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Evaluation of variation in genes of the arylhydrocarbon receptor pathway for an association with multiple sclerosis

Narin Zorlu[a,b,1], Sabine Hoffjan[a,*,1], Aiden Haghikia[c], Igor V. Deyneko[d,e], Jörg T. Epplen[a,b]

[a] Department of Human Genetics, Ruhr-University Bochum, Germany
[b] Faculty for Health, ZBAF, University of Witten/Herdecke, Germany
[c] Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Germany
[d] Institute of Microbiology, Technical University Braunschweig, Braunschweig, Germany
[e] Group of Functional Genomics, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Russia

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ABSTRACT

The aryl hydrocarbon receptor (AhR) contributes to immune regulation in autoimmune diseases such as multiple sclerosis (MS). Analysis of selected polymorphisms in AhR pathway genes in 805 MS patients and 1023 controls revealed a modest association of a *CYP1B1* polymorphism with secondary progressive MS that became more pronounced in combination with other SNPs in the pathway, suggesting interactive effects. Additionally, first evidence for an interaction with smoking was found, but due to small sample sizes statistical significance was only nominal. Confirmation of these results in independent cohorts is recommended, since targeting the AhR constitutes a therapeutic option for autoimmune diseases.

1. Introduction

Multiple sclerosis (MS) is an autoimmune-related disease of the central nervous system affecting over 2.5 million people worldwide (Steinman, 2014). It is the most common cause of permanent neurological disability in young adults (Ramagopalan and Sadovnick, 2011). MS is known to be multifactorial in etiology, with both genetic and environmental factors influencing disease risk (Thompson et al., 2018). Besides the HLA region as strongest genetic risk factor, > 200 susceptibility loci have been discovered to date, each contributing only very modestly to the overall risk of the disease (Baranzini and Oksenberg, 2017). On the other hand, both gene-gene and gene-environment interactions have been implicated in MS pathogenesis (Baranzini and Oksenberg, 2017), which could potentially elucidate more of the genetic background, but such analyses have not been comprehensively integrated in the genetic analyses so far.

The arylhydrocarbon receptor (AhR) is a ligand-activated transcription factor that is involved in the regulation of gene activity in response to hydrophobic halogenated aromatic hydrocarbons such as 2,3,7,8-Tetrachlorodibenzodioxin (TCDD; dioxin), polycyclic aromatic hydrocarbons (PAHs) and other ligands in order to promote the metabolism and clearance of xeniobiotics. The role of this receptor in cellular responses against multiple endogenous and physiologic ligands has long been known; in the past few years, however, evidence accumulated that it may additionally play an important role in the regulation of immune responses in autoimmune diseases such as MS (Wheeler et al., 2017). It could be demonstrated that AhR is widely expressed within the immune system where it regulates the control of regulatory T cells (Treg) and Th17 cell differentiation (Quintana et al., 2008). Further, it was shown that AhR activation by TCDD can suppress experimental autoimmune encephalomyelitis (EAE) in the mouse model of MS by upregulating Treg cells (Quintana et al., 2008). Kaye et al. recently demonstrated that laqinimod, an oral drug evaluated for treatment of MS and other autoimmune diseases, abrogated EAE by activation of the AhR pathway (Kaye et al., 2016), suggesting a therapeutic role of this pathway for MS. AHR signaling has also been implicated in vitamin D

Abbreviations: AHR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator protein; EAE, experimental autoimmune encephalomyelitis; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; MS, multiple sclerosis; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; TCDD, tetrachlorodibenzodioxin

* Corresponding author at: Ruhr-University, Department of Human Genetics, Universitätsstrasse 150, 44801 Bochum, Germany.
E-mail address: sabine.hoffjan@rub.de (S. Hoffjan).

[1] These authors contributed equally.

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homeostasis (Takami et al., 2015), and vitamin D deficiency has long been known as a pathogenic factor for MS (Munger et al., 2014). Further, a next-generation sequencing study of circulating exosomes revealed four differently expressed miRNAs in RRMS patients compared to controls; with AHR being one of the putative overlapping targets of these miRNAs (Selmaj et al., 2017). Overall, therefore, evidence is accumulating that AhR and its signaling pathway may play an important role in MS pathogenesis.

