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CLINICAL REPORT

Loss-of-function polymorphisms in NQO1 are not associated with the development of subacute myelo-optico-neuropathy

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Abstract

Background: Subacute myelo-optico-neuropathy (SMON) is a neurological disorder associated with the administration of clioquinol, particularly at very high doses. Although clioquinol has been used worldwide, there was an outbreak of SMON in the 1950s–1970s in which the majority of cases were in Japan, prompting speculation that the unique genetic background of the Japanese population may have contributed to the development of SMON. Recently, a possible association between loss-of-function polymorphisms in *NQO1* and the development of SMON has been reported. In this study, we analyzed the relationship between *NQO1* polymorphisms and SMON in Japan.

Methods: We analyzed 125 Japanese patients with SMON. *NQO1* loss-of-function polymorphisms (rs1800566, rs10517, rs689452, and rs689456) were evaluated. The allele frequency distribution of each polymorphism was compared between the patients and the healthy Japanese individuals (Human Genomic Variation Database and Integrative Japanese Genome Variation Database), as well as our in-house healthy controls.

Results: The frequencies of the loss-of-function *NQO1* alleles in patients with SMON and the normal control group did not differ significantly.

Conclusion: We conclude that known *NQO1* polymorphisms are not associated with the development of SMON.

KEYWORDS

clioquinol, Japanese, NQO1, subacute myelo-optico-neuropathy

1 | INTRODUCTION

Subacute myelo-optico-neuropathy (SMON) is a neurological disorder associated with the administration of clioquinol (Nakae et al., 1973; Tateishi et al., 1971). It mainly affects the optic nerves, spinal cord, and peripheral nerves (Kono, 1971). The pharmacological mechanism underlying the neurotoxicity of clioquinol has been analyzed from various perspectives and is gradually being elucidated (Katsuyama et al., 2012, 2021; Kawamura et al., 2014; Kimura et al., 2011; Schaumburg & Herskovitz, 2008). However, the genetic factors involved in the pathogenesis of SMON have not yet been revealed. Although SMON occurs worldwide, the vast majority of cases have been reported in Japan, most of which were in an outbreak that occurred between 1957 and 1970 (Meade, 1975). Although an excessive dose of clioquinol is thought to be the main etiological factor (Sobue et al., 1973), it is hypothesized that unique genetic factors in the Japanese population may contribute to the development of SMON. Perez et al. suggested a possible relationship between the development of SMON and *ABCC4* (OMIM: 605250) and *ABCC11* (OMIM: 607040) polymorphisms (Perez et al., 2019). *ABCC4* and *ABCC11* are related to the cAMP transport pump. The *ABCC4* rs3765534 polymorphism (c.2269G>A, p.Glu757Lys) dramatically reduces transporter function by disrupting the localization of the ABCC4 protein in the plasma membrane (Krishnamurthy et al., 2008). The *ABCC11* rs17822931 polymorphism (c.538G>A, p.Gly180Arg) is involved in apocrine gland secretion and is associated with a certain type of earwax and the onset of axillary osmidrosis (Super Science High School Consortium, 2009; Toyoda et al., 2009). Both the *ABCC4* rs3765534 and the *ABCC11* rs17822931 polymorphisms are more common in Japanese people than in Europeans. However, in our previous study, we did not detect an association between the development of SMON and these polymorphisms in the Japanese population (Matsumoto et al., 2022).

Recently, on the basis of an in vitro functional assay, Chhetri et al. reported that the loss of function of *NQO1* (OMIM: 125860, NM_000903.3) may be associated with the development of SMON (Chhetri et al., 2022). In particular, the *NQO1* rs1800566 polymorphism (c.559C>T, p. Pro187Ser) (Lienhart et al., 2014), which is common in the Japanese population (Table 1), may be associated with the development of SMON. In this study, we analyzed the *NQO1* rs1800566 polymorphism and other previously reported loss-of-function polymorphisms in *NQO1*, rs10517 (c.*1119 T>C; 3′ UTR variant) (Tian et al., 2021), rs689452 (c.8-27G>C; intron variant) (Hemissi et al., 2021), and rs689456 (c.-1221G>A; upstream transcript variant) (Reddy et al., 2009), using genomic data for patients with SMON.

