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ORIGINAL ARTICLE

Impact of *CYBA* genotypes on severity and progression of multiple sclerosis

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Abstract

Background and purpose: The NOX2 enzyme of myeloid cells generates reactive oxygen species (ROS) that have been implicated in the pathology of multiple sclerosis (MS). We aimed to determine the impact of genetic variation within *CYBA*, which encodes the functional CYBA/p22^{phox} subunit of NOX2, on MS severity and progression.

Methods: One hundred three MS patients with up to 49 (median = 17) years follow-up time from first MS diagnosis were genotyped at the single nucleotide polymorphisms rs1049254 and rs4673 within *CYBA*. Results were matched with disease severity and time to diagnosis of secondary progressive MS (SPMS). NOX2-mediated formation of ROS was measured by chemiluminescence in blood myeloid cells from healthy donors ($n = 55$) with defined genotypes at rs1049254 and rs4673.

Results: The rs1049254/G and rs4673/A *CYBA* alleles were associated with reduced formation of ROS and were thus defined as low-ROS alleles. Patients carrying low-ROS alleles showed reduced multiple sclerosis severity score ($p = 0.02$, $N = 103$, linear regression) and delayed onset of SPMS ($p = 0.02$, hazard ratio [HR] = 0.46, $n = 100$, log-rank test). In a cohort examined after 2005, patients carrying low-ROS *CYBA* alleles showed >20 years longer time to secondary progression ($p = 0.003$, HR = 0.29, $n = 59$, log-rank test).

Conclusions: These results implicate NOX2 in MS, in particular for the development of secondary progressive disease, and point toward NOX2-reductive therapy aiming to delay secondary progression.

KEYWORDS
multiple sclerosis, NOX2, rs1049254, rs4673, single nucleotide polymorphism

INTRODUCTION

In the majority of patients with multiple sclerosis (MS), the phase of recurrent neurological symptoms (relapsing–remitting MS [RRMS]) is followed by progressive neurological disability (secondary progressive MS [SPMS]) within years or decades. The currently available disease-modifying therapies (DMTs) efficiently reduce the frequency of MS episodes [1] but modestly, if at all, delay the onset of SPMS [2,3]. Genome-wide association studies (GWAS) have identified genes of relevance to MS risk, including human leukocyte

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antigen genes and genes linked to other immune-related or neural pathways [4,5], but genes that may herald the long-term course of disease remain to be defined [3].

MS is considered an autoimmune disease involving T and B cell-mediated immunity, but central nervous system (CNS)-resident microglia and infiltrating myeloid cells have also been implicated in MS pathophysiology [4]. Activated microglia thus produce inflammatory mediators such as cytokines, chemokines, and reactive oxygen species (ROS) that may inflict direct or indirect neurotoxicity [4]. NOX2, a member of the family of nicotinamide adenine dinucleotide phosphate oxidases, is almost exclusively expressed by circulating and tissue-resident myeloid cells, including microglia [6]. Its only known function is to generate ROS, which are reduced tissue-toxic species of oxygen [7] and often referred to as oxygen radicals. Upon activation, the membrane-bound NOX2 subunits gp91^{phox} (also called CYBB) and p22^{phox} (CYBA) associate with cytosolic subunits to form a superoxide-generating NOX2 complex [7]. The assembly of functional NOX2 occurs at phagosomal membranes or at other internal membranes of myeloid cells to generate intracellular ROS, or at the plasma membrane to generate extracellular ROS [7]. NOX2-derived ROS are pivotal in defense against microbial infection [8] and have been implicated in kidney and lung disease, in triggering cancer-related mutations, and in regulating lymphocyte-mediated immunity [9].

NOX2 is a dominant source of enzyme-derived ROS in the CNS [7,10]. In patients with MS, ROS-producing microglia are present in active lesions and in adjacent tissue [11]. Magnetic resonance imaging typically shows a slowly expanding iron-containing ring around chronic MS lesions that is assumed to reflect activated microglia [12]. In addition, MS patients frequently show elevated markers of oxidative stress, defined as a redox imbalance in favor of oxidation, in serum and cerebrospinal fluid [13]. In the murine experimental autoimmune encephalomyelitis model designed to mimic human MS, genetic deletion of NOX2 resulted in less severe disease [14,15]. Additionally, studies of in vitro cocultures of microglia and oligodendrocytes imply that NOX-derived ROS released from glia cells are significant mediators of oligodendrocyte toxicity [16,17].