Ligand binding to AhR induces a conformational change that allows the receptor to migrate into the nucleus where it can form a heterodimer with the AhR nuclear translocator protein (ARNT) to generate an aryl hydrocarbon receptor complex (AHRC, see Fig. 1). Subsequently, transcription of target genes such as *CYP1A1*, *CYP1A2, CYP1B1* and *NQO1* (NAD(P)H quinone dehydrogenase 1) is induced. AHRR serves as a negative regulator within this pathway through inhibition of AhR-mediated signal transduction (Vogel and Haarmann-Stemmann, 2017). In a recent study, common and potentially functional single nucleotide polymorphisms (SNPs) in seven genes of the AhR pathway were investigated in relation to essential hypertension (Polonikov et al., 2017). The authors found both single-gene associations as well as evidence for gene-gene and gene-environment interactions, including cigarette smoking. Given the emerging importance of the AhR pathway for MS pathogenesis and/or therapy, the current study was aimed at evaluating SNPs in the AhR pathway for an association with MS. Considering the importance of cigarette smoking as an environmental risk factor for MS, we also included an interaction analysis of AhR variants in the subgroup of patients for which smoking data were available.

2. Materials and methods

2.1. Study individuals

The investigated case-control cohort included 805 patients with a diagnosis of MS according to the McDonald criteria (Thompson et al., 2018) that were recruited at the St. Josef-Hospital (Bochum, Germany) between 2001 and 2011 (Table 1). The smoking history of each patient was adjusted retrospectively based on the discharge documents; complete data were available for 199 MS patients. Smoking status was defined as follows: non-smokers were classified as never having smoked throughout their lives. Smokers were defined as current smokers on the most recent consultation, and ex-smokers were defined as patients with a past history of smoking. The control cohort contained DNA samples from 1023 healthy individuals, mainly blood donors, recruited in Essen, Hamburg and Gladbeck, Germany. Information regarding the smoking status in the control cohort was available for 272 individuals. An overview outlining the characteristics of the study participants is given in Table 1. Written informed consent was obtained for each participant prior to enrolment in the study, which was approved by the Ethics Committee of the Ruhr-University Bochum. The Declaration of Helsinki protocols were followed.

2.2. SNP selection and genotyping

Seven potentially functional SNPs in AhR pathway genes were selected for genotyping as given in Polonikov et al., 2017. Additionally, we chose another SNP in the *AHR* gene (rs7796976) that had shown an

