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ORIGINAL ARTICLE

Myeloperoxidase genetic polymorphisms and susceptibility to Kawasaki disease in Taiwanese children

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KEYWORDS
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Abstract *Background/purpose:* The aim of this study was to investigate the myeloperoxidase (MPO) -463G>A polymorphism in Kawasaki disease (KD) patients, and the relationship between gene polymorphism and MPO levels.
Methods: A total of 334 KD children and 492 sex-matched controls were assayed for polymorphism analysis. TaqMan assays were used for genotyping. MPO was measured in 37 KD patients and 42 febrile controls.
Results: A significant linear trend of KD risk was found to be related to the G/G genotype ($p_{\text{linear trend}} = 0.032$). The combined genotypes (G/A and A/A) of MPO -463G>A were associated with a significantly decreased KD risk compared to the G/G genotype [adjusted odds ratios (AOR) = 0.71, 95% confidence interval (CI): 0.52–0.99, $p = 0.040$]. In addition, KD patients with

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A allele were associated with a significantly decreased KD risk as compared to those with G allele (AOR = 0.73, 95% CI: 0.54—0.98, $p = 0.033$). MPO levels were significantly elevated in KD patients in preintravenous immunoglobulin (pre-IVIG) stage compared to febrile controls ($p = 0.002$). KD patients in pre-IVIG stage had significantly higher MPO levels than febrile controls in terms of G/G genotype ($p = 0.003$) and G allele ($p < 0.001$). KD patients with A allele had significantly lower MPO levels than those with G allele in post-IVIG acute stage ($p = 0.042$). However, there was no significant difference of individual MPO change for KD patients from pre- to post-IVIG stage in terms of genotypes ($p = 0.837$) or alleles ($p = 0.631$).

Conclusion: Our results suggest that G allele of MPO -463G>A polymorphism is a potential genetic marker for KD risk in Taiwanese children.

Introduction

Kawasaki disease (KD), an acute systemic vasculitis with a predilection for the Asian race, occurs mainly in infants and children under 5 years of age. It is frequently complicated by the development of coronary artery lesions (CALs) and recognized as the leading cause of acquired heart disease in children.[1] Treatment with a single high dose of intravenous immunoglobulin (IVIG) is effective in reducing the incidence of CALs.[2,3] Its pathogenesis remains unclear.

Oxidative stress is a disturbance in the balance of reactive oxygen species (ROS) and antioxidants, leading to damage of lipids, proteins, and nucleic acids. Niwa and Sohmiya[4] found that oxygen intermediates increased during the early stage of KD. Shen and Wang[5] reported a KD infant with IVIG resistance who showed dramatic responsiveness to the addition of antioxidants (a-tocopherol and ascorbic acid). Cheung et al's[6] findings suggest that oxidative stress is increased in KD patients with coronary aneurysms and is associated with carotid intima-media thickening and stiffening. Takatsuki et al[3] found that KD patients in acute stage suffered from obvious hyperoxidant stress, which improved in response to IVIG treatment in most patients. Kaneko et al[7] demonstrated that children with acute KD experienced oxidative stress characterized by increased ROS and decreased biological antioxidant potential. Straface et al's[8] observations further lead to hypothesize that the simultaneous oxidative and nitrative stress may play a pathogenic role in the coronary arteritis associated with KD. These studies[3—8] suggest that oxidative stress plays an important role in the pathogenesis of KD in both the acute and chronic stages.

Myeloperoxidase (MPO), a highly oxidant enzyme, generates ROS and is a key biomarker of vascular inflammation.[9,10] Elevated MPO levels have been detected in acute KD patients.[8,11] A recent study with MPO-deficient mice did not show vasculitis by using a murine model of KD.[12] These limited data on humans or animals[8,11,12] suggest potential mechanisms for MPO in the development of KD. Research into MPO polymorphism has largely focused on a single nucleotide polymorphism -463G>A (Genbank IDs: rs2333227).[13] G allele binds the SP1 transcription factors more strongly and is associated with 25-fold higher MPO levels than A allele.[14] Therefore, it is biologically plausible that MPO -463G>A polymorphism may modulate the risk of KD. MPO -463G>A polymorphism is reported to be associated with susceptibility to several vascular diseases, including coronary artery disease,[15—17], lupus nephritis,[18] and MPO-ANCA-associated vasculitis in women.[19] There has been no research on MPO polymorphism and risk of KD. The purpose of this study was to investigate MPO -463G>A polymorphism in KD patients. In addition, the relationship of genotypic and allelic types with MPO levels in KD was also evaluated.