Here, we report the results of a long-term follow-up study implying that genetic variation at *CYBA*, which encodes a functional subunit of NOX2, is linked to the severity and risk of progression in MS.

METHODS

Study approval

The study was approved by the local ethics committee, and written informed consent was obtained from all participants (approval numbers S 196-96, Ad 361-96, and 222-04).

Patients and controls

The study comprised two cohorts of MS patients fulfilling the Poser diagnostic criteria (18), one examined in 1996–1997 ($n = 43$; Cohort 1) and another examined after 2005 ($n = 60$; Cohort 2). The investigators N.M., O.A., and S.H., who are specialist MDs in neurology, performed a complete medical journal review and personal neurological examination of MS patients in Cohorts 1 and 2. Based on this, they determined the type of course: RRMS, SPMS, or primary progressive MS (PPMS). Additionally, they determined the year of conversion from RRMS to SPMS. SPMS was defined according to the consensus Lublin and Reingold criteria [18], and the severity of MS was assessed by the Expanded Disability Status Scale (EDSS) [19]. The EDSS score was converted to the Multiple Sclerosis Severity Score (MSSS), which takes disease duration into account [20]. As indicated in Table 1, three patients had PPMS, 61 had RRMS, and 39 had SPMS at the time of evaluation. Thirteen patients in Cohort 2 had received interferon beta or glatiramer acetate therapy. Further patient information is available in previous publications [21,22]. Peripheral blood from these patients was frozen as whole blood or cell lysates and used for the present study in a fully blinded fashion. Peripheral blood was concomitantly collected from 108 healthy volunteers. In addition, peripheral blood from 55 healthy blood donors, collected in 2020, was used as allele frequency controls. We also utilized allele frequency data extracted for Europeans from the National Center for Biotechnology Information (NCBI) dbSNP database [23,24] as a comparator for genotypes of patients with MS and the genotyped healthy controls ($n = 163$).

Peripheral blood mononuclear cell isolation and ROS measurement

In experiments aimed at determining the impact of *CYBA* single nucleotide polymorphism (SNP) genotypes on the formation of NOX2-derived ROS, peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donor buffy coats ($n = 55$) provided by the Sahlgrenska Blood Center. Mononuclear cells were separated by density centrifugation on Lymphoprep (Alere Technologies) as described elsewhere [25]. PBMCs were either immediately analyzed for ROS production or cryopreserved for later SNP analysis and ROS quantification. Superoxide anion was quantified by chemiluminescence as described elsewhere [26]. In the assay, PBMCs were added to 96-well plates (2–5 million cells/ml) in the presence of 10 μg/ml isoluminol (Sigma-Aldrich) and 4 U/ml horseradish peroxidase (EMD Millipore), and stimulated or not with 10^{-7} M of the NOX2-specific ROS inducer fMLF (Sigma-Aldrich) [27]. Light emission was recorded continuously for 8.5 min using a FLUOstar plate reader (BMG Labtech). ROS were quantified as area under the curve for the duration of the measurement.

DNA extraction

DNA was extracted from samples using a high-throughput DNA extraction device (MagnaPure LC, Roche Molecular Systems) with a Total NA (serum, plasma, blood) kit or manually using the DNEasy

TABLE 1 Study patient characteristics

Characteristic	All	RRMS phase	SPMS phase	PPMS phase
Cohort 1 + Cohort 2				
Patients, *n*	103	61	39	3
Females (%)	77 (75%)	52 (85%)	25 (64%)	0 (0%)
Median age, years (range)	48 (21–79)	43 (21–65)	53 (32–79)	58 (27–66)
Median MS duration, years (range)	17 (1–49)	14 (1–38)	25 (5–49)	23 (8–29)
Median EDSS (range)	3.5 (0–9)	3 (0–4.5)	7 (3–9)	4 (2.5–6.5)
Median MSSS (range)	3.5 (0.25–9.8)	2 (0.25–7.9)	7 (1.2–9.8)	5 (1.3–5.6)
Cohort 1				
Patients, *n*	43	23	18	2
Females (%)	28 (65%)	19 (83%)	9 (50%)	0 (0%)
Median age, years (range)	46 (21–62)	43 (21–59)	47.5 (32–62)	42.5 (27–58)
Median MS duration, years (range)	15 (1–36)	9 (1–26)	21.5 (5–36)	18.5 (8–29)
Median EDSS (range)	3.5 (1.5–8.5)	2.5 (1.5–4)	7 (4.5–8.5)	5 (3.5–6.5)
Median MSSS (range)	4.35 (1.2–9.8)	4.9 (1.8–8.0)	7.5 (1.2–9.8)	5.3 (4.9–5.6)
Patients who had received DMF, *n*	0	0	0	0
Cohort 2				
Patients, *n*	60	38	21	1
Females (%)	49 (82%)	33 (87%)	16 (76%)	0 (0%)
Median age, years (range)	51.5 (33–79)	44 (35–65)	58 (33–79)	66
Median MS duration, years (range)	20 (1–49)	15.5 (1–38)	28 (6–49)	23
Median EDSS (range)	3 (0–9)	2 (0–4.5)	6 (4–9)	2.5
Median MSSS (range)	2.7 (0.25–9.8)	1.65 (0.25–6.6)	3.1 (2.7–9.8)	1.3
Patients who had received DMF, *n*	13	11	1	1