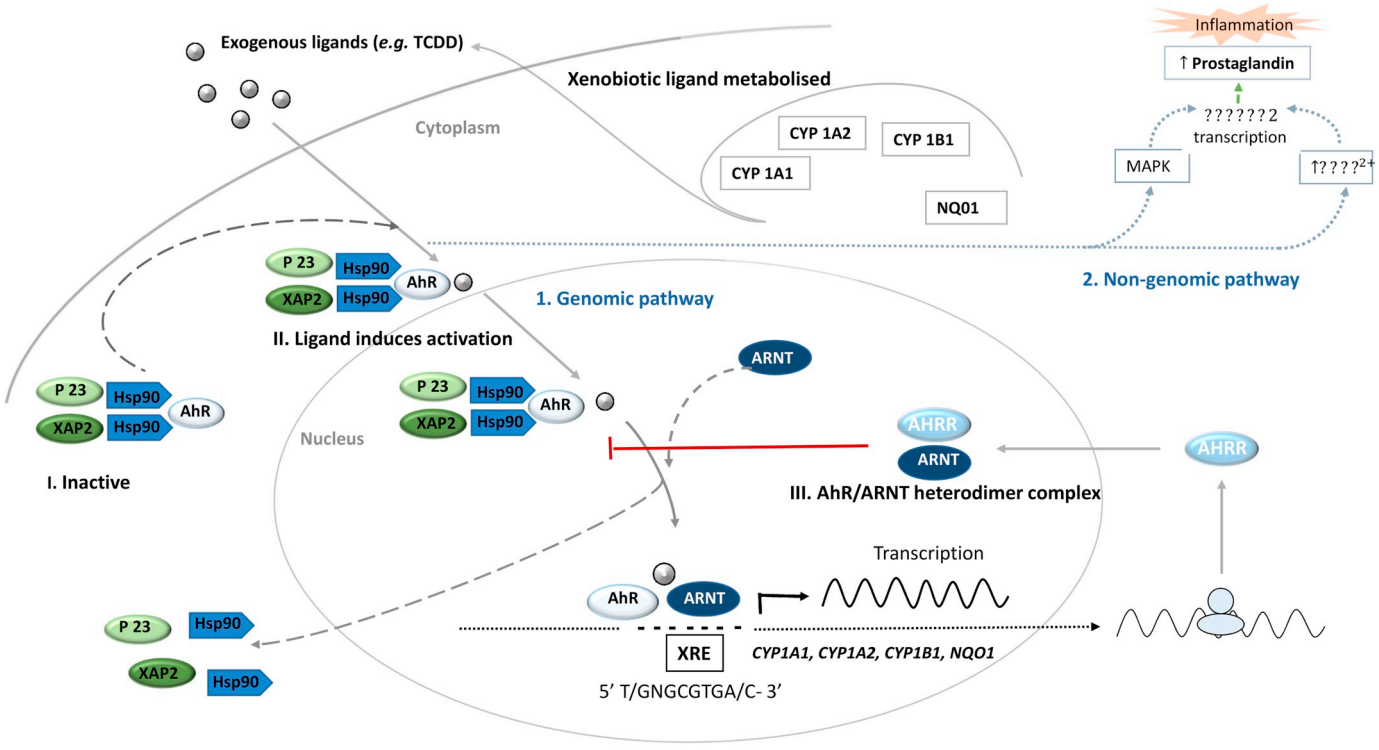

Fig. 1. The AhR pathway and its negative feedback loop with AhRR.
1. Genomic pathway: I. AhR is inactive in the cytoplasm in the absence of a ligand and surrounded by different proteins (Hsp90,p23 and XAP2). II. Ligands that activate the receptor induce a conformational change that allows the AhR to migrate into the nucleus. III. AhR can form a heterodimer with ARNT and bind to the xenobiotic response element (XRE) and introduce the transcription of XMEs (CYP1A1, CYP1A2, CYP1B1 etc.). AhR signaling is regulated by AHRR, which prevents AhR-ARNT complex formation.
2. Non-genomic pathway: TCDD can also lead to increased release of intracellular calcium concentration as well as activation of MAPK via the tyrosine kinase SrC (not shown). MAPK activation leads to the transcription of COX2. These mechanisms can contribute to inflammation by the production of prostaglandins (Larigot et al., 2018).
AHRR: aryl hydrocarbon receptor repressor; ARNT: aryl hydrocarbon receptor nuclear translocator; COX2: cyclooxygenase 2; MAPK: mitogen-activated protein kinase; SrC: proto–oncogene c-SrC; TCDD: tetrachlorodibenzo-p-dioxin; XME - xenobiotics-metabolizing enzyme. Image adapted from Polonikov et al., 2017.

Table 1
Overview outlining the characteristics of the investigated cohorts.

Disease course	Patients with MS					Controls
	in total	RRMS	SPMS	PPMS	CIS	–
Number (%)	805 (100)	456 (56.6)	178 (22.1)	151 (18.8)	13 (1.6)	1023
Sex	m:265	m:133	m:65	m:59	m:5	m:528
	f: 530	f:322	f:113	f:83	f:8	f:470
	u:10	u:1	u:0	u:9	u:0	u:25
Data on smoking status available	199	126	63	7	3	272
Mean age at blood sampling (years)	41.3 ± 11.6	36.56 ± 9.69	46.94 ± 10.01	49.91 ± 11.21	33.62 ± 8.36	48.45 ± 15.23
Mean age of onset (years)	30.1 ± 9.9	28.86 ± 8.92	31.42 ± 10.59	42.14 ± 12.38*	32.0 ± 7.57	n/a

f: female, m: male, u: unknown; MS: multiple sclerosis; RRMS: relapsing-remitting MS; SPMS: secondary-progressive MS; PPMS: primary-progressive MS; CIS: clinically isolated syndrome; n/a: non-applicable. * Age of onset data is available only for 21 patients.