Materials and methods

Patients studied

We performed a study at the Department of Pediatrics, Kaohsiung Veterans General Hospital (KVGH), Kaohsiung, Taiwan. Medical records of all children who fulfilled the diagnostic criteria for KD[1] in our hospital between 1990 and 2013 were reviewed. Medical records were reviewed for age, sex, presenting symptoms, complications, and laboratory data within 7 days of illness. A total of 334 unrelated Han-Chinese children who met the established criteria of acute KD and had no evidence of viral or bacterial infection were enrolled for polymorphism analysis. Patients were treated with a high dose IVIG (2 g/kg) as a single infusion for 10—12 hours without concomitant aspirin therapy[1] at KVGH. Patients who had defervescence within 48 hours after the completion of IVIG treatment were categorized as the IVIG-responsive group, and those whose fever persisted or recrudesced for at least 48 hours but not longer than 7 days after completion of IVIG treatment were categorized as the IVIG-resistant group. A total of 250 patients who received initial IVIG treatment within 10 days of fever were recruited for analysis of IVIG resistance, excluding 84 patients who had missing clinical data ($n = 22$), did not receive IVIG treatment ($N = 55$), or received initial IVIG treatment beyond 10 days of fever ($n = 7$). Acute and chronic CALs are defined, respectively, as CALs within 2 weeks of illness and beyond 1 year. CALs were defined as follows: (1) internal lumen diameter $\geq$ 3 mm in children younger than 5 years; (2) internal lumen diameter $\geq$ 4 mm in children older than 5 years, (3) internal lumen diameter

Table 1 Comparisons of demographic data in KD patients and controls.

Factor/category	MPO polymorphism analysis			Combined MPO level and polymorphism analysis		
	KD children (n = 334)	Controls (n = 492)	p	KD children (n = 37)	Febrile controls (n = 42)	p
	n (%)	n (%)		n (%)	n (%)	
Age (mo; Mean ± SD)	28.4 ± 28.8	615.5 ± 120.8	**< 0.001**[a]	24.7 ± 26.5	25.2 ± 15.7	0.920[a]
≤ 12	103 (30.8)	0 (0)	**< 0.001**[b]	12 (32.4)	11 (26.2)	0.533[c]
12–60	196 (58.7)	0 (0)		24 (64.9)	31 (73.8)	
> 60	35 (10.5)	492 (100.0)		1 (2.7)	0 (0.0)	
Sex			0.999[b]			0.957[b]
Female	131 (39.2)	193 (39.2)		13 (35.1)	15 (35.7)	
Male	203 (60.8)	299 (60.8)		24 (64.9)	27 (64.3)	
Clinical data						
With/without IVIG resistance/excluded	29/221/84			3/34/0		1.000[d]
With/without acute CALs/excluded	125/186/23			20/17/0		0.366[e]
With/Without chronic CALs/excluded	33/278/23			2/35/0		1.000[f]

Values in bold font were statistically significant.
[a] p value was estimated by Mann–Whitney U test.
[b] p value was estimated by Chi-square test.
[c] p value was estimated by Fisher's exact test.
[d] p value was estimated by Fisher's exact test after 84 KD children were excluded.
[e] p value was estimated by Chi-square test after 23 KD children were excluded.
[f] p value was estimated by Fisher's exact test after 23 KD children were excluded.
CAL = coronary artery lesion; KD = Kawasaki disease; MPO = myeloperoxidase; SD = standard deviation.

1.5 times that of an adjacent segment; or (4) clearly irregular coronary artery lumen.[20] A total of 311 patients were recruited for analysis of CALs, excluding 23 patients with missing clinical data. For polymorphism analysis, the control group comprised 492 sex-matched Han-Chinese controls without individual histories of KD, and autoimmune, allergic, or inflammation-associated diseases. For the combined MPO level and polymorphism analysis, we enrolled 37 KD children with similar demographic [age (p = 0.239), sex (p = 0.875)] and clinical data to the 334 KD children (Table 1). Forty-two febrile Han-Chinese controls with a clinically viral syndrome as well as without a history of KD were recruited and matched to 37 KD cases on age and sex.

Blood samples were collected for the analysis of MPO levels and polymorphism. This study was approved by the Institutional Review Board of KVGH. All written informed consents were obtained from guardians on the behalf of the children involved in this study and from all adult control individuals.