2 | MATERIALS AND METHODS

2.1 | Recruitment of patients with SMON and DNA extraction

This study was based on samples used in a previous report (Matsumoto et al., 2022). The participants were Japanese SMON patients who participated in the "SMON health examination by the SMON research group members" (health screening specifically for SMON patients

TABLE 1 Frequency distribution of *NQO1* polymorphisms among the populations reported in gnomAD (data set: v2.1.1).

	Allele frequency (allele count)							
Population	**rs1800566**		**rs10517**		**rs689452**		**rs689456**	
Japanese	0.3158	(48)	nd	nd	0.6458	(93)	nd	nd
Korean	0.4175	(1594)	nd	nd	0.6307	(2408)	nd	nd
Other East Asian	0.4668	(6730)	0.6208[a]	(966)[a]	0.6261	(8979)	0.6184[a]	(956)[a]
South Asian	0.3143	(9587)	nd	nd	0.8320	(25140)	nd	nd
Latino/Admixed American	0.3896	(13770)	0.9269	(786)	0.9394	(32888)	0.9304	(789)
European (non-Finnish)	0.1886	(23857)	0.8637	(13319)	0.8800	(111292)	0.8767	(13513)
European (Finnish)	0.183	(4591)	0.8440	(2932)	0.8632	(21309)	0.8627	(2997)
Ashkenazi Jewish	0.1839	(1903)	0.8345	(242)	0.8489	(8542)	0.8345	(242)
African/African-American	0.1857	(4632)	0.8548	(7449)	0.8616	(21344)	0.8568	(7447)
Other	0.2297	(1651)	0.8490	(922)	0.8621	(6133)	0.8566	(932)
Total	0.2468	(69078)	0.8480	(26616)	0.8595	(239092)	0.8572	(26876)

[a]Overall of East Asian.

Abbreviation: nd, no data.

provided through a national policy) from 2016 to 2020 (total number of patients screened: 297). All patients were administered clioquinol, but accurate records of dosage and duration of administration do not exist. All patients presented with typical symptoms of SMON and were excluded from other diseases. Peripheral blood samples of 125 SMON patients who gave written informed consent were collected from collaborating institutions. The details of the DNA extraction and a summary of the clinical information for patients with SMON enrolled in this study can be found in this previous study.

2.2 | Details of in-house normal controls

In total, 101 DNA samples collected for another study about genetic analysis with approval for secondary use conducted at Gifu University (Protocol number: 29-322) were included as normal controls (non-SMON). These 101 samples were used after written informed consent for the secondary use of DNA was obtained.

2.3 | Single nucleotide polymorphism genotyping assay using the Sanger sequencing method

The following primers were used for the single nucleotide polymorphism (SNP) analysis: *NQO1* rs1800566, forward 5′-AAGCCCAGACCAACTTCT-3′ and reverse 5′-GCGTTTCTTCCATCCTTC-3′; *NQO1* rs10517, forward 5′-TGCCAAAACTGTTCACCAAA-3′ and reverse 5′-AAATCCACACCGTACATCTGC-3′; *NQO1* rs689452, forward 5′-CATGGCATAGAGGTCCGACT-3′ and reverse 5′-CCCTGTAGCTGAAGGTTTGC-3′; *NQO1* rs689456, forward 5′-GGGTGACGAAGTGAGACTCC-3′ and reverse 5′-ACTTTTGCACTGGAGGGACA-3′. The sequences were analyzed using a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA; Cat. No. 4337450) and an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems).

2.4 | SNP genotyping assay using the TaqMan PCR method

PCR was performed using a StepOne RealTime PCR System (48-well format) (Applied Biosystems), TaqMan GTXpress Master Mix (Applied Biosystems; Cat. No. 4401892), and TaqMan SNP genotyping assay mix (*NQO1* rs1800566 polymorphism, Assay ID C___2091255_30, Cat. No. 4362691, *NQO1* rs10517 polymorphism, Assay ID C___8729256_30, Cat. No. 4362691, *NQO1* rs689452 polymorphism, Assay ID C___1035493_1_, Cat. No. 4351379, *NQO1* rs689456 polymorphism, Assay ID C___1035499_10, Cat. No. 4351379; Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's instructions.