Table 2
Investigated SNPs in the AHR pathway.

Gene	Localization	SNP	AA exchange[b]	Genotyping method	Primer	Enzyme
AHR	7p21.1	rs2066853	R554K	RFLP	F: TGGCAAGATAATACTGCACCG R: TCTGAAGTCAACCTCACCAGA	*Hpy*188I
AHR	7p21.1	rs7796976	Promoter	RFLP	F: GGCGGGAAGCACCCAGGATTT R: TAGAATCCTGGCCTGGGTCG	Van91I
AHRR	5p15.33	rs2292596	P185A	TaqMan Assay[a]	–	–
ARNT	1q21.3	rs2228099	–	TaqMan Assay[a]	–	–
CYP1A1	15q24.1	rs1048943	I462V	RFLP	F: CAAGCGGAAGTGTATCGGTG R: AGCTGCATTTGGAAGTGCTC	Taa1
CYP1A2	15q24.1	rs762551	Promoter	RFLP	F: TGATGTGTGGAGGAGAGAGC R: GCTGAGGGTTGAGATGGAGA	BsuRI
CYP1B1	2p22.2	rs1056836	V432 L	TaqMan Assay[a]	–	–
NQO1	16p22.1	rs1800566	P187S	RFLP	F: TGAGAAGCCCAGACCAACTT R: TCTCCAGGCGTTTCTTCCAT	*Hin*fI

[a] TaqMan SNP genotyping assay, conducted according to the manufacturer's protocol.
[b] Known functional effects of these polymorphisms are summarized in Polonikov et al., 2017.

interactive effect with smoking on intestinal permeability in Crohn's disease (Prager et al., 2016). Genotyping was performed with restriction fragment length polymorphism (RFLP) analysis or TaqMan assays according to the manufacturer's protocol (Table 2).

2.3. Statistical analysis

Student's *t*-test and χ^2 test were used to compare age and sex distributions between patients and controls. Hardy–Weinberg equilibrium (HWE) was calculated using the PLINK program. The reported MS disease prevalence in Germany is estimated to be 0.32% (Holstiege et al., 2017). However, there is a high level of regional heterogeneity in the prevalence of MS within Germany. The selected sample size of the study allowed to detect genetic effects with an OR ≥ 1.35 for a minor allele frequency (MAF) of ≈0.1 and with an OR ≥ 1.22 for more frequent SNPs (MAF ≈ 0.3). Here the significance level was set to 5% and the power to 80% (CaTS program). Genotype frequencies were compared using logistic regression analysis and were adjusted for age at onset, sex, smoking status and disease course where appropriate. The following genetic models were tested: additive, dominant and recessive. Odds ratios (OR), 95% confidence intervals (CI) and *p*–values were calculated as measures of associations. Bonferroni correction for multiple testing was applied where appropriate by multiplying by the number of independent comparisons. *P*–values < 0.05 (after correction, where applicable) were considered as significant for all tests. The combined effects of the SNP pairs on disease risk were analyzed using Fisher exact test for all possible SNP combinations using dominant, recessive and over-dominant genetic models in R, as previously described (Varzari et al., 2015). To adjust for sex, cases were weighted to match the male/female ratio of the controls. Correlations between age at disease onset and individual SNPs were assessed using logistic regression analysis in the subgroup of patients with early disease onset (≤28 years) and late disease onset (≥29 years) (for more details see (Varzari et al., 2018). Linkage disequilibrium (LD) between polymorphisms was examined using the HaploView software, generating D′ and r^2 coefficients.