Polymorphism genotyping

MPO (-463G>A, rs2333227) was performed by the TaqMan real-time polymerase chain reaction assay using the ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Factor City, CA, USA) in the 96-well format. Polymerase chain reactions were carried out in a reaction volume of 10 μL containing 10 ng DNA, 5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 0.5 μL 20× primer/probe mixture, and ddH_2O (added to a final volume of 10 μL). The allelic-specific fluorescence data from each plate were analyzed using the SDS versin 1.3.1 software (Applied Biosystems, 2005) to automatically determine the genotype of each sample.

MPO measurements

MPO level in four different stages was measured in 37 KD patients. Plasma sample was collected in pre-IVIG acute (5.1 ± 1.9 days from fever onset), post-IVIG acute (9.3 ± 2.6 days from fever onset), subacute (36.2 ± 15.8 days from fever onset), and convalescent (186.2 ± 160.4 days from fever onset) stages in these KD patients. MPO level was also measured in 42 age- and sex-matched febrile controls with a clinically viral syndrome (4.9 ± 1.8 days from fever onset). The MPO standards, background, and samples were measured in random duplicate using human Cardiovascular Disease Panel 2 Magnetic Bead Kit (cat. # HCVD2MAG-67K, Luminex, magnetic bead) precisely according to the guideline of MILLIPLEX MAP assay.

Statistical analysis

Demographic and clinical data between patients with/without CALs, those with/without IVIG resistance, and controls were compared by the Chi-square test, Student t test, or Mann–Whitney U test. For each tested single nucleotide polymorphism (SNP), departure from Hardy–Weinberg equilibrium in adult controls was evaluated by comparing the observed genotypic frequencies with the expected ones using a goodness-of-fit Chi-square test.

Allelic and genotypic frequencies of MPO were compared between patients with/without CALs, those with/without IVIG resistance, and controls using simple and multiple logistic regression, respectively. In addition, multiple logistic regression was used to evaluate the association between allelic types, and genotypes of SNPs in MPO with the risk of CALs, IVIG resistance, and KD, by adjusting various confounders, such as sex, age, etc. The crude odds ratios, adjusted odds ratios (AORs), and 95% confidence intervals (CIs) were all estimated using multiple logistic regression models. Expression levels of MPO were presented as median (range). The difference in demographic and clinical data between KD cases and controls were evaluated by the Chi-square test. Student *t* test, Mann–Whitney *U* test, and Kruskal–Wallis one-way analysis of variance were used to evaluate the differences of MPO expression levels and laboratory data between KD patients and controls, or between patients of various genotypes and allelic types. Wilcoxon sign rank test was used to evaluate KD patients' individual differences in MPO expression levels between pre- and post-IVIG stages. A p value <0.05 was considered statistically significant. The statistical software package of SPSS (version 17.0; IBM SPSS Inc., Chicago, IL, USA) and SAS/Genetics (version 9.1.3; SAS Institute Inc., Cary, North Carolina, USA) were used for all of the statistical analysis.

Results

Baseline patient demographic data are summarized in Table 1. A total of 334 KD patients (M/F 203/131, mean age 28.4 ± 28.8 months) and 492 sex-matched controls (M/F 299/193, mean age 615.5 ± 120.8 months) were assayed for polymorphism analysis. The proportion of older age (> 60 months) in KD patients was significantly less than that in controls ($p < 0.001$). There were significant differences between two groups in terms of age ($p < 0.001$). However, no significant differences were observed between two groups in terms of sex. There were 28 patients with IVIG resistance and 212 without IVIG resistance. The remaining 94 patients who had missing clinical data ($n = 32$), did not receive IVIG treatment ($N = 55$), or received initial IVIG treatment beyond 10 days of fever ($n = 7$) were excluded from the analysis of IVIG resistance. A total of 301 patients were recruited for the analysis of CALs, excluding 33 patients with missing clinical data.

A total of 37 KD patients (M/F 24/13, mean age 24.7 ± 26.5 months) and 42 age- and sex-matched febrile controls (M/F 27/15, mean age 25.2 ± 15.7 months) were recruited for combined MPO level and polymorphism analysis (Table 1). Of the 37 KD patients, three patients had IVIG resistance, 20 acute CALs, and two chronic CALs (Table 1).

