised McDonald criteria, or for RIS according to Okuda et al., 2009[7] based on Barkhof criteria.[1,2,7]

Patients were included and assigned to one of three cohorts according to the following criteria. In the "OCB neg." cohort, patients who underwent lumbar puncture and did not have CSF-OCBs were included. The "Familial" group comprised patients who reported a positive family history of MS with at least two further blood relatives affected. If patients fulfilled both above mentioned criteria, they were included in the "Familial OCB neg." cohort. Clinical data were retrospectively obtained from medical records.

OCB detection
Results for OCBs were retrospectively retrieved from in-house analysis results during standard clinical testing. Detection of OCBs in CSF/serum pairs was done through isoelectric focusing and silver staining. CSF-OCBs are defined as having no correspondent band in the serum sample. At our clinic and for this study positivity of CSF-OCBs is defined as the detection of ≥3 OCBs exclusively in the CSF.

Gene panel composition
We defined a comprehensive gene panel of 495 genes (see supplementary Table A1) known to cause monogenic diseases that might clinically or on magnetic resonance imaging (MRI) mimic MS. A PubMed search in November 2020 identified genes associated with hereditary ataxias, spastic paraplegias, leukoencephalopathies, mitochondriopathies, metabolic or autoinflammatory disorders, and vasculopathies affecting the central nervous system.[5,6,8–10] Additionally, a search in HPO was performed using the search term "CNS demyelination" to detect further genes that can mimic MS. Finally, in-house diagnostic unit gene panels from Tübingen (for HSP, ataxia, leukodystrophy, demyelinating syndromes) were used.

Whole exome sequencing and genetic analysis
WES of genomic DNA samples from our local biobank was performed by Regeneron Genetics Center (Tarrytown, New York, USA) according to previously described methods.[11] A 20-fold coverage in > 80% of target bases was needed for the generated sequences. Moreover, quality control was performed by assessing contamination score (contamination <5% via verifyBamID software & heterozygous/homozygous ratio), sample duplication, gender concordance, and exome-genotype concordance, as described previously.

Analysis of WES data was performed as previously described.[12] In short, the megSAP pipeline (https://github.com/imgag/megSAP) and subsequently the GSvar graphical user interface (https://github.com/imgag/ngs-bits/tree/master/doc/GSvar) applying the comprehensive gene panel described above were used for automated variant analysis using in-house standard predefined criteria regarding zygosity, allelic frequency, impact on transcript/protein, and disease-association on HGMD and ClinVar databases.[13,14] Mitochondrial DNA was included in this analysis workflow. Additionally, copy number variants, structure variants, and repeat expansions were evaluated. The resulting list of variants was then in a second step manually reviewed guided by the patient's

phenotype. Based on the American College of Medical Genetics and Genomics (ACMG) guidelines pathogenicity was determined.[15]

Results

Patient characteristics

Exome data and clinical data including family history were available in 1739 patients in total. Exome data and clinical data including CSF-OCB status were available in 990 patients in total. Based on all eligible patients for this study, the proportion of patients with a positive family history with at least one further affected blood relative was 15.6% and with at least two further affected blood relatives was 2.9%. The proportion of CSF-OCB negative patients was 23.6% out of all eligible patients. A total of 278 patients that did not have CSF-OCBs ("OCB neg.", n = 228) or had a positive family history with at least two further affected blood relatives ("Familial", n = 44) or both ("Familial OCB neg.", n = 6) were analyzed. Sex distribution with 62.6% of patients being female was typical of MS. Patient characteristics are depicted in Table 1.

Definite monogenic disease: a case of CADASIL mimicking MS

In one 40-year-old patient from the "OCB neg." group (patient 1, see Tables 2 and 3), we detected an underlying monogenic disease mimicking the MS phenotype. The detected heterozygous missense variant c.544C > T (p.Arg182Cys) in NOTCH3 is annotated in HGMD and ClinVar to be pathogenic for CADASIL.

Patient 1 initially presented with recurrent hypoesthesia, dysesthesia, as well as vertigo and multiple FLAIR hyperintense lesions periventricular and associated with the corpus callosum on cerebral MRI. CSF-OCBs were negative on repeated testing. A follow-up MRI scan showed new, multiple juxtacortical and periventricular lesions, some with contrast-enhancement, leading to the diagnosis of RRMS. Further follow-up MRIs repeatedly showed progression with new periventricular, subcortical, and infratentorial lesions as well as in the basal ganglia. The patient's symptoms initially responded only partially to methylprednisolone (MPS) pulse therapy. After the MS diagnosis was made, immunomodulatory therapy with interferon-beta was initiated. However, after two clinical "relapses" with hemiparesis and dysarthria, only limited response to MPS pulse therapy and even plasmapheresis, and continuous MRI progression, the immunomodulatory therapy was escalated to natalizumab and later, due to a positive JC-virus antibody index, to rituximab. Despite complete B-cell depletion the patient repeatedly exhibited MR-tomographic disease progression with progressive confluent cerebral lesions. These lesions were markedly symmetrical, confluent, also affected non-MS typical regions including the thalamus or basal ganglia, and were increasingly pronounced in the temporal poles. Additionally, singular microbleeds cortical and pontine were detected. MRI findings are

Table 1. Patient characteristics at blood sample collection date according to subgroups.