Abbreviations: DMF, disease-modifying therapy (interferon-beta or glatiramer acetate); EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; MSSS, Multiple Sclerosis Severity Score; PPMS, primary progressive MS; RRMS, relapsing–remitting MS; SPMS, secondary progressive MS.

Blood & Tissue Kit (Qiagen). The latter procedure utilizes proteinase K for cell lysis, after which the suspension is placed on a filter column, where DNA is washed in two steps and then eluted.

SNP genotyping

We used real-time quantitative polymerase chain reaction (PCR) predesigned TaqMan genotyping assays for the rs4673 and rs1049254 SNPs (Thermo Fisher Scientific). Assays were run on a 7500 Fast Real-Time PCR machine (Applied Biosystems). Data were analyzed using the 7500 SDS software version 1.4 (Applied Biosystems) with an automatic allele calling to define SNP genotypes. Examples of allelic discrimination plots are shown in Figure S1.

Statistical analysis

Data were analyzed using Prism 8.4.2 software (GraphPad Software), SPSS Statistics 27 (IBM), and R version 3.4.2. To assess the effect of *CYBA* SNP variants on MS incidence, we used logistic regression, whereas the impact of *CYBA* SNP genotypes on ROS production and MSSS was analyzed using linear regression. Genotypes and groups with combined genotypes were coded 0, 1, or 2 for use in linear regression models. Parameter estimates and *p*-values related to linear regression models are found in Table S1. The impact of *CYBA* SNP genotypes on time to diagnosis of SPMS among patients with RRMS was analyzed using the log-rank test where two groups were compared, or the log-rank test for trend when comparing more than two groups. Patients with PPMS and those with progression within <1 year after initial diagnosis were not included in the analysis of time to SPMS. The effect of potential confounders (age at onset, sex) on disease parameters was assessed in multivariate analysis that included parameters with a p-value < 0.1 in univariate analysis. GraphPad Prism 8.4.2 software was used for generating plots.

RESULTS

CYBA variation versus NOX2-derived ROS formation by myeloid cells from healthy blood donors

We first defined the impact of variation at *CYBA* on the NOX2-dependent formation of ROS from myeloid cells in healthy donors ($n = 55$). The production of superoxide anion, which is the initial ROS

formed by NOX2 [28], was measured by chemiluminescence in the presence or absence of the NOX2-activating peptide fMLF and related to genotypes at rs1049254 and rs4673. In this assay, ROS are entirely contributed by NOX2$^+$ myeloid cells [27,29].

The fMLF-induced ROS formation from PBMCs was significantly reduced in donors carrying the rs1049254/G *CYBA* allele (Figure 1a). Similarly, the constitutive ROS production, that is, ROS generated in the absence of fMLF, by PBMCs from donors carrying the rs10492547/G allele was significantly reduced (Figure 1d). The fMLF-induced ROS production remained significantly reduced in donors carrying the rs1049254/G allele after correction for constitutive ROS formation, that is, ROS generated in the absence of fMLF ($p = 0.03$). Carriers of rs4673/A tended to produce lower levels of NOX2-derived ROS (Figure 1b,e). Analysis of the combined impact of genetic variation at *CYBA* showed a stronger association with ROS formation. Hence, the lowest ROS production was noted in donors carrying rs1049254/GG and rs4673/AA and the highest ROS production was observed in donors carrying rs1049254/AA and rs4673/GG (Figure 1c). We thus defined rs1049254/G and rs4673/A as "low-ROS alleles," whereas rs1049254/A and rs4673/G were defined as "high-ROS alleles."