3. Results

All investigated polymorphisms were in HWE (at $p > .01$) in patients and controls (Table 3). When single SNPs were evaluated for an association with MS, we did not find any significant associations. One exception is rs762551 (*CYP1A2* gene), which gave a nominally significant result under an additive model and only after adjustment for sex, age at investigation, disease course and smoking status ($p = .015$, Table 4). Unfortunately, such adjustment reduced the number of individuals and led to a greater CI for OR crossing one, making this association only marginal. Stratification for disease course and smoking status revealed several significant associations. The most significant was rs1056836 (*CYP1B1*) (OR = 1.7, $p = .006$ under a dominant model) for patients with spMS (Table 5). Only nominal significance was shown for rs7796976 (*AHR*) under a dominant model (OR = 0.7, $p = .031$). No significant associations were found for rrMS or ppMS patients. When only smokers were evaluated, nominally significant results were achieved for rs7796976 (*AHR*) under a recessive model (OR = 0.2, $p = .029$) and rs1800566 (*NQO1*) under a dominant model (OR = 0.4, $p = .014$; Table 5). However, all of these results became insignificant after Bonferroni correction. Analysis of age-at-onset specific subgroups did not reveal any dependencies between the time of the disease onset and genetic variations in AhR genes. Analysis of haplotypes identified one weak linkage disequilibrium block between rs2066853 and rs7796976 located in the *AHR* gene (D` = 0.68). No

Table 3
Genotype distribution of the studied polymorphisms.

Gene	Polymorphism	Genotype	Cases n (%)	HWE *p*-value	Controls n (%)	HWE *p*-value
AHR	rs2066853	AA	11 (1.4)	0.10	17 (1.7)	0.03
		AG	127 (16.2)		167 (17.1)	
		GG	647 (82.4)		794 (81.2)	
AHR	rs7796976	AA	49 (6.3)	0.32	62 (6.6)	0.60
		AG	272 (34.7)		348 (36.9)	
		GG	462 (59.0)		533 (56.5)	
AHRR	rs2292596	GG	136 (17.3)	0.51	157 (16.1)	0.55
		GC	371 (47.1)		456 (46.8)	
		CC	280 (35.6)		361 (37.1)	
ARNT	rs2228099	CC	105 (13.4)	0.27	104 (10.7)	0.72
		CG	344 (43.9)		436 (44.9)	
		GG	335 (42.7)		432 (44.4)	
CYP1A1	rs1048943	GG	0 (0.0)	1.00	0 (0.0)	0.40
		GA	53 (6.8)		77 (7.9)	
		AA	729 (93.2)		900 (92.1)	
CYP1A2	rs762551	CC	73 (9.2)	0.74	95 (9.8)	0.94
		CA	343 (43.4)		416 (42.8)	
		AA	374 (47.3)		460 (47.4)	
CYP1B1	rs1056836	CC	151 (19.4)	0.83	204 (21.1)	0.03
		CG	387 (49.8)		444 (45.8)	
		GG	239 (30.8)		321 (33.1)	
NQO1	rs1800566	TT	26 (3.3)	0.91	20 (2.1)	0.03
		TC	238 (30.6)		298 (31.0)	
		CC	514 (66.1)		644 (66.9)	

HWE - Hardy-Weinberg equilibrium.

significant association with haplotypes was identified.

SNP-SNP interaction analysis revealed interactive effects at a significance level of $p < .01$ for five SNP combinations (Table 6). The strongest association was observed between MS and genotypes AA for rs1048943 (*CYP1A1*) and CG for rs1056836 (*CYP1B1*) for spMS patients, with an uncorrected *p*-value of 0.0005. Further, the combination of genotype GG for rs2228099 (*ARNT*) and GG for rs1056836 (*CYP1B1*) was also significantly associated with MS risk in this group (uncorrected $p = .007$). These two results remained significant after Bonferroni correction ($p_c = 0.014$ and 0.0196, resp.). None of the other combinations passed the significance threshold after correction.

4. Discussion

We herein present first evidence for both single-gene association as well as gene-gene interactive effects in the AhR signaling pathway in

Table 4
Association analysis of single polymorphisms with MS risk.