Patient characteristics				
	Total	OCB neg.	Familial	Familial OCB neg.
Number of patients	278	228	44	6
Female	174 (62.6%)	138 (60.5%)	31 (70.5%)	5 (83.3%)
Male	104 (37.4%)	90 (39.5%)	13 (29.5%)	1 (16.7%)
Diagnosis				
RRMS	187 (67.3%)	145 (63.6%)	37 (84.1%)	5 (83.3%)
SPMS	22 (7.9%)	16 (7%)	6 (13.6%)	0 (0%)
PPMS	18 (6.5%)	18 (7.9%)	0 (0%)	0 (0%)
CIS	45 (16.2%)	44 (19.3%)	0 (0%)	1 (16.7%)
RIS	6 (2.2%)	5 (2.2%)	1 (2.3%)	0 (0%)
Median age in years (range)	37.5 (16–74)	38 (16–74)	34 (16–72)	42 (37–57)
Median disease duration[a] in years (range)	1 (0–36)	1 (0–36)	3 (0–29)	0 (0–17)

RRMS: remitting relapsing multiple sclerosis (MS); SPMS: secondary progressive MS; PPMS: primary progressive MS; CIS: clinically isolated syndrome; RIS: radiologically isolated syndrome.
[a]calculated from first manifestation to blood sample collection date.

Table 2. Clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype.

Clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype

Patient	Sex	Age[a]	Type of MS	Clinical symptoms regarded as typical for MS	Additional phenotypic features associated with the genetic variant	cMRI WMH	sMRI lesions
Cohort: "OCB neg."							
1	m	36	RRMS	Hypoesthesia, dysesthesia, vertigo, hemiparesis and dysarthria	family history of dementia, episodic headaches, sleep disorder, slight depressive symptoms	Confluent, symmetrical (pv, c/jc, inf, basal ganglia, thalamus, temporal lobes)	no
2	f	25	RRMS	NNO, INO, abducens nerve palsy	none	Two separated WMH (pv, jc temporopolar), N. opticus T2 hyperintense	no
3	m	38	RRMS	Paresis, hypoesthesia	none	Multiple separated WMH (pv, sc, inf)	2
4	f	61	PPMS	Atactic gait, vertigo, nystagmus	none	Multiple separated WMH (pv, jc) and lacunar defects in centrum semiovale	2
5	f	25	RRMS	Hypoesthesia, Lhermitteś sign	none	Multiple (>5) separated WMH (pv, c/jc)	1
6	m	46	RRMS	Hypoesthesia, paraparesis	renal cysts, pontine micro hemorrhage or telangectasy	Multiple (> 20) separated WMH (pv, c/jc), N. opticus T2 hyperintense	no
7	f	53	RRMS	Multiple NNOs	chronic kidney disease, GFR 58 ml/min, microalbuminuria, cataract	Multiple (>9) separate WMH (pv, c/jc, sc), N. opticus T2 hyperintense	no
8	f	33	SPMS	Spastic tetraparesis, NNO	depression	Multiple (>17) confluent WMH (pv, c/jc, inf, sc, temporal), thickened N. opticus, atrophy	n/a
9	f	43	RRMS	NNO, hypoesthesia, urinary dysfunction	GFR 81 ml/min	Multiple separated WMH (pv, c/jc, sc), N. opticus T2 hyperintense	2–3
10	m	31	RRMS	Hypoesthesia	none	Several WMH (pv, c/jc, sc)	2–3
11	f	55	SPMS	Hypoesthesia, NNO, spastic-atactic gait, fatigue	spastic-atactic gait, saccadic eye movements, pos. Babinski sign	Multiple separated (> 11) WMH (pv, c/jc, inf, sc), N. opticus T2 hyperintense	5
12	m	36	CIS	NNO	none	Multiple separated WMH (pv, jc, sc), N. opticus T2 hyperintense	no
13	m	34	RRMS	Hypoesthesia, hemiparesis	none	Multiple separated WMH (pv, jc)	no
14	f	29	RRMS	NNO	none	Multiple (>11) separated WMH (pv, c/jc, sc), N. opticus T2 hyperintense	no

(continued)

Table 2. Continued.

Clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype

Patient	Sex	Age[a]	Type of MS	Clinical symptoms regarded as typical for MS	Additional phenotypic features associated with the genetic variant	cMRI WMH	sMRI lesions
15	m	33	PPMS	Tetraparesis, spastic-atactic gait	none	Multiple (>11) separated WMH (pv, c/jc, sc, inf)	Medulla-Th10
16	f	59	CIS	NNO, eye pain	none	Multiple (>9) separated WMH (pv, inf, sc)	no
Cohort: "Familial"							
17	f	35	RRMS	Para-/hemiparesis, hypo-esthesia, gait disturbance	none	Multiple separated WMH (pv temporal, 5 jc, inf), brain atrophy	5
18	f	25	RRMS	Hypoesthesia, paresis, vertigo	none	Multiple separated WMH (pv, sc, inf)	7
19	f	48	SPMS	Spastic paraparesis, hypoesthesia, gait disturbance, urinary symptoms	"relapses" with spastic-atactic gait, saccadic eye movements, diplopia, decreased distal vibration sense, limb ataxia, hyperreflexia, pos. Babinski sign, urinary symptoms	Multiple confluent WMH (pv, jc, sc, splenic), cerebellar ischemia	n/a

m: male; f: female; NNO: neuritis nervi optici; INO: internuclear ophthalmoplegia; GFR: glomerular filtration rate; cMRI: cerebral magnetic resonance imaging; WMH: white matter hyperintensities; pv: periventricular; c/jc: cortical/ juxtacortical; inf: infratentorial; sc: subcortical; sMRI: spinal magnetic resonance imaging; n/a: data not available.
[a]Age indicated at first manifestation of MS.

Table 3. Genetic and clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype.

Genetic and clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype

| Patient	Gene (Transcript, Protein)	Related disorder, Inheritance	Variant: cDNA change, amino acid change (domain), zygosity	MAF[a] (total)	ACMG class[b]	HGMD[c]	Clin-Var[d]	CADD[e]	ESM 1b	Poly Phen-2[f]	SIFT[g]	PAN-THER[h]	PhD-SNP[i]	fathmm[k]	PRO-VEAN[l]	Pheno-type[m]	MRI[n]
Cohort: "OCB neg."																	
1	*NOTCH3* (ENST00000263388, Q9UM47)	CADASIL1, AD	c.544C>T, p.Arg182Cys (EGF-like domain 4), het	0.0031%	5	DM	p	28.60	−10.90	pd, 0.946	d, 0.01	pd, 0.74	d	d, −2.27	d, −4.944	yes	yes
2			c.964G>A, p.Val322Met (EGF-like domain 8), het	0.022%	3	DM	lb	27.60	−9.49	pd, 0.925	t, 0.11	pd, 0.57	n	d, −2.95	n, −1.339	none	no
3			c.1774C>A, p.Arg592Ser (EGF-like domain 15), het	0.0057%	3	DM?	u	24.20	−10.09	b, 0.421	t, 0.59	pb, 0.27	n,	d, −2.43	d, −2.767	none	no
4			c.5353C>T, p.Arg1785Cys, het	0.0016%	3	n/a	n/a	32.00	−10.19	pd, 1.000	t, 0.10	pd, 0.74	d	t, 0.52	d, −7.465	none	no
5	*HTRA1* (ENST00000368984, Q92743)	CADASIL2, AD	c.1208G>A, p.Arg403Gln, het	0.0032%	3	n/a	n/a	23.80	−4.62	b, 0.233	t, 0.70	pod, 0.5	n	t, 2.46	n, −0.765	none	no
6	*COL4A1* (ENST00000375820, P02462)	Brain small vessel disease, AD	c.2587C>T, p.Pro863Ser, het	0%	3	n/a	n/a	24.40	−7.47	pd, 0.987	t, 0.06	pd, 0.57	d	d, −2.73	d, −5.284	some	yes
7	*GLA* (ENST00000218516, P06280)	Fabry disease, X-linked	c.427G>A, p.Ala143Thr, het	0.05%	3	DM?	u	24.90	−9.51	pod, 0.853	d, 0.05	pd, 0.85	d	d, −8.23	d, −3.119	some	yes
8			c.427G>A, p.Ala143Thr, het	0.05%	3	DM?	u	24.90	−9.51	pod, 0.853	d, 0.05	pd, 0.85	d	d, −8.23	d, −3.119	some	yes
9			c.937G>T, p.Asp313Tyr, het	0.3%	3	DM?	u	21.80	−3.94	pod, 0.786	d, 0.01	pb, 0.19	d	d, −8.15	d, −3.183	some	yes
10			c.937G>T, p.Asp313Tyr, hemizygous	0.3%	3	DM?	u	21.80	−3.94	pod, 0.786	d, 0.01	pb, 0.19	d	d, −8.15	d, −3.183	none	no
11	*CACNA1G* (ENST00000359106.5, O43497)	SCA42, AD	c.2110G>A, p.Asp704Asn, het	0.0021%	3	n/a	n/a	23.80	−9.73	b, 0.322	d, 0.00	pd, 0.57	n	d, −4.10	d, −3.064	yes	no
12	*IFIH1* (ENST00000263642.2, Q9BYX4)	Aciardi Goutier syndrome, spastic paraparesis, white matter disease, AD	c.1558A>G, p.Thr520Ala, het	0.01%	3	n/a	u	25.70	−10.42	pd, 0.980	d, 0.02	pd, 0.57	d	t, 3.21	d, −2.920	none	no
13			c.1365T>G, p.Asn455Lys, het	0%	3	n/a	n/a	23.00	−12.32	pd, 1.000	t, 0.06	pd, 0.85	d	t, 1.45	d, −5.787	none	no
14			c.1222G>A, p.Asp408Asn, het	0.0036%	3	n/a	u	25.90	−8.64	pd, 0.972	t, 0.11	pd, 0.74	n	t, 3.34	d, −3.706	none	yes