Patient and disease characteristics

Neurological status and physical disability were independently scored according to EDSS [19]. At the time of evaluation, the median disease duration was 17 years (range = 1–49 years). To compensate for variable follow-up time, patients were scored according to MSSS [20]. Three patients were diagnosed with PPMS, and the remaining 100 patients were in the phase of RRMS at onset. At the time of evaluation, 39 of 100 of patients diagnosed with RRMS had converted to SPMS, and the interval between the initial diagnosis of MS and diagnosis of SPMS was retrieved from patient records. Further details of patients and disease characteristics are shown in Table 1.

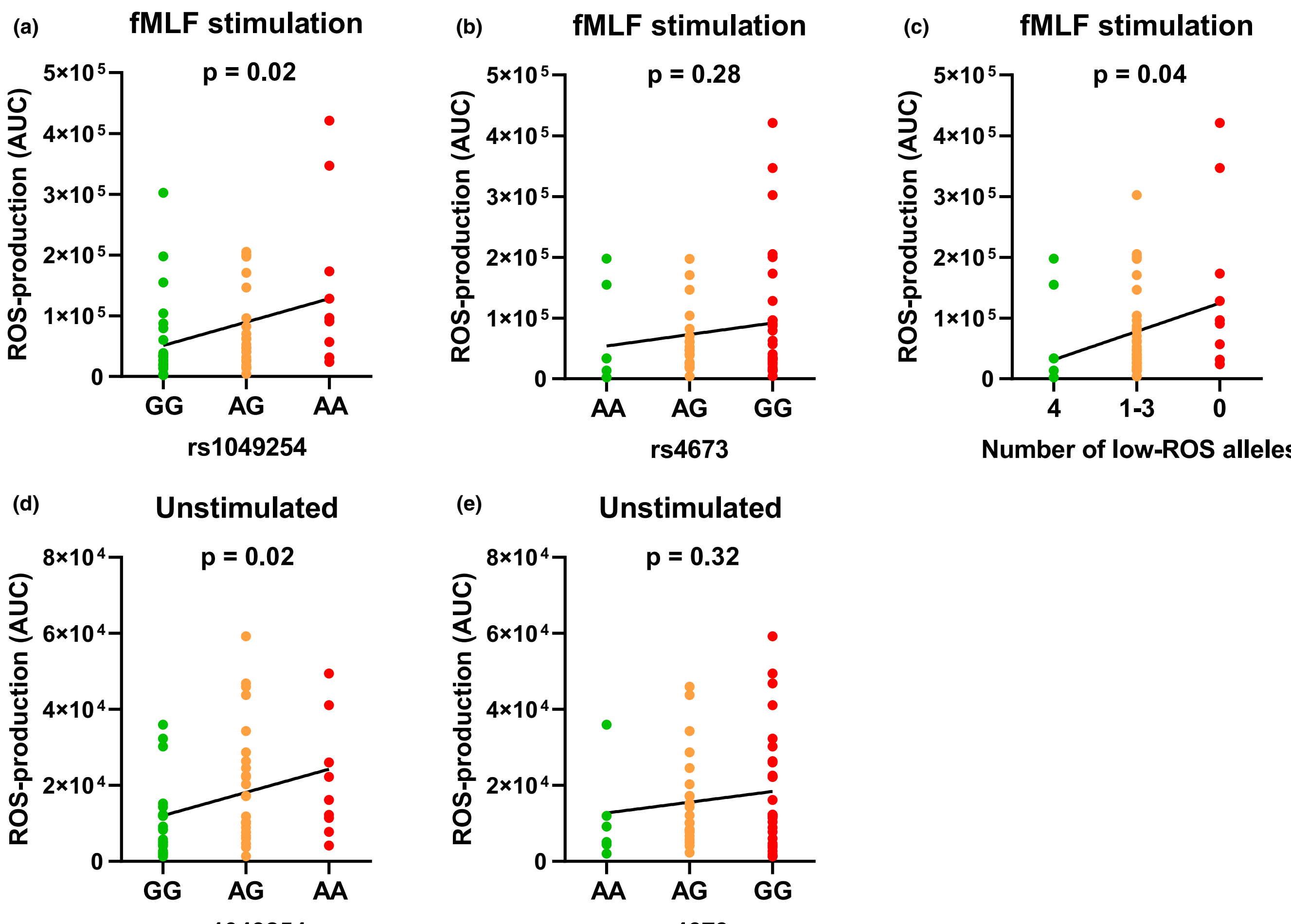

FIGURE 1 *CYBA* polymorphisms determine NOX2-derived reactive oxygen species (ROS) production by myeloid cells. Peripheral blood mononuclear cells from healthy donors ($n = 55$) were analyzed for production of extracellular ROS by chemiluminescence in the presence (a–c) or absence (d, e) of the NOX2 inducer fMLF (10^{-7} M). The total ROS production (area under curve [AUC]) is shown for each donor. For the combined genotypes (c), 0 constitutes patients with the rs1049254 AA and rs4673 GG genotype (high-ROS alleles only) and 4 constitutes patients with the rs1049254 GG and rs4673 AA genotype (low-ROS alleles only); remaining patients formed an intermediate group. Statistical analysis was by linear regression

TABLE 2 Genotype frequencies among patients with multiple sclerosis, healthy controls, and the European population

Genotype frequencies, *n* (%)	rs1049254			rs4673		
	Patients	Control[a]	European[b]	Patients	Control	European
AA	23 (22.3%)	25 (15.4%)	3378 (14.9%)	8 (7.8%)	19 (11.7%)	35,493 (11.7%)
AG	56 (54.4%)	75 (46.3%)	8569 (37.8%)	34 (33%)	65 (39.9%)	137,725 (45.4%)
GG	24 (24.3%)	62 (38.3%)	10,655 (47%)	61 (59.2%)	79 (48.5%)	131,354 (43.3%)
HWE[c]	0.67	0.96		0.58	0.61	
p[d]	0.014			0.049		

Abbreviation: HWE, Hardy–Weinberg equilibrium.

[a]The control group comprised 55 healthy blood donors and 108 healthy controls.

[b] Genotypes were retrieved from the National Center for Biotechnology Information dbSNP database [23,24].

[c]A value of <0.05 indicates deviation from HWE.

[d]Probability values refer to logistic regression analysis comparing patients and the control group.

Distribution of *CYBA* genotypes among patients with MS