Genotypic and allelic frequencies of MPO polymorphisms in KD patients and controls

The genotypic and allelic frequencies of MPO -463G>A polymorphisms in KD patients and controls are shown in Table 2. Genotype distributions of controls ($n = 492$) were in Hardy–Weinberg equilibrium ($p = 0.759$). Compared to G/A or A/A genotypes, there was no significant association between KD risk and the G/G genotype, but a significant linear trend of KD risk was found to be related to the G/G genotype ($p_{\text{linear trend}} = 0.032$). Further analysis revealed that the combined genotypes (G/A and A/A) of MPO -463G>A were associated with a significantly decreased KD risk than the G/G genotype (AOR = 0.71, 95% CI: 0.52–0.99, $p = 0.040$). In addition, patients with A allele were associated with a 1.37-fold increased protection of KD as compared to those with G allele (AOR = 0.73, 95% CI: 0.54–0.98, $p = 0.033$).

Age, sex, times of IVIG treatment, laboratory findings, and genotypic and allelic frequencies of MPO polymorphisms in KD patients with and without CALs in acute and chronic stages

A total of 311 patients with and without CALs in acute and chronic stages were recruited for analysis (data not shown).

Table 2 Distribution and odds ratios for risk of KD by various genotypic and allelic frequencies of MPO.

SNP of MPO (-463G>A) rs2333227	Genotype or allelic type	334 KD patients versus 492 controls					
		KD children	Controls	COR (95% CI)	p[a]	AOR (95% CI)	p[b]
		n (%)	*n* (%)				
	G/G	259 (77.5)	350 (71.1)	1.00		1.00	
	G/A	71 (21.3)	131 (26.6)	0.73 (0.53–1.02)	0.065	0.73 (0.53–1.02)	0.065
	A/A	4 (1.2)	11 (2.2)	0.49 (0.16–1.56)	0.228	0.49 (0.15–1.56)	0.228
				p for linear trend	**0.032**	p for linear trend	**0.032**
	G/A + A/A	75 (22.5)	142 (28.8)	**0.71 (0.52–0.985)**	**0.040**	**0.71 (0.52–0.99)**	**0.040**
	G allele	589 (88.2)	831 (84.5)	1.00		1.00	
	A allele	79 (11.8)	153 (15.5)	**0.73 (0.54–0.98)**	**0.033**	**0.73 (0.54–0.98)**	**0.033**
	χ^2		0.09				
	HW p value		0.759				

Values in bold font were statistically significant.
[a] p value was estimated by logistic regression.
[b] AOR adjusted for sex; p value was estimated by logistic regression.
AOR = adjusted odds ratio; COR = crude odds ratio; KD = Kawasaki disease; HW= Hardy-Weinberg; SNP=single nucleotide polymorphism; MPO = myeloperoxidase.

Male patients tended to have a higher risk of the acute CALs ($p = 0.033$). Compared to KD patients without acute CALs, those with acute CALs received more times of IVIG treatment ($p < 0.001$). KD patients with chronic CALs received more times of IVIG treatment than those without chronic CALs ($p = 0.032$). In case of acute CALs, there were significant differences between two groups in terms of white blood cell count ($p = 0.006$), neutrophil count ($p = 0.003$), and lymphocyte count ($p = 0.003$).

Genotype distributions of KD children without acute CALs ($n = 186$) or without chronic CALs ($n = 273$) were in Hardy–Weinberg equilibrium (among those without acute CALs, $p = 0.916$; among those without chronic CALs, $p = 0.930$). After adjusting confounders, no significant association was found between CALs and MPO -463G>A polymorphisms (data not shown).

Age, sex, laboratory findings, and genotypic and allelic frequencies of MPO polymorphisms in KD patients with and without IVIG resistance

A total of 250 patients who received initial IVIG treatment within 10 days of fever were recruited for analysis (data not shown). After chart review, 29 patients (M/F 19/10, mean age 32.3 ± 31.5 months) were identified as being IVIG resistant and the remaining 221 patients (M/F 132/89, mean age 25.8 ± 26.2 months) as being IVIG responsive. There were significant differences between the two groups in terms of white blood cell count ($p = 0.036$) and hemoglobin count ($p = 0.008$).

Genotype distributions of KD patients without IVIG resistance were in Hardy–Weinberg equilibrium ($p = 0.783$). After adjusting for confounders, no significant association was found between the risk of IVIG resistance and MPO -463G>A (data not shown).