2.5 | Statistical analysis of polymorphisms

Allele frequencies of *NQO1* rs1800566 (Ref: C/Alt: T), *NQO1* rs10517 (Ref: T/Alt: C), *NQO1* rs689452 (Ref: G/Alt: C), and *NQO1* rs689456 (Ref: G/Alt: A) for different ethnicities were collected from the Genome Aggregation Database (gnomAD) data set: v2.1.1 (Karczewski et al., 2020). The frequency distributions of the four polymorphisms in patients with SMON and healthy Japanese people, based on the Human Genomic Variation Database (HGVD) (Higasa et al., 2016; Narahara et al., 2014), the Japanese Multi Omics Reference Panel (jMorp) data set: ToMMo 54KJPN (Tadaka et al., 2023), and in-house normal controls, were evaluated. Dominant and recessive genetic models were used for comparisons of allele frequencies. In the analysis of the *NQO1* rs1800566 polymorphism, the SMON group was further divided into two groups according to the severity at onset, one with relatively severe symptoms (ocular or neurological symptoms) and the other with relatively mild symptoms (same as above), and the frequency of each allele was compared between the SMON and normal control groups (subgroup analysis). The group with more severe visual impairment included patients with total blindness, light/dark only, hand motion, and counting fingers. The group with mild visual impairment included patients with mildly reduced or almost normal vision. The group with more severe gait disturbance included patients who were unable to walk. The mild gait disturbance group included patients with the following: wheelchair, needing assistance, clinging, crutches, single cane, unsteady walking, and slight unsteady walking. Statistical analyses were performed using Prism 9 (GraphPad Software, LLC, San Diego, CA, USA). Differences were evaluated using Pearson's chi-squared test. Statistical significance was set at $p < 0.05$.

3 | RESULTS

The frequencies of *NQO1* rs1800566, *NQO1* rs10517, *NQO1* rs689452, and *NQO1* rs689456 polymorphisms in different populations registered in the gnomAD are shown in Table 1. The frequency of the alternative (Alt) T allele at *NQO1* rs1800566 was much higher in the Japanese population than in other populations. The allele frequencies of *NQO1* rs10517 reference (Ref) (C) and Alt (T), *NQO1*

rs689452 (Ref) (G) and Alt (C), and *NQO1* rs689456 (Ref) (G) and Alt (A) were reversed in the East Asian population, including Japanese people, compared with those in other populations (European, Ashkenazi Jewish, African, and Other). The allele and genotype frequency distributions of *NQO1* rs1800566, *NQO1* rs10517, *NQO1* rs689452, and *NQO1* rs689456 polymorphisms in patients with SMON and healthy Japanese people (HGVD, jMorp databases, and in-house normal controls) are shown in Tables 2 and 3, respectively. We did not detect a significant difference in the frequencies of these four polymorphisms between patients with SMON and healthy Japanese people. Note that *NQO1* rs689452 and rs689456 had the same frequencies in the SMON group and the in-house normal control group. Focusing on the rs1800566 polymorphism, we further divided the SMON group into two groups, one with relatively severe ocular or neurological symptoms and the other with relatively mild symptoms. Table 4 shows the results of the subgroup analysis, comparing allele frequencies under dominant and recessive models in patients with SMON with severe or mild symptoms and normal controls. Even when the analysis was limited to patients with SMON with severe ocular or neurological symptoms, we did not detect a significant difference in frequency between patients and the healthy Japanese population.

4 | DISCUSSION

In this study, we evaluated *NQO1* variants that were recently identified as possible genetic susceptibility factors for SMON. The frequencies of the *NQO1* rs1800566, *NQO1* rs10517, *NQO1* rs689452, and *NQO1* rs689456 polymorphisms did not differ significantly between patients with SMON and healthy Japanese people. Therefore, we could not verify the association between the development of SMON and these four polymorphisms, at least in terms of allele frequencies.

Various studies have provided insight into the pharmacological mechanism of the neurotoxicity of clioquinol. Although clioquinol affects cellular function via multiple pathways, it has a well-established role as a chelator of iron, copper, and zinc ions (Mao & Schimmer, 2008). Clioquinol causes increased iron uptake into neural cells as well as lipid peroxidation and cell death (Yagi et al., 1985), a copper deficiency (Kimura et al., 2011; Schaumburg & Herskovitz, 2008), and zinc influx and copper accumulation (Katsuyama et al., 2021). The acceleration of copper excretion by clioquinol suppresses the decomposition of reactive oxygen species by decreasing the activity of SOD1, which requires copper and zinc in its active center. This action induces oxidative stress, which has been proposed as a mechanism underlying neurological damage (Kawamura et al., 2014). The factors involved in the metabolism of reactive oxygen species are thought to be important in the development of SMON.