Patients were analyzed for *CYBA* variants at rs1049254 and rs4673. The genotype distribution was compared with that of 163 healthy controls sampled at the Sahlgrenska University Hospital and also with gene frequencies reported for Europeans in the NCBI dbSNP database [23,24]. The low-ROS G allele at rs1049254 and the low-ROS A allele at rs4673 were significantly less common among patients with MS compared with the healthy controls ($p = 0.014$ and $p = 0.049$, respectively, logistic regression). Control subjects showed a *CYBA* SNP genotype distribution similar to that of the general European population. The rs1049254 and rs4673 loci are situated 3408 base pairs apart on chromosome 16 and were in linkage disequilibrium ($D' = 0.98$, $r^2 = 0.29$; Table S2). The genotype distributions among patients with MS and controls did not significantly deviate from Hardy–Weinberg equilibrium (Table 2).

Polymorphisms within *CYBA* determine MS severity

CYBA genotypes at rs1049254 and rs4673 significantly determined disease severity as measured by MSSS (Figure 2a,b). Hence, patients carrying low-ROS-associated rs1049254/G or rs4673/A alleles showed lower severity scores. The lowest MSSS was noted in patients homozygous for low-ROS *CYBA* alleles at both loci (Figure 2c). The association between *CYBA* genotypes and MSSS remained significant in multivariate analysis correcting for age at onset and sex (Table S3). A similar albeit nonsignificant gene association was observed when analyzing the impact of *CYBA* polymorphisms on EDSS (Figure S2).

Polymorphisms within *CYBA* determine time to secondary MS progression

We determined the potential impact of *CYBA* variation on secondary progression of MS by calculating the time to diagnosis of SPMS for patients in RRMS. Patients carrying at least one low-ROS G allele at rs1049254 showed a median of 12 years prolongation to SPMS ($p = 0.02$, hazard ratio [HR] = 0.37, 95% confidence interval [CI] = 0.16–0.86, log-rank test). For rs4673, presence of at least one low-ROS A allele tended to be associated with prolonged time to SPMS (median prolongation = 18 years, $p = 0.09$). A combined analysis of the impact of variation at rs1049254 and rs4673 revealed a favorable impact of carrying at least one low-ROS allele at either of these positions (median prolongation of RRMS = 12 years, $p = 0.02$, HR = 0.37, 95% CI = 0.16–0.86, $n = 100$, log-rank test).