Association between MPO level and MPO -463G > A polymorphisms in KD children and febrile controls

KD patients in pre-IVIG stage had significant higher MPO levels than febrile controls [254.0 (44–1459) ng/mL vs. 138.0 (53–547) ng/mL, $p = 0.002$, Figure 1]. MPO levels in KD patients decreased immediately after IVIG treatment [254.0 (44–1459) ng/mL vs. 131.0 (31–388) ng/mL, $p < 0.001$, Figure 1]. MPO levels in post-IVIG stage did not differ significantly from those in febrile controls [131.0 (31–388) ng/mL vs. 138.0 (53–547) ng/mL, $p = 0.420$, Figure 1]. MPO levels were further compared between pre-IVIG KD patients and febrile controls in terms of G/G and G/A+A/A genotypes. These levels in pre-IVIG KD patients were significantly higher than those in febrile controls in terms of G/G genotype ($p = 0.003$), but the above expression differences were not observed between two groups in terms of G/A+A/A genotypes ($p = 0.448$; Figure 2). Similarly, MPO levels of pre-IVIG KD patients and febrile controls were compared in terms of G and A alleles. These levels in pre-IVIG KD patients were significantly higher than those in febrile controls in terms of G allele ($p < 0.001$), but the above expression differences were not observed between two groups in terms of A allele ($p = 0.275$, Figure 2). Compared to febrile controls, upregulation of MPO levels was found to be correlated with G/G genotype ($p = 0.003$) and G allele ($p < 0.001$) in pre-IVIG KD patients.

Association of MPO levels with MPO -463G > A polymorphisms in KD children at various stages

Patients with GA or AA genotype had lower MPO levels than those with GG genotype in post-IVIG acute stage, but there was no statistical significance ($p = 0.099$, Table 3). Patients with A allele had significantly lower MPO levels than those with G allele in post-IVIG acute stage ($p = 0.042$, Table 3). However, there was no significant difference of individual

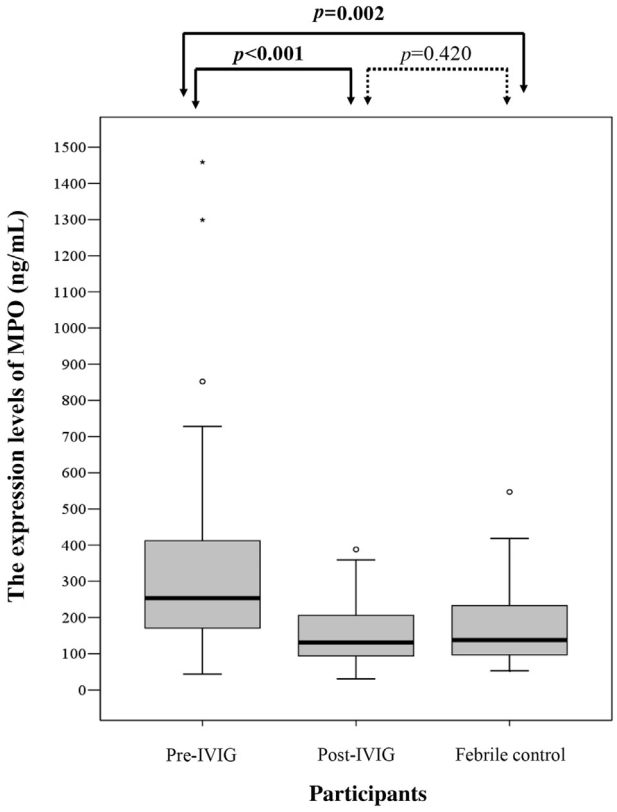

Figure 1. MPO levels of KD patients in acute stage and febrile controls. KD patients in pre-IVIG acute stage had significantly higher MPO levels than febrile controls. MPO levels in KD patients decreased immediately after IVIG treatment and did not differ significantly from those in febrile controls. Central box shows values from lower to upper quartile (25–75 percentile). In the box plots, the middle line represents the median. An outside value is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range (inner fences). These values are plotted with a round marker. A far out value is defined as a value that is smaller than the lower quartile minus three times the interquartile range, or larger than the upper quartile plus three times the interquartile range (outer fences). These values are plotted with an asterisk marker. IVIG = intravenous immunoglobulin; KD = Kawasaki disease; MPO = myeloperoxidase.