(continued)

Table 3. Continued.

Genetic and clinical findings in patients with pathogenic variant/VUS possibly mimicking the MS phenotype

Patient	Gene (Transcript, Protein)	Related disorder, Inheritance	Variant: cDNA change, amino acid change (domain), zygosity	MAF (total)[a]	ACMG class[b]	HGMD[c]	ClinVar[d]	CADD[e]	ESM 1b	PolyPhen-2[f]	SIFT[g]	PANTHER[h]	PhD-SNP[i]	fathmm[k]	PROVEAN[l]	Phenotype[m]	MRI[n]
15	*GFAP* (ENST00000253408, P14136)	adult onset Alexander disease, AD	c.893C>T, p.Ser298Phe, het	0.0036%	3	n/a	u	23.80	−13.82	b, 0.178	d, 0.00	pod, 0.5	d	d, −3.84	d, −3.273	none	yes
16	*OPA1* (ENST00000392438, O60313)	Optic atrophy plus syndrome, AD	c.344C>T, p.Ala115Val, het	0.022%	3	DM?	u	22.50	−6.86	pod, 0.646	d, 0.00	pd, 0.85	n	d, −3.33	n, −0.312	none	no
Cohort: "Familial"																	
17	*NOTCH3* (ENST00000263388, Q9UM47)	CADASIL1, AD	c.2437G>A, p.Glu813Lys (EGF-like domain 21), het	0.0053%	3	n/a	u	29.00	−13.70	pd, 1.000	d, 0.04	pd, 0.85	d	d, −5.18	d, −3.574	none	no
18	*GLA* (ENST00000218516, P06280)	Fabry disease, X-linked	c.427G>A, p.Ala143Thr, het	0.05%	3	DM?	u	24.90	−9.51	pod, 0.853	d, 0.05	pd, 0.85	d	d, −8.23	d, −3.119	none	no
19	*CACNA1G* (ENST00000359106.5, O43497)	SCA42, AD	c.2455G>A, p.Val819Met, het	0.00051%	3	n/a	n/a	32.00	−10.55	pd, 0.994	d, 0.01	pd, 0.78	n	d, −4.99	d, −2.701	some	No

AD: autosomal dominant; cDNA: coding position; het: heterozygous; n/a: data not available.

[a]MAF: minor allele frequency according to gnomAD.

[b]ACMG class: 5 pathogenic; 3 uncertain significance.

[c]HGMD: DM: disease-causing mutation; DM?= likely disease-causing, but with questionable pathogenicity.

[d]ClinVar: p: pathogenic; u: uncertain significance; lb: likely benign.

[e]CADD: score according to Combined Annotation Dependent Depletion.

[f]PolyPhen-2: pd: probably damaging; pod: possibly damaging; b: benign.

[g]SIFT: d: deleterious; t: tolerated.

[h]PANTHER: pd: probably damaging; pod: possibly damaging; pb: probably benign.

[i]PhD-SNP: d: disease; n: neutral.

[k]fathmm: d: damaging; t: tolerated.

[l]PROVEAN: d: deleterious; n: neutral.

[m]Phenotype: specific phenotypic features compatible with defect in gene with detected variant.