We next analyzed the impact of *CYBA* variation on SPMS in Cohorts 1 and 2 separately. In Cohort 1 (patients examined in 1996–1997), low-ROS *CYBA* alleles were not significantly associated with prolonged time to SPMS (rs1049254: $p = 0.32$; rs4673: $p = 0.19$, log-rank test for trend). In the more recently analyzed Cohort 2 (patients examined after 2005), there was a striking prolongation of the time to SPMS for each individual *CYBA* low-ROS allele as well as in combined analysis of the impact of low-ROS alleles (Figure 2d–f). Similar results were obtained when the analysis was restricted to patients who had not received DMTs (Figure S3). The prolongation of the time to diagnosis of SPMS in Cohort 2 remained significant for rs1049254 ($p = 0.01$) and for combined low-ROS alleles ($p = 0.01$) after correction for potential confounders (age at onset of MS, sex) in multivariate Cox regression analysis (Table S3).

DISCUSSION

This study aimed to define the potential impact of variants of NOX2-related genes on the severity of MS and, for patients with RRMS, the time to diagnosis of secondary progressive disease. We thus analyzed MSSS and the time from MS diagnosis to SPMS versus genetic variation at rs4673 and rs104954, which are SNPs within *CYBA* that encode the functional p22^{phox} component of the ROS-producing enzyme NOX2 of myeloid cells. We utilized healthy donor myeloid cells to identify SNP variants that were associated with reduced formation of NOX2-derived ROS, that is, the G alleles at rs1049254 and

(a) rs1049254

p = 0.04

MSSS: 0, 5, 10, 15

GG AG AA

(b) rs4673

p = 0.04

MSSS: 0, 5, 10, 15

AA AG GG

(c) Number of low-ROS alleles

p = 0.03

MSSS: 0, 5, 10, 15

4 1-3 0

(d) rs1049254

Patients with non-progressive MS (%)

GG AG AA

p = 0.001

Duration (Years): 0 10 20 30 40 50

Number at risk

Years	0	10	20	30
AA:	15	6	4	0
AG:	29	21	10	6
GG:	15	12	8	1

(e) rs4673

Patients with non-progressive MS (%)

AA AG GG

p = 0.02

Duration (Years): 0 10 20 30 40 50

Number at risk

Years	0	10	20	30
GG:	33	18	11	4
AG:	22	18	8	4
AA:	4	3	3	0

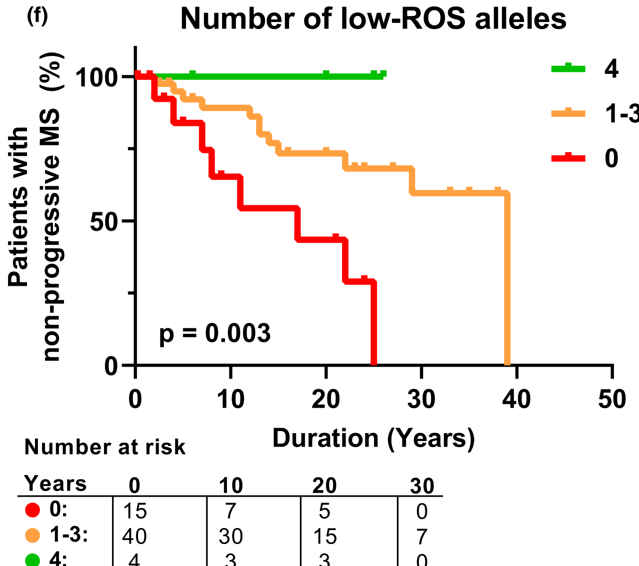

FIGURE 2 *CYBA* polymorphisms affect multiple sclerosis (MS) severity and progression. The dot plots in a–c show severity of disease according to Multiple Sclerosis Severity Score (MSSS) for patients with MS ($N = 103$) separated by genotype at rs1049254 (a), rs4673 (b), and combined genotypes at rs1049254 and rs4673 (c). The Kaplan–Meier curves in d–f show the time to diagnosis of secondary progressive MS for patients in the relapsing–remitting MS phase examined after 2005 ($n = 59$), where patients are separated based on genotype at rs1049254 (d), rs4673 (e), and combined genotypes at rs1049254 and rs4673 (f). The number of patients at risk at 0, 10, 20, and 30 years is given below each graph. For the combined genotypes (c, f), 0 constitutes patients with the rs1049254 AA and rs4673 GG genotype (high-reactive oxygen species [ROS] alleles only) and 4 constitutes patients with the rs1049254 GG and rs4673 AA genotype (low-ROS alleles only); the remaining patients formed an intermediate group. (a–c) Analyzed using linear regression. (d–f) Analyzed using the log-rank test for trend

the A alleles at rs4673 ("low-ROS alleles"). These latter findings are in agreement with previous studies, although partially divergent results have been reported [30–33].