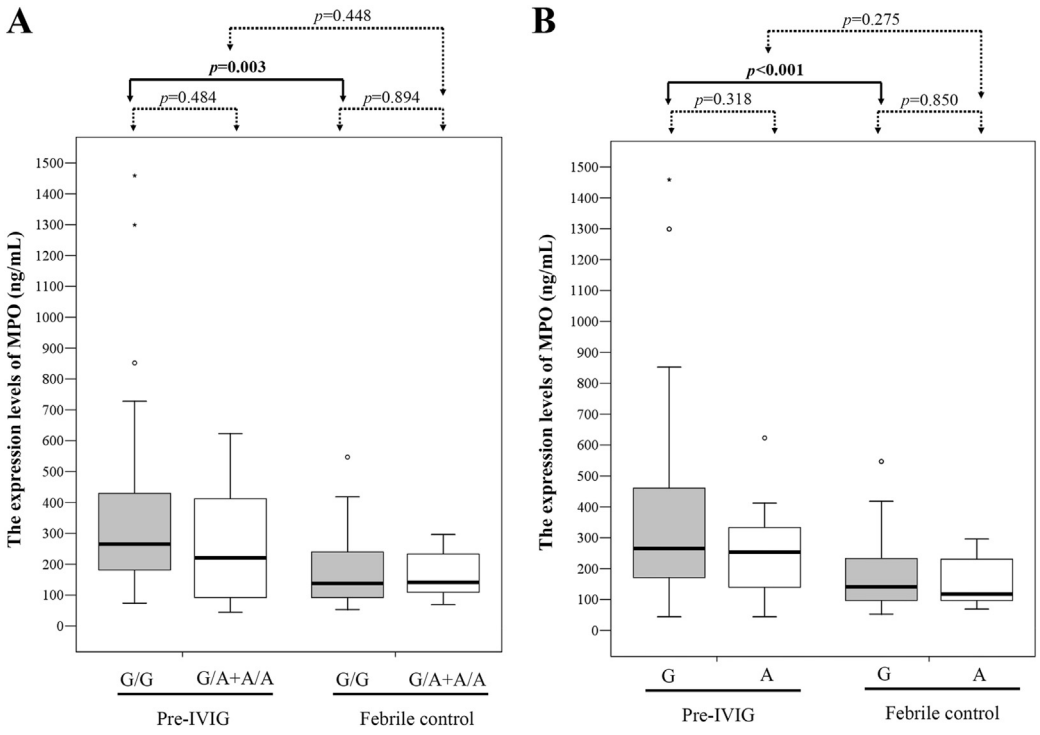

Figure 2. Expression of MPO in pre-IVIG KD patients and febrile controls according to the SNP of MPO (-463G>A) rs2333227. (A) Expression of MPO in pre-IVIG KD patients and febrile controls according to genotype of MPO (-463G>A). (B) Expression of MPO in pre-IVIG KD patients and febrile controls according to allelic type of MPO (-463G>A). IVIG = intravenous immunoglobulin; KD = Kawasaki disease; MPO = myeloperoxidase; SNP=single nucleotide polymorphism.

MPO change for KD patients from pre- to post-IVIG stage in terms of genotypes ($p = 0.837$) or alleles ($p = 0.631$).

Association of neutrophil number and MPO level with IVIG resistance, acute CALs, and chronic CALS in KD patients at various stages

There was no significant association of neutrophil number with IVIG resistance and chronic CALs at the four different stages (all $p > 0.05$; Table 4). There was a significant association between neutrophil number and acute CALS in pre-IVIG stage ($p = 0.006$), but no significant association between neutrophil number and acute CALS in the other three stages (all $p > 0.05$; Table 4). There was no significant association of MPO levels with IVIG resistance, acute CALs, and chronic CALs at the four different stages (all $p > 0.05$; Table 4).

Discussion

Our study showed that there was a significant association between MPO -463G>A polymorphism and susceptibility of KD in Taiwanese KD children. To the best of our knowledge,

Table 3 Comparisons of MPO level for KD patients by MPO -463G>A genotypes and alleles at various stages.

Stage	MPO -463G>A			MPO -463G>A		
	GG	GA+AA	p^b	G allele	A allele	p^b
	n (%)	*n* (%)		*n* (%)	*n* (%)	
	31 (83.8)	6 (16.2)		67 (90.5)	7 (9.5)	
	Median (range)	Median (range)		Median (range)	Median (range)	
Pre-IVIG	265.0 (74–1459)	220.8 (44–623)	0.484	265.0 (44–1459)	254.0 (44–623)	0.518
Post-IVIG	137.0 (31–388)	72.0 (51–239)	0.099	137.0 (31–388)	63.0 (51–239)	**0.042**
Subacute	89.0 (28–223)	67.5 (34–208)	0.434	89.0 (28–223)	59.0 (34–208)	0.196
Convalescent	58.5 (26–217)	63.5 (44–185)	0.578	58.5 (26–217)	74.0 (44–185)	0.276
Post-IVIG–pre-IVIG[a]	−116.0 (−1278 to 101)	−151.8 (−384 to 7)	0.837	−116.0 (−1278–101)	−197.0 (−384–7)	0.631

Values in bold font were statistically significant.
[a] Change in individual MPO levels between post-IVIG and pre-IVIG stages.
[b] *p* value was estimated by Mann–Whitney U test.
IVIG = intravenous immunoglobulin; KD = Kawasaki disease; MPO = myeloperoxidase.