[n]MRI: MRI findings support disease associated with VUS.
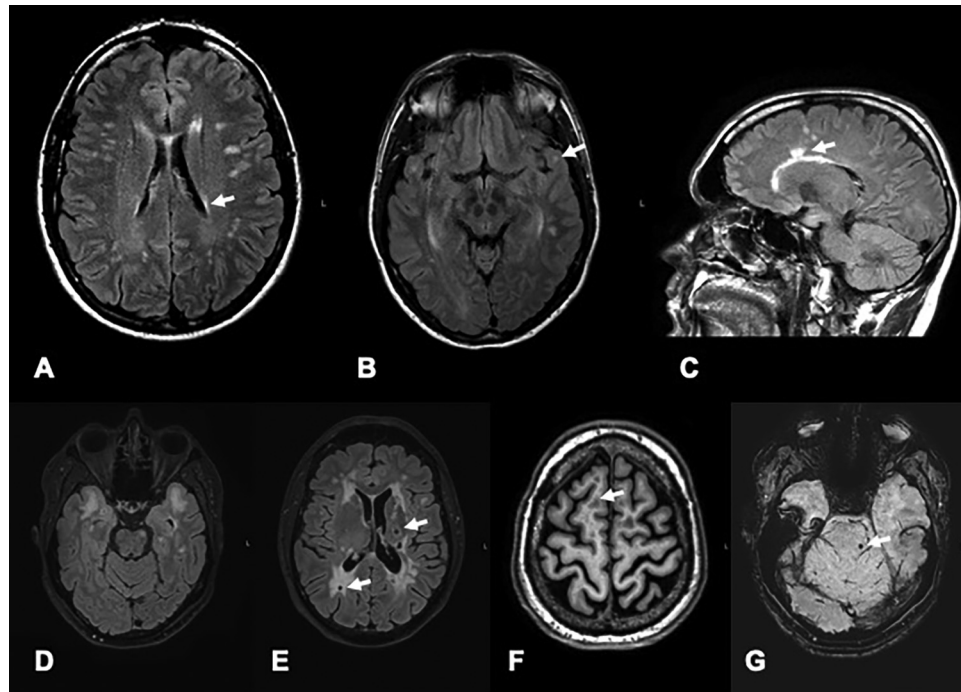
Figure 1. MRI findings in the CADASIL patient 1. A-C. FLAIR sequences upon initial presentation of the patient showing lesions in periventricular (arrow) and unspecific subcortical (A) and juxtacortical (arrow, B) location, as well as in the corpus callosum (arrow, C); D-G. Latest follow-up MRI sequences depicting now CADASIL-typical findings with marked FLAIR-hyperintense, confluent lesions increased in both temporal poles (D), lacunar post-ischemic defects in the white matter and the basal ganglia (arrows, E), spared cortical ribbon (arrow) in subcortical lesions on T1w sequence (F), and exemplary, unspecific microbleed (arrow) in SWI sequence (G). L = left, P = posterior.

depicted in Figure 1. Spinal MRI scans showed no lesions and evoked potentials were normal. Reevaluation of the family history disclosed that the patient's father, paternal aunt, and paternal grandparents were diagnosed with dementia at a relatively young age of around 50 years. Although the patient did not fulfill diagnostic criteria for migraine, episodic headaches were reported. Regarding mood disorders, the patient reported sleeping problems and restlessness, as well as mild depressive symptoms.

Detected variants of unclear significance
In 18 patients (6.5% of total, 6.6% of "OCB neg.", 6.8% of "Familial", 0% of "Familial OCB neg."), we detected 15 different variants of unclear significance (VUS) in 8 different genes. Detailed variant and patient data can be found in Tables 2 and 3. In particular, a considerable number of VUS were identified in genes associated with cerebral small vessel disease (*NOTCH3*, 4 patients; *GLA*, 5 patients) and spastic syndromes (*IFIH1*, 3 patients; *CACNA1G*, 2 patients). However, upon re-evaluation of the available clinical and MRI phenotype of the respective patients and comparison with the disease associated

with the gene carrying the detected VUS, the pathogenicity of the detected variants still remained unclear. Moreover, we employed multiple freely available computational tools for variant pathogenicity prediction of the VUS.[16–23] These results along with summarized clinical and MRI data are visualized in Table 3. Due to the limitations imposed by the patients' consent forms of our biobank, which prohibited the reporting of unclear results such as VUS back to the patients, we were prevented from re-phenotyping the patients or performing further diagnostic testing to further clarify the pathogenicity of the respective VUS.

Incidental finding
In one 42-year-old female patient with RRMS from the "OCB neg." cohort a heterozygous duplication encompassing the entire *PMP22* gene (chr17: 14095285–15472415) was detected by copy number variant analysis. Heterozygous duplications of *PMP22* constitute the most common cause of Charcot-Marie-Tooth neuropathy (CMT), namely CMT1A. This patient suffered from sensory impairment of the left arm lasting two weeks but did not

show clinical symptoms of peripheral neuropathy. However, nerve conduction studies showed marked demyelination with a motor velocity of the median nerve of 20 m/s. Family history was positive as the father was affected by a peripheral neuropathy. Therefore, a neuropathy was already suspected concomitant with the MS disease.