Gene	Polymorphism	Association analyses using logistic regression under the respective genetic model						
		Genetic model[a]	OR (95% CI)	*p*-value	OR (95% CI)[b]	*p*-value[b]	OR (95% CI)[c]	*p*-value[c]
AHR	rs2066853	A	0.92(0.72–1.18)	0.453	0.92 (0.73–1.15)	0.450	0.63 (0.09–4.42)	0.639
		D	0.85(0.59–1.22)	0.505	0.94 (0.73–1.22)	0.634	0.66 (0.08–5.50)	0.698
		R	1.00(0.83–1.21)	0.575	0.62 (0.28–1.39)	0.246	1.00 (0.01–99)	0.992
AHR	rs7796976	A	1.12(0.91–1.37)	0.347	0.94 (0.80–1.10)	0.427	0.87 (0.24–3.08)	0.826
		D	1.07(0.88–1.30)	0.299	0.90 (0.74–1.11)	0.318	0.56 (0.11–2.90)	0.486
		R	0.90(0.75–1.09)	0.789	1.01 (0.67–1.51)	0.983	2.52 (0.29–21.6)	0.399
AHRR	rs2292596	A	0.99(0.86–1.14)	0.431	1.07 (0.92–1.23)	0.384	1.62 (0.58–4.55)	0.362
		D	1.08(0.90–1.29)	0.520	1.11 (0.90–1.36)	0.328	1.65 (0.32–8.64)	0.551
		R	1.07(0.89–1.30)	0.515	1.05 (0.81–1.37)	0.708	2.23 (0.42–11.7)	0.343
ARNT	rs2228099	A	1.10(0.96–1.27)	0.174	1.15 (0.99–1.33)	0.064	1.18 (0.39–3.52)	0.770
		D	1.02(0.89–1.16)	0.471	1.15 (0.94–1.40)	0.175	2.19 (0.42–11.3)	0.353
		R	1.06(0.92–1.21)	0.084	1.32 (0.97–1.79)	0.076	1.00 (0.01–99)	0.788
CYP1A1	rs1048943	A	1.04(0.85–1.27)	0.380	0.91 (0.62–1.33)	0.615	1.00 (0.01–99)	0.804
		D	1.29(0.97–1.72)	0.380	0.91 (0.62–1.33)	0.615	1.00 (0.01–99)	0.804
		R	0.90(0.71–1.15)	0.380	0.91 (0.58–1.46)	0.615	1.00 (0.01–99)	0.804
CYP1A2	rs762551	A	1.09(0.85–1.40)	0.870	0.99 (0.85–1.16)	0.934	3.93 (1.30–11.8)	***0.015***
		D	0.95(0.64–1.40)	0.989	0.99 (0.81–1.21)	0.917	5.41 (0.65–45.2)	0.119
		R	0.80(0.37–1.73)	0.700	1.00 (0.71–1.40)	0.995	6.87 (1.49–31.6)	0.013
CYP1B1	rs1056836	A	0.93(0.80–1.08)	0.828	1.04 (0.90–1.19)	0.616	1.01 (0.36–2.84)	0.992
		D	0.92(0.74–1.14)	0.292	1.14 (0.92–1.41)	0.223	3.18 (0.38–26.6)	0.287
		R	0.85(0.59–1.22)	0.404	0.94 (0.73–1.20)	0.599	1.00 (0.01–99)	0.769
NQO1	rs1800566	A	0.98(0.78–1.22)	0.405	1.08 (0.89–1.30)	0.442	2.19 (0.63–7.60)	0.216
		D	0.94(0.68–1.29)	0.700	1.03 (0.83–1.27)	0.795	2.98 (0.66–13.4)	0.157
		R	1.63(0.90–2.94)	0.106	1.76 (0.94–3.29)	0.076	1.00 (0.01–99)	0.970

OR - odds ratio; CI - confidence interval;
[a] Genetic models: A – additive, D – dominant, R – recessive.
[b] OR and p-values adjusted for sex and age at investigation.
[c] OR and *p*-values adjusted for sex, age at investigation, disease course and smoking status.

Table 5
Stratification analysis of the polymorphisms within smokers and patients with secondary progressive disease course (spMS). No associations were identified within non-smokers and groups of patients with rrMS or ppMS (data not shown).