In 2022, Chhetri et al. reported that oxidative stress is elicited in the process of neuropathy induced by clioquinol in cultured cells and zebrafish and that the enzymatic activity of NADPH-quinone-oxidoreductase 1 (NQO1; EC 1.6.5.2) suppresses this oxidative stress. NQO1 is a cytoplasmic flavin adenine dinucleotide (FAD)-dependent flavoprotein and acts as a multifunctional antioxidant enzyme. The main functions of NQO1 are thought to include the reduction of free radicals in cells and the detoxification of xenobiotics. Oxidative stress induces NQO1 via NF-E2 p45-related factor 2 (Nrf2) and the aromatic hydrocarbon receptor (AhR), thereby preventing the formation of reactive semiquinones and exerting a protective effect against oxidative stress. NQO1 can also directly scavenge superoxide radicals and protect against oxidative stress (Ross & Siegel, 2021). Several polymorphisms in *NQO1* have been reported to reduce NQO1 enzyme activity. The most prominent loss-of-function polymorphism is rs1800566 (Lienhart et al., 2014). The enzyme activity of NQO1 is reduced by almost half that of the wild-type genotype in

TABLE 2 Frequency distribution of *NQO1* polymorphisms in patients with SMON and databases of healthy Japanese individuals.

		Genotype				Statistical analysis	
SNP	**Population**	**Ref/Ref**	**Ref/Alt**	**Alt/Alt**	**Total**	**Dominant model**	**Recessive model**
rs1800566	SMON patients	39	68	18	125	$p=0.091$	$p=0.586$
	HGVD	471	542	197	1210		
rs10517	SMON patients	8	62	50	120	$p=0.381$	$p=0.145$
	Normal controls	10	39	52	101		
rs689452	SMON patients	8	60	52	120	$p=0.532$	$p=0.099$
	Normal controls	9	37	55	101		
rs689456	SMON patients	8	60	52	120	$p=0.532$	$p=0.099$
	Normal controls	9	37	55	101		

Abbreviation: HGVD, healthy Japanese individuals (HGVD).

TABLE 3 Frequency distribution of *NQO1* polymorphisms in patients with SMON and databases of healthy Japanese individuals (HGVD and jMorp data set: ToMMo 54KJPN).

		Allele number			Statistical analysis		
SNP	Population	Ref	Alt	Total	Comparing SMON patients with HGVD	Comparing SMON patients with jMorp	Comparing SMON patients with normal controls
rs1800566	SMON patients	146	104	250	$p=0.321$	$p=0.159$	nd
	HGVD	1484	936	2420			
	jMorp	68111	40493	108604			
rs10517	SMON patients	78	162	240	$p=0.429$	$p=0.997$	$p=0.456$
	HGVD	2270	4220	6490			
	jMorp	35310	73294	108604			
	Normal controls	59	143	202			
rs689452	SMON patients	76	164	240	$p=0.596$	$p=0.815$	$p=0.309$
	HGVD	1112	2224	3336			
	jMorp	33630	74974	108604			
	Normal controls	55	147	202			
rs689456	SMON patients	76	164	240	nd	$p=0.820$	$p=0.309$
	jMorp	33651	74953	108604			
	Normal controls	55	147	202			

Abbreviations: HGVD, healthy Japanese individuals (HGVD); jMorp, healthy Japanese individuals (jMorp data set: ToMMo 54KJPN); nd, no data.

the heterozygous genotype (C/T) and is almost zero for the homozygous genotype (T/T) (Siegel et al., 1999). The frequency of the *NQO1* rs1800566 T/T genotype is 4% to 20%, depending on the ethnic group, with the highest prevalence in Asian populations (Kelsey et al., 1997), especially in Japan. *NQO1* rs10517, like the *NQO1* rs1800566 polymorphism, affects warfarin metabolism by affecting NQO1 function (Tian et al., 2021). *NQO1* rs689452 is an intronic polymorphism located in a protein-binding motif with the potential to modulate NQO1 protein activity. The *NQO1* rs689452 polymorphism is involved in the progression of muscle-invasive bladder cancer by reducing p53 stability (Hemissi et al., 2021). *NQO1* rs689456 is located in the promoter region of *NQO1* and has protective effects against acute lung injury by downregulating NQO1 (Reddy et al., 2009). Like the rs1800566 polymorphism, these three polymorphisms are also expected to reduce NQO1 enzyme activity by decreasing NQO1 expression or affecting its function.