Table 4 Comparisons of neutrophil number and MPO level of 37 KD patients by at various stages.

Factor/category	Pre-IVIG			Post-IVIG			Subacute			Convalescent		
	n (%)	Median (range)	*p*[a]	*n* (%)	Median (range)	*p*[a]	*n* (%)	Median (range)	*p*[a]	*n* (%)	Median (range)	*p*[a]
Neutrophil number ($\times 10^3/mm^3$)												
IVIG resistance												
No	34 (91.9)	5.8 (0.4–17.1)	0.373	34 (91.9)	2.7 (0.5–11.9)	0.541	26 (89.7)	2.3 (0.2–7.4)	0.474	15 (83.3)	2.0 (0.7–8.3)	0.953
Yes	3 (8.1)	4.8 (3.6–7.3)		3 (8.1)	4.3 (1.8–9.1)		3 (10.3)	3.2 (1.9–4.1)		3 (16.7)	2.0 (1.1–3.0)	
Acute CALs												
No	17 (45.9)	4.8 (0.4–12.9)	**0.006**	17 (45.9)	2.6 (0.6–11.9)	0.670	12 (41.4)	1.7 (0.2–7.4)	0.063	7 (38.9)	2.1 (1.3–3.2)	0.821
Yes	20 (54.1)	8.7 (3.6–17.1)		20 (54.1)	2.9 (0.5–9.6)		17 (58.6)	2.7 (1.0–4.9)		11 (61.1)	2.0 (0.7–8.3)	
Chronic CALs												
No	35 (94.6)	5.7 (0.4–17.1)	0.946	35 (94.6)	2.7 (0.5–11.9)	0.788	27 (93.1)	2.3 (0.2–7.4)	0.931	16 (88.9)	2.0 (0.7–8.3)	0.399
Yes	2 (5.4)	6.1 (4.8–7.3)		2 (5.4)	5.5 (1.8–9.1)		2 (6.9)	2.5 (1.9–3.2)		2 (11.1)	1.6 (1.1–2.1)	
MPO level												
IVIG resistance												
No	34 (91.9)	254 (44–1459)	0.978	34 (91.9)	129 (31–388)	0.540	34 (91.9)	83.5 (28–233)	0.436	34 (91.9)	63.3 (26–217)	0.330
Yes	3 (8.1)	289 (92–509)		3 (8.1)	223 (63–263)		3 (8.1)	88 (76–220)		3 (8.1)	45 (44–72)	
Acute CALs												
No	17 (45.9)	221 (79–1459)	0.464	17 (45.9)	107 (31–359)	0.190	17 (45.9)	95 (30–223)	0.796	17 (45.9)	58 (26–217)	0.927
Yes	20 (54.1)	300 (44–1299)		20 (54.1)	151.5 (50–388)		20 (54.1)	83 (28–220)		20 (54.1)	63.3 (26–185)	
Chronic CALs												
No	35 (94.6)	254 (44–1459)	0.523	35 (94.6)	131 (31–388)	0.840	35 (94.6)	89 (28–223)	0.840	35 (94.6)	58.5 (26–217)	0.687
Yes	2 (5.4)	190.5 (92–289)		2 (5.4)	143 (63–223)		2 (5.4)	82 (76–88)		2 (5.4)	58.5 (45–72)	

Values in bold font were statistically significant.

[a] *p* value was estimated by Mann–Whitney *U* test.

CAL = coronary artery lesion; IVIG = intravenous immunoglobulin; KD = Kawasaki disease; MPO = myeloperoxidase.

this was the first study to investigate the association between KD risk and MPO polymorphisms. Genotypic and allelic frequencies did not, however, differ between patients with and without CALs. There was also no significant association between the risk of IVIG resistance and MPO -463G>A. Compared to febrile controls, KD patients had significantly higher MPO levels in the pre-IVIG acute phase. In addition, patients with A allele had significantly lower MPO levels than those with G allele in the post-IVIG acute phase.