Discussion

This study screened a cohort of MS patients without CSF-OCBs and/or with a positive family history of MS for an underlying monogenic disease that might mimic their MS phenotype. Genetic diseases were not frequently mistaken in these cohorts. In one patient of our cohort of 228 MS patients without CSF-OCBs, we identified a clearly pathogenic *NOTCH3* variant causing CADASIL, which mimicked the MS phenotype. Although dual pathology of concomitant MS and CADASIL disease cannot ultimately be excluded, we consider it very unlikely. Throughout the disease course, the patient never developed spinal lesions, did not have CSF-OCBs, and had no signs of demyelinating disease when repeatedly testing evoked potentials. Furthermore, the patient demonstrated consistent progression under immunomodulatory therapy, even when completely B-cell depleted and alleged relapses only showed limited response to cortisone pulse therapy or even plasmapheresis. Therefore, we corrected the diagnosis from MS to CADASIL in this patient. Several other patients with CADASIL initially misdiagnosed as MS have already been reported.[24–26] The heterozygous missense variant p.Arg182Cys detected in our patient 1 is one of the most common mutations causing CADASIL and genotype-phenotype correlation analysis showed a broader phenotypic spectrum.[27] Our case highlights that CADASIL is an important genetic disease that should be considered in patients with suspected MS, particularly when CSF-OCBs are negative, there are no spinal cord lesions, and no response to highly active treatment.

No further clearly pathogenic variants in other genes from our comprehensive gene panel were detected, which could definitively explain the respective patient's MS phenotype. However, several VUS in eight different genes were identified. We frequently detected VUS in *NOTCH3* (n = 4, in patients 2–4 and 17). The temporopolar region, typically affected in CADASIL, was only altered in patients 2 and 17.[28] The clinical picture did match CADASIL in patients 3 and 17, but not perfectly in patient 2 due to isolated neuritis nervi optici (NNO) and patient 4 due to a chronic progressive disease course. In

patient 2 the variant p.Val322Met was detected; the clinical relevance of this variant is unclear. While described as likely benign on ClinVar, it has been reported in a patient with CADASIL and skin biopsies revealing CADASIL-specific deposits of granular osmiophilic material in the vascular smooth-muscle cells.[28] Patient 3 carried the VUS p.Arg592Ser, which is located in EGF-like 15 domain close to a cysteine residue, thereby possibly disturbing the domain's structure and has previously been described as a VUS for CADASIL.[29] The VUS p.Glu813Lys detected in patient 17 was modeled to result in a significant alteration in protein structure.[30]

In addition to *NOTCH3* variants, we detected VUS in seven further genes associated with either cerebral small vessel disease (*HTRA1*, *COL4A1*, *GLA*), spastic syndromes (*CACNA1G*, *IFIH1*, *GFAP*), or optic nerve atrophy (*OPA1*) in a total of 14 patients. Noteworthy, in five patients two different VUS in *GLA* in the heterozygous/hemizygous state were detected (patients 7–10 and 18, four females). It is now widely accepted that also females with heterozygous *GLA* variants can develop symptoms of Fabry disease.[31] The VUS p.Ala143Thr has previously been reported in a female patient initially misdiagnosed with MS, and it was postulated that p.Asp313Tyr causes a milder form of Fabry disease with predominantly neurologic manifestations including stroke.[32,33] Nevertheless, the pathogenicity of both variants remains unclear. Only patient 7 showed further symptoms typical of Fabry disease, such as significantly decreased kidney function.[31] Regarding spastic syndromes, we found a VUS in *IFIH1* (p.Thr520Ala, p.Asn455Lys and p.Asp408Asn) in three patients (patients 12–14). Heterozygous *IFIH1* variants can cause a spectrum of neuroimmune phenotypes including spastic paraparesis and white matter disease, but there are also clinically asymptomatic carriers.[34] Additional information on other detected VUS can be found in Supplement 1. In conclusion, the pathogenicity of the detected VUS remains unclear as existing data on the variants and/or phenotypic features of the patients were inconclusive.