Gene	Polymorphism	Stratified association analyses using logistic regression under the respective genetic model				
		Genetic model[#]	Smokers only[a]		Patients with spMS[b]	
			OR (95% CI)	*p*-value*	OR (95% CI)	*p*-value*
AHR	rs2066853	A	1.2 (0.4–3.5)	0.687	1.1(0.8–1.6)	0.440
		D	1.2 (0.4–3.6)	0.760	1.1(0.8–1.7)	0.536
		R	1.1 (0.3–1.9)	0.981	1.5(0.5–4.1)	0.445
AHR	rs7796976	A	0.5 (0.3–1.0)	0.064	0.8(0.6–1.0)	0.054
		D	0.6 (0.3–1.4)	0.249	**0.7(0.5–1.0)**	**0.031**
		R	**0.2 (0.1–0.9)**	**0.029**	0.8(0.4–1.7)	0.629
AHRR	rs2292596	A	0.9 (0.5–1.5)	0.609	1.0(0.8–1.2)	0.851
		D	0.6 (0.3–1.5)	0.287	0.9(0.6–1.3)	0.529
		R	1.2 (0.4–3.6)	0.681	1.1(0.7–1.7)	0.636
ARNT	rs2228099	A	1.3 (0.7–2.4)	0.387	1.2(0.9–1.5)	0.153
		D	1.4 (0.6–3.2)	0.379	1.3(0.9–1.8)	0.170
		R	1.3 (0.4–4.7)	0.668	1.2(0.8–2.0)	0.397
CYP1A1	rs1048943	A	0.4 (0.1–1.8)	0.225	0.7(0.4–1.4)	0.326
		D	0.4 (0.1–1.8)	0.225	0.7(0.4–1.4)	0.326
		R	0.9 (0.5–2.4)	0.933	1.3(0.3–2.8)	0.870
CYP1B1	rs1056836	A	1.0 (0.6–1.7)	0.966	1.2(1.0–1.5)	0.083
		D	1.4 (0.6–3.2)	0.485	**1.7(1.2–2.5)**	**0.006**
		R	0.6 (0.2–1.7)	0.368	1.0(0.7–1.5)	0.920
CYP1A2	rs762551	A	1.1 (0.6–2.0)	0.818	1.0(0.7–1.2)	0.720
		D	1.0 (0.4–2.4)	0.959	1.0(0.7–1.4)	0.967
		R	1.3 (0.4–4.4)	0.695	0.8(0.4–1.4)	0.388
NQO1	rs1800566	A	0.5 (0.3–1.0)	0.062	1.0(0.7–1.3)	0.878
		D	**0.4 (0.2–0.8)**	**0.014**	0.9(0.7–1.3)	0.714
		R	1.6 (0.3–10.2)	0.595	1.4(0.5–3.8)	0.533

bold: (nominally) significant results
* p-values adjusted for sex and age at investigation.
[a] association tests were performed between smokers in patient and control groups.
[b] association tests were performed between patients with respective disease course and all controls.

Table 6
Pairwise genotype - genotype interaction effects on MS risk as revealed by Fisher test. All possible pairwise combinations were tested independently in the whole sample and subgroups of smokers and patients with (spMS, rrMS and ppMS). Only associations with a nominal *p*-value < .01 are shown.

1st locus	Genotype	2nd locus	Genotype	Cases n (%)	Controls (%)	OR (95% CI)	*p*-Value	*p*-Value*	p_c-value**	Dataset
rs1048943	AA	rs1056836	CG	94 (55%)	402 (42%)	1.79 (1.28–2.51)	0.0022	0.0005	0.014	spMS_only
rs2228099	GG	rs1056836	GG	9 (5.3%)	144 (15%)	0.35 (0.16–0.67)	0.0006	0.0007	0.0196	spMS_only
rs2066853	GG	rs1056836	GG	29 (17%)	261 (27%)	0.50 (0.32–0.78)	0.0039	0.0013	n.s.	spMS_only
rs2228099	CC	rs1056836	CC	29 (3.8%)	15 (1.6%)	2.44 (1.25–4.93)	0.0042	0.0053	n.s.	all patients
rs7796976	GG	rs762551	AC	214 (27%)	204 (22%)	1.27 (1.01–1.60)	0.0093	0.0355	n.s.	all patients