There are growing data on the involvement of oxidative stress in the overproduction of ROS in KD.[3,4,6–8] However, whether ROS is a cause or consequence of KD is still an unsolved issue. Furthermore, ROS are composed of abundant molecules, so their specificity as a signaling molecule has been questioned in a previous report.[21] These cross-sectional studies[3,4,6–8] need to be re-evaluated with other biomarkers for exploring the effects of oxidative stress on subsequent development of KD. MPO can generate an array of diffusible oxidants and contribute to oxidative stress.[22] It is abundant in granules of human inflammatory cells such as activated neutrophils, etc.[22] Neutrophil activation has been suggested to play an important role in KD pathogenesis.[23,24] In this series, the significant association between neutrophil number and acute CALs in KD patients provides partial evidence of the involvement of neutrophils in the pathogenesis of KD. The effect of MPO in vasculitis might be related to direct production of ROS, facilitation of neutrophil priming or activation, elevation of extracellular proteolytic activity, or contribution to endothelial dysfunction.[25] Therefore, MPO may be a potential key biomarker involved in the development of KD. Rider et al[11] reported that MPO levels in acute KD patients ($n = 14$) were significantly higher than those in adult normal controls. Straface et al[8] demonstrated that there was an increase in MPO level in acute KD ($n = 8$). As the basis for clinical studies, Su et al's[12] results further showed that MPO is important in the development of KD and can be a key functional imaging biomarker for assessing vasculitis in a murine model of KD. In this series, KD patients had significantly higher MPO levels than febrile controls. These findings including ours suggest that MPO may play an important role in the inflammation induced by KD. Our study further demonstrated that there was a significant association between MPO -463G>A polymorphism and susceptibility of KD. The effect of MPO on KD might be explained by the MPO -463G>A gene polymorphism in this series.

We observed a significant association between MPO -463G>A polymorphism and susceptibility of KD in this series. The combined genotypes (G/A and A/A) of MPO -463G>A were associated with a significantly decreased KD risk than the G/G genotype. In addition, KD patients with A allele were associated with a significantly decreased KD risk as compared to those with G allele. Increasing evidence suggests that G allele in MPO polymorphism may determine the MPO level and play a crucial role in inflammation.[14,26–28] Our results showed that KD patients in the pre-IVIG stage had significantly higher MPO levels than febrile controls in terms of GG genotype and G allele. This may partially explain the increased risk of KD associated with the presence of the G allele observed in the present study. Our results were compatible with the association of G allele with susceptibility to disease, such as coronary artery disease,[15,16] MPO-ANCA-associated vasculitis in women,[19] acute promyelocytic leukemia,[26] diabetic nephropathy,[27] lung cancer,[29] and digestive tract cancer.[30] There is a wide variation in the A allele frequency of the MPO -463G>A polymorphism across different ethnicities. The A allele frequency was 22.8% in European population, but approximately 14.7% in Asian population.[31] Compatible with a previous study conducted in Asian population,[31] our results showed that A allele frequency was 11.8% in KD patients and 15.5% in normal controls. Whether the different MPO A allele frequencies between Asian and Western countries influence the incidence of KD is an interesting issue. A replication study performed in Caucasian countries may further elucidate the role of MPO polymorphism in KD.

In this series, KD patients with A allele had significantly lower MPO levels than those with G allele in the post-IVIG acute phase, implicating a possible allele-modulating effect on the efficiency of IVIG treatment. Kaneko et al[7] reported that the mechanism of IVIG treatment in KD is associated with scavenging ROS. The mechanism of IVIG in reducing inflammation caused by KD is not clearly understood. Modulation of the production of cytokines, especially tumor necrosis factor alpha and interleukin 1, is an important mechanism.[32] Tumor necrosis factor alpha can activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in a wide variety of cells and lead to generation of ROS.[33] Two previous studies have also suggested that IVIG may have antioxidative effects.[3,34] These reports[3,7,34] may provide some evidence for the mechanism of IVIG in reducing oxidative stress. However, there was no significant difference of individual MPO change for KD patients from pre- to post-IVIG stage in terms of genotypes or alleles. MPO level was also not associated with IVIG resistance. The antioxidant efficiency of IVIG might not be related to MPO polymorphism and level. Further studies are required to elucidate the role of MPO polymorphism in IVIG resistance.

Several aspects of the present study warrant attention. First, there is no direct evidence to prove that the MPO level could play an important role in KD. MPO was not measured in all participants. ROS and neutrophil activation were also not evaluated. Measurements of various biomarkers involved in oxidative stress are required to elucidate the role of MPO in KD. Second, this study was a single-center investigation of KD patients with modest sample sizes. Therefore, replication studies with independent large cohorts are suggested.

In conclusion, our results suggest that G allele of MPO -463G>A polymorphism is a potential genetic marker for KD risk in Taiwanese children.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jmii.2015.05.004.

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