No relevant variants were identified in genes associated with hereditary spastic paraplegia, as previously detected in a phenocopy study in progressive MS patients.[6] Notably, we did not detect any relevant variants in genes causing autoinflammatory syndromes, which would constitute a relevant differential diagnosis, especially in familial MS cases. Although mitochondrial DNA was included in our analysis and the sex distribution of our cohort was shifted

towards females, no relevant genetic variant causing a mitochondrial disorder, which is increasingly described as mimickers of MS,[5] was detected. Moreover, we cannot exclude that other diseases than monogenic ones mimicking MS might be present in our cohort. Further limitations of this study are its retrospective approach with further family members not being available for genetic testing and the nature of WES data, as mutations in introns or regulatory regions might be overlooked. Moreover, repeat expansions – being the genetic cause, e.g., of several spinocerebellar ataxias – and intronic variants might have been missed by exome sequencing. Due to the selected patient cohorts, the study methods preclude any conclusions about applying genetic testing in typical cohorts of MS patients, e.g., with positive CSF-OCBs or in patients first presenting for MS evaluation. Moreover, the patients' consent forms did not allow for further biochemical or lab testing to investigate the pathogenicity of detected VUS. Lastly, the small sample size and monocentric design with samples from our local biobank cannot entirely exclude a possible selection bias. The slightly higher proportion of CSF-OCB negative patients out of all eligible patients, compared to published MS cohorts,[35] is attributed to the fact that a high rate of CIS/RIS patients and mostly early MS cases at initial evaluation at our center participate in the local biobank and thus could be included in our study. The proportion of patients with a positive family history out of all eligible patients was in accordance with previous studies.[36]

In summary, a definitive underlying monogenic disease in our MS cohorts was rare with 0.44% in our "OCB neg." and 0% in our "Familial" and "Familial OCB neg." cohorts. However, several further VUS that cannot conclude or exclude an underlying monogenic disease were identified in 6.5% of our total cohort. The detected pathogenic *NOTCH3* variant (n = 1, 0.36% of total) and, subordinately, the multiple VUS in *NOTCH3* (n = 4, 1.4% of total) highlight the importance of CADASIL as a relevant disease causing an MS-like phenotype. As familial clustering in MS is well known and given the current findings, genetic screening for all familial MS cases should be decided on a case-by-case basis considering atypical clinical presentation or negative CSF-OCBs. Although in CSF-OCB negative cases physicians should be more doubtful regarding the MS diagnosis, our results also show that testing of all CSF-OCB negative patients for CADASIL is low yield and would not be cost-effective.

Nevertheless, genetic testing must be considered in selected cases with clinical and radiographic "red flags" of CADASIL, such as our patient. The applied testing strategy might be modified based on the specificity of phenotypic features, available sequencing platforms, and reimbursement regulations. In our setting, we currently recommend exome or genome sequencing in MS patients who meet a combination of the following criteria: (1) atypical clinical course, such as a lack of response to highly active MS therapy, (2) abnormal MRI findings, such as the absence of spinal lesions or a specific distribution and shape of lesions, (3) a positive family history, not only of MS but exceeding symptoms such as dementia, and (4) negative CSF-OCBs. These criteria are also part of the further refined and recently published clinical and paraclinical red flags and diagnostic approach recommendations in suspected MS patients.[37] Nevertheless, it is important to note that family history can be normal, as many monogenic diseases such as CADASIL may arise from *de novo* mutations, and even CSF-OCBs have been described in single CADASIL patients.[25,26] To supplement the diagnostic process and verify the pathogenicity of detected variants, biochemical tests and histochemical analyses should be considered. By identifying an underlying monogenic disease in a suspected MS patient, the administration of unnecessary and ineffective immunomodulatory therapies, along with the respective potentially harmful side effects, can be prevented. Furthermore, if available, a specific therapy might be initiated. Moreover, genetic counseling of patients and their families could be offered.

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Supplemental material

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References

1. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018; 17: 162–173.

2. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.

3. Katsarogiannis E, Landtblom A-M, Kristoffersson A, et al. Absence of oligoclonal bands in multiple sclerosis: a call for differential diagnosis. *J Clin Med* 2023; 12: 4656.

4. Solomon AJ, Naismith RT and Cross AH. Misdiagnosis of multiple sclerosis. *Neurology* 2019; 92: 26–33.

5. Weisfeld-Adams JD, Katz Sand IB, Honce JM, et al. Differential diagnosis of Mendelian and mitochondrial disorders in patients with suspected multiple sclerosis. *Brain* 2015; 138: 517–539.

6. Jia X, Madireddy L, Caillier S, et al. Genome sequencing uncovers phenocopies in primary progressive multiple sclerosis. *Ann Neurol* 2018; 84: 51–63.

7. Okuda DT, Mowry EM, Beheshtian A, et al. Incidental MRI anomalies suggestive of multiple sclerosis. *Neurology* 2009; 72: 800–805.

8. Köhler W, Curiel J and Vanderver A. Adulthood leukodystrophies. *Nat Rev Neurol* 2018; 14: 94–105.

9. Beaudin M, Klein CJ, Rouleau GA, et al. Systematic review of autosomal recessive ataxias and proposal for a classification. *Cerebellum Ataxias* 2017; 4: 3.