* p-values adjusted for sex.
** *p*-values after Bonferroni correction for multiple tests (in total 28 combinations possible).

relation to MS. Analysis of common and potentially functional SNPs within the AHR pathway identified the SNP rs1056836 in the *CYP1B1* gene, which showed consistent association with MS in patients with the secondary progressive disease course. The C allele at rs1056836 increased the risk of spMS, whilst homozygosity for the G allele had a protective effect. This result became more pronounced when rs1056836 was combined with other SNPs within the AhR pathway. The most promising combination was rs1056836 (*CYP1B1*) with rs1048943 (*CYP1A1*), which remained significant after Bonferroni correction and comprised a significant number of patients and controls, making the results valuable for further investigation. Polymorphic variants of cytochrome P450 have been widely studied for various cancer types, including lung and breast cancer (Daly, 2015). Further, an influence on neurodegenerative disorders such as Parkinson's disease had been suggested, but was not confirmed as a strong risk factor (Alonso-Navarro et al., 2014). To our knowledge, however, the *CYP1B1* and *CYP1A1* genes have not been evaluated for an association with MS yet. Our data suggest for the first time a role of these genes in spMS, yet the effect was not seen for rrMS or ppMS patients.

Interaction between genetic variants, also called epistasis, has long been discussed as an important pathogenic mechanism in autoimmune diseases including MS (Rose and Bell, 2012). Despite > 200 susceptibility loci described to date (Baranzini and Oksenberg, 2017), only few studies have evaluated interactive phenomena so far. In a first attempt to analyze GWAS data for interactive effects within known biological contexts, Bush et al. (2011) found gene-gene interaction between three pairs of genes in the field of calcium-signaled cytoskeletal regulation. Our result of an epistatic effect between SNPs in two genes within the AhR signaling cascade further underlines the importance of analyzing pathways comprehensively and within their biological contexts, otherwise modest or epistatic effects may remain undetected. Additional studies in larger cohorts are needed to replicate the current results.

In addition to gene-gene interactive effects, there are gene-environment interactions that complicate genetic investigations. Even large-scale GWAS studies, including thousands of patients and controls worldwide, cannot explain significant parts of MS heritability (Canto and Oksenberg, 2018; Manolio et al., 2009). Besides infections, vitamin

D and diet, among others, smoking is an important environmental trigger that has long been discussed for MS pathogenesis. A recent meta-analysis supported a causal involvement of smoking both in the development and the progression of MS (Degelman and Herman, 2017). Interestingly, an interaction between AhR variation and smoking was recently shown for risk of lung cancer, with smokers carrying the SNP rs2066853 in the *AHR* gene showing a decreased risk (Budwhar et al., 2018). In the present study, we only found modest evidence for an association of rs7796976 (*AHR*) and rs1800566 (*NQO1*) with MS in smokers that did not withstand Bonferroni correction. This may be explained by the fact that smoking data were only available for a small subgroup (~200 each) of the MS patients and controls, hampering profound statistical analyses. In an attempt to better understand gene-environment interactions, Mechelli et al. (2013) performed a so-called "candidate-interactome" analysis of GWAS data in MS. Here, AHR did not belong to the three of 13 interactomes that showed a statistical enrichment of associations; however, this analysis was primarily centered on viral interactions. Additional prospective studies in larger cohorts are therefore warranted to further evaluate the role of an interaction between smoking and SNPs in AhR signaling genes.

5. Conclusion

In conclusion, our data give a first hint that both single-gene association as well as gene-gene interactive effects in the AhR signaling pathway may contribute to MS pathogenesis, but confirmation of these results in larger independent cohorts is recommended. Profound knowledge about genetic variation in the AhR pathway appears mandatory assuming that targeting of the AhR as a therapeutic treatment has already been proposed (reviewed in Roman et al., 2018). However, issues of safety and potential toxicity will have to be further addressed before AHR agonists can be considered as therapeutic option in chronic inflammatory diseases such as MS (Ehrlich and Kerkvliet, 2017).

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