We analyzed previously reported loss-of-function polymorphisms in *NQO1*, particularly rs1800566, a common SNP in the Japanese population; however, we did not find any difference in allele frequencies between patients with SMON and healthy Japanese controls. There are three possible explanations for this result. The lack of a relationship may be explained by the small sample size. Although our cohort included the largest number of patients with SMON evaluated to date, it is difficult to increase the sample size further, as this would require new cases of SMON. Second, it is possible that another SNP is associated with the loss of NQO1 function, and this additional SNP may be significantly more common in the Japanese population than in other populations. A third possibility is that NQO1 is not involved in the development of SMON alone but interacts with other factors associated with the metabolism of clioquinol. Chhetri et al. reported an association between loss of NQO1 function and the development of SMON in cultured cells and in visual experiments of zebrafish, but our study did not show the same conclusion in humans. There are several possible reasons for this result. One is the possibility that the metabolism of clioquinol in cultured cells and zebrafish differs from that in humans in vivo. It is also possible that there are other antioxidant capabilities that compensate for the reduced function of NQO1 in humans in vivo. Third, factors other than oxidative stress may be more significant in clioquinol toxicity. There are two major hypotheses for the pathogenesis of SMON: one is that factors related to the oxidative stress pathway have a strong influence, and the other is that drug-sensitivity genes have a strong influence. In the future, genetic analysis of drug-sensitivity genes should also be conducted.

To prevent drug-induced disorders similar to SMON in the future, it is important to understand the pathogenesis of SMON. Therefore, we will continue to search for other genetic factors associated with SMON development using the

TABLE 4 Subgroup analysis of the degree of ocular and neurological symptoms and the *NQO1* rs1800566 polymorphism in patients with SMON.

Symptom	Group	Genotype				Statistical analysis		Allele number			Statistical analysis	
		Ref/Ref	Ref/Alt	Alt/Alt	Total	Dominant model	Recessive model	Ref	Alt	Total	Comparing SMON patients with HGVD	Comparing SMON patients with jMorp
Ocular symptoms	SMON (severe)	9	16	3	29	$p=0.466$	$p=0.429$	34	22	56	$p=0.926$	$p=0.757$
	SMON (mild)	30	51	14	95	$p=0.156$	$p=0.694$	111	79	190	$p=0.430$	$p=0.221$
	HGVD	471	542	197	1210	–	–	1484	936	2420	–	–
	jMorp	nd	nd	nd	nd	–	–	68111	40493	108604	–	–
Neurological symptoms	SMON (severe)	17	30	8	55	$p=0.232$	$p=0.733$	64	46	110	$p=0.509$	$p=0.326$
	SMON (mild)	22	38	10	70	$p=0.210$	$p=0.659$	82	58	140	$p=0.516$	$p=0.311$
	HGVD	471	542	197	1210	–	–	1484	936	2420	–	–
	jMorp	nd	nd	nd	nd	–	–	68111	40493	108604	–	–

Abbreviations: HGVD, healthy Japanese individuals (HGVD); jMorp, healthy Japanese individuals (jMorp data set: ToMMo 54KJPN); SMON (severe), severe symptoms group of SMON patients; SMON (mild), mild symptoms group of SMON patients; nd, no data.

largest database of patients with SMON. Comprehensive analyses, including analyses of all SNPs in *NQO1*, and identification of drug-sensitivity genes are required in future research. This study had limitations similar to those reported previously (Matsumoto et al., 2022). In particular, data for the total dose and duration of clioquinol administration in patients with SMON were lacking.

5 | CONCLUSION

No clear association was found between known loss-of-function polymorphisms in *NQO1* (rs1800566, rs10517, rs689452, and rs689456) and the development of SMON. It will be necessary to investigate other genetic factors involved in the development of SMON in the future.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICAL CONSIDERATIONS

This study was approved by the ethics review committee on medical research at Gifu University (Protocol number: 29-209). Institutions of collaborations also provided ethical approval.

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