10. Taieb G, Kaphan E, Duflos C, et al. Hemophagocytic lymphohistiocytosis gene mutations in adult patients presenting with CLIPPERS-like syndrome. *Neurol Neuroimmunol Neuroinflamm* 2021; 8: e970.

11. Van Hout CV, Tachmazidou I, Backman JD, et al. Exome sequencing and characterization of 49,960 individuals in the UK biobank. *Nature* 2020; 586: 749–756.

12. Falb RJ, Müller AJ, Klein W, et al. Bi-allelic loss-of-function variants inKIF21Acause severe fetal akinesia with arthrogryposis multiplex. *J Med Genet* 2023; 60: 48–56.

13. Stenson PD, Mort M, Ball EV, et al. The human gene mutation database (HGMD®): optimizing its use in a clinical diagnostic or research setting. *Hum Genet* 2020; 139: 1197–1207.

14. Landrum MJ, Lee JM, Benson M, et al. Clinvar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res* 2018; 46: D1062–D1067.

15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015; 17: 405–424.

16. Rentzsch P, Witten D, Cooper GM, et al. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res* 2019; 47: D886–D894.

17. Brandes N, Goldman G, Wang CH, et al. Genome-wide prediction of disease variant effects with a deep protein language model. *Nat Genet* 2023; 55: 1512–1522.

18. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010; 7: 248–249.

19. Ng PC and Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res* 2001; 11: 863–874.

20. Thomas PD, Ebert D, Muruganujan A, et al. PANTHER: making genome-scale phylogenetics accessible to all. *Protein Sci* 2022; 31: 8–22.

21. Capriotti E, Calabrese R and Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. *Bioinformatics* 2006; 22: 2729–2734.

22. Shihab HA, Gough J, Cooper DN, et al. Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden markov models. *Hum Mutat* 2013; 34: 57–65.

23. Choi Y, Sims GE, Murphy S, et al. Predicting the functional effect of amino acid substitutions and indels. *PLoS ONE* 2012; 7: e46688.

24. Khan A, Abedi V, Li J, et al. CADASIL Vs. Multiple sclerosis: is it misdiagnosis or concomitant? A case series. *Front Neurol* 2020; 11: 860.

25. Joutel A, Dodick DD, Parisi JE, et al. De novo mutation in the Notch3 gene causing CADASIL. *Ann Neurol* 2000; 47: 388–391.

26. Ampuero I, Alegre-Abarrategui J, Rodal I, et al. On the diagnosis of CADASIL. *JAD* 2009; 17: 787–794.

27. Mukai M, Mizuta I, Watanabe-Hosomi A, et al. Genotype–phenotype correlations and effect of mutation location in Japanese CADASIL patients. *J Hum Genet* 2020; 65: 637–646.

28. Abramycheva N, Stepanova M, Kalashnikova L, et al. New mutations in the Notch3 gene in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *J Neurol Sci* 2015; 349: 196–201.

29. Ungaro C, Mazzei R, Conforti FL, et al. CADASIL: extended polymorphisms and mutational analysis of the NOTCH3 gene. *J of Neuroscience Research* 2009; 87: 1162–1167.

30. Vlachakis D, Tsaniras SC, Ioannidou K, et al. A series of Notch3 mutations in CADASIL; insights from 3D molecular modelling and evolutionary analyses. *J Mol Biochem* 2014; 3: 134.

31. Germain DP, Levade T, Hachulla E, et al. Challenging the traditional approach for interpreting genetic variants: lessons from fabry disease. *Clin Genet* 2022; 101: 390–402.

32. Böttcher T, Rolfs A, Tanislav C, et al. Fabry disease – underestimated in the differential diagnosis of multiple sclerosis? *PLoS ONE* 2013; 8: e71894.

33. Palaiodimou L, Stefanou M-I, Bakola E, et al. D313y variant in fabry disease: a systematic review and meta-analysis. *Neurology* 2022; 99: e2188–e2200.

34. Rice GI, Park S, Gavazzi F, et al. Genetic and phenotypic spectrum associated with IFIH1 gain-of-function. *Hum Mutat* 2020; 41: 837–849.

35. Dobson R, Ramagopalan S, Davis A, et al. Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: a meta-analysis of prevalence, prognosis and effect of latitude. *Journal of Neurology. Neurosurgery & Psychiatry* 2013; 84: 909–914.

36. Ehtesham N, Rafie MZ and Mosallaei M. The global prevalence of familial multiple sclerosis: an updated systematic review and meta-analysis. *BMC Neurol* 2021; 21: 246.

37. Solomon AJ, Arrambide G, Brownlee WJ, et al. Differential diagnosis of suspected multiple sclerosis: an updated consensus approach. *Lancet Neurol* 2023; 22: 750–768.