Our study comprised two cohorts of patients with MS, one analyzed in 1996–1997 ($n = 43$) and one analyzed after 2005 ($n = 60$). In a combined analysis of all study patients, we observed that patients carrying low-ROS alleles at *CYBA* showed lower MSSS and delayed onset of SPMS by >10 years (median). The impact of *CYBA* variation on secondary progression was pronounced among the more recently examined patients, where patients carrying low-ROS alleles showed prolongation of the time to SPMS by >20 years.

This study allowed for long follow-up of MS progression, but we did not observe significant results in both cohorts when they were analyzed separately. The older cohort comprised a lower number of patients, which may limit detection of a significant impact of *CYBA* variation, but the reason for the discrepancy between these cohorts is otherwise unknown. MSSS and EDSS were higher in the 1996–1997 cohort compared with the more recent cohort ($p = 0.001$ and 0.03, respectively, Mann–Whitney *U*-test), which is in line with studies showing that MS severity has declined in the past decades [34]. Additionally, 13 of 60 patients in Cohort 2 had received DMT for relapse prevention. Exploratory analyses within this cohort suggested that low-ROS alleles at rs1049254 and rs4613 predicted reduced risk of MS progression also when only including untreated patients (Figure S3). Overall, these results highlight the need for studies that further define the impact of *CYBA* variation on secondary progression in contemporary cohorts of MS patients. Notably, CYBA is a subunit also of NOX1, NOX3, and NOX4 [7]. Although NOX2 is considered the dominant source of ROS in the CNS [10], our results do not formally exclude alternative effects of polymorphisms within *CYBA*, including effects on ROS formation from other NOX enzymes.

We also observed a significantly different SNP distribution at rs1049254 and rs4673 between patients with MS and healthy controls or the general European population. The low-ROS rs1049254/G and rs4673/A alleles were thus less prevalent among patients with MS than in healthy controls or in the European population. Although these findings imply that individuals carrying low-ROS alleles are at reduced risk for MS, larger confirmatory studies are warranted to confirm this association. Notably, *CYBA* is not listed among genes that significantly herald MS risk in GWAS [5].

In summary, the results of this study imply that allelic variation at *CYBA*, which encodes a functional component of NOX2, may be associated with the severity of MS and the time to secondary progression and disability. Our findings may be useful for prognostication and point toward MS therapy that specifically targets NOX2.

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CONFLICT OF INTEREST

Nothing to report.

AUTHOR CONTRIBUTIONS

Andreas Törnell: Data curation (equal), formal analysis (lead), investigation (lead), methodology (equal), visualization (lead), writing–original draft (lead), writing–review & editing (equal). **Roberta Kiffin:** Conceptualization (equal), data curation (supporting), formal analysis (supporting), investigation (supporting), methodology (equal), project administration (supporting), supervision (equal), writing–review & editing (equal). **Sara Haghighi:** Data curation (lead), investigation (supporting), resources (equal), writing–review & editing (supporting). **Natalia Mossberg:** Data curation (equal), investigation (supporting), resources (supporting), writing–review & editing (supporting). **Oluf Andersen:** Data curation (lead), investigation (lead), resources (equal), writing–review & editing (equal). **Kristoffer Hellstrand:** Conceptualization (equal), formal analysis (supporting), funding acquisition (equal), project administration (equal), resources (equal), supervision (equal), writing–original draft (lead), writing–review & editing (equal). **Anna Martner:** Conceptualization (lead), formal analysis (equal), funding acquisition (equal), methodology (equal), project administration (equal), resources (equal), supervision (lead), writing–original draft (equal), writing–review & editing (equal).

DATA AVAILABILITY STATEMENT

Deidentified patient data can be found in an open access repository at https://doi.org/10.17605/OSF.IO/FKT6H.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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