ASSOCIATION BETWEEN ORAL IRON SUPPLEMENTATION AND RETINAL OR SUBRETINAL HEMORRHAGE IN THE COMPARISON OF AGE-RELATED MACULAR DEGENERATION TREATMENTS TRIALS

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Purpose: Because patients often take iron supplements without medical indication, and iron can accumulate in vascular endothelial cells, the authors evaluated the association of oral iron supplementation with retinal/subretinal hemorrhage in patients with neovascular age-related macular degeneration.

Methods: A post hoc secondary data analysis of comparison of age-related macular degeneration treatments trials was performed. Participants were interviewed for use of oral iron supplements. Trained readers evaluated retinal/subretinal hemorrhage in baseline fundus photographs. Adjusted odds ratios from multivariate logistic regression models assessed the association between iron use and baseline hemorrhage adjusted by age, sex, smoking, hypertension, anemia, and use of antiplatelet/anticoagulant drugs.

Results: Among 1,165 participants, baseline retinal/subretinal hemorrhage was present in the study eye in 71% of 181 iron users and in 61% of 984 participants without iron use (adjusted odds ratio = 1.47, $P = 0.04$), and the association was dose dependent (adjusted linear trend $P = 0.048$). Iron use was associated with hemorrhage in participants with hypertension (adjusted odds ratio = 1.87, $P = 0.006$) but not without hypertension. The association of iron use with hemorrhage remained significant among hypertensive participants without anemia (adjusted odds ratio = 1.85, $P = 0.02$).

Conclusion: Among participants of comparison of age-related macular degeneration treatments trials, the use of oral iron supplements was associated with retinal/subretinal hemorrhage in a dose–response manner. Unindicated iron supplementation may be detrimental in patients with wet age-related macular degeneration.

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Age-related macular degeneration (AMD) is a common eye condition and a leading cause of vision loss among people aged 50 years or older.[1] Early stage (dry AMD) is diagnosed by the presence of medium-sized drusen. One of the late stages, neovascular AMD (wet AMD), is characterized by abnormal blood vessel growth underneath the retina. These vessels can leak fluid and blood, causing swelling and damage to the macula. Neovascular AMD is the most common disease associated with retinal/subretinal hemorrhage.[2]

Oral iron supplements are widely used by patients with anemia, even after the anemia is cured, whereas iron-containing multivitamins/minerals are commonly used by nonanemic, well-nourished individuals without concern for potential risks. Such extensive use of iron raises questions regarding safety and any

unintended side effects. Previous studies have shown that both local and systemic iron overload contribute to AMD-like retinal degeneration in mice[3–5] and humans.[6–8] In mice, intravenous iron elevates retinal vascular endothelial cell iron,[3] which could cause dysfunction of vascular endothelial cells, leading to retinal hemorrhage.

The present study investigated the association of oral iron supplementation with retinal/subretinal hemorrhage among participants in the comparisons of AMD treatments trials (CATT), a multicenter clinical trial of anti-VEGF treatments for neovascular AMD.

Methods

Details on the study design and methods have been reported in previous publications[9,10] and on ClinicalTrials.gov (identifier NCT00593450). Only the major features related to this study are described here.

Study Participants and Study Procedure

The institutional review board associated with each participating center approved the study protocol, and written consent was obtained from each participant.[10]

Participants from 43 clinical centers in the United States were enrolled and randomized to 1 of 4 treatment groups: 1) ranibizumab monthly, 2) bevacizumab monthly, 3) ranibizumab as needed (pro re nata [PRN]), and 4) bevacizumab PRN. The study enrollment criteria included patients who were above 50 years old, diagnosed with AMD and active choroidal neovascularization (CNV) in the study eye, and have not been treated previously; and visual acuity in the study eye was from 20/25 to 20/320. The presence of active CNV was defined by lesion of CNV shown on fluorescein angiography, fluid seen on optical coherence tomography, located within or below the retina or below the retinal pigment epithelium. Participants with vitreous hemorrhage or diabetic retinopathy that may need medical or surgical intervention in the study eye were not eligible for the study.

At enrollment, participants provided information on demographic characteristics and medical history, including history of smoking, cardiovascular diseases, hypertension, and anemia (classified as none, past, or ongoing). The participants were interviewed by the study coordinator about the use of iron supplements at baseline including iron multivitamins/minerals, name of the iron supplement, and administration dose and frequency. The fundus photographs and fluorescein angiograms of the macula were submitted to the CATT reading center for grading.

Evaluation of Retinal or Subretinal Hemorrhage

As described previously,[11] color fundus photographs and fluorescein angiographs were graded by two certified graders independently, who were masked to the participants' iron use status, for the presence and size of retinal/subretinal hemorrhage ($\leq$1, $>$1 to $\leq$2, or $>$2 disk areas [DAs]). Discrepancies between the two graders were adjudicated by a third grader or the CATT reading center's principal investigator (J.E.G). The reproducibility results of grading of a random sample of 84 image sets were published previously.[11] Specifically, 80% grade–regrade agreement (weighted kappa, 0.72) and 85% inter-grader agreement (weighted kappa, 0.74) were achieved in the grading for the presence and size of retinal/subretinal hemorrhage.

Statistical Analysis

The two sample *t*-test and the Fisher exact test were used to compare for mean values and proportions of characteristics between participants' use versus no use of iron supplements at baseline. The associations between any iron supplement use (yes/no), iron dosage per day (no use, $<$18 mg [typical iron dose in multivitamins], 18–36 mg, $>$36 mg), and retinal/subretinal hemorrhage were assessed among all CATT participants, among those without a history or ongoing anemia, and stratified by hypertension status and number of risk alleles for the complement factor H (*CFH*). The odds ratio (OR) and its 95% confidence interval (95% CI) for their association were calculated from univariate and multivariate logistic regression models. In the multivariate logistic regression models, we adjusted for the same baseline covariates as our previous study of antiplatelet/anticoagulant drugs and retinal/

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subretinal hemorrhage in CATT[12] including age, sex, smoking status, diabetes, dietary supplement use, medical history of cardiovascular disease, and CNV in the fellow eye. In addition, we also adjusted for use of antiplatelet or anticoagulant that was previously found to be associated with retinal/subretinal hemorrhage[9] and the anemia status (no, past, ongoing) that was associated with iron use. The association between use of iron supplements and the size of retinal/subretinal hemorrhage at baseline was evaluated using the chi-square test and Cochran–Armitage trend test. All data analyses were performed using SAS version 9.4 (SAS Institute, Inc. Cary, NC), and 2-sided $P < 0.05$ was considered to be statistically significant.

Results

Characteristics of Participants With and Without Iron Use at Baseline

Among 1,185 CATT participants, 20 participants were excluded because of unreadable fundus photographs. Of the remaining 1,165 participants, 984 (84.5%) did not use iron supplements at baseline and 181 (15.5%) used either iron-containing multivitamins/minerals (n = 163, 14.0%) or prescriptions (n = 18, 1.5%). The most common iron prescription was ferrous sulfate (n = 11, 61.1%).

The comparisons of characteristics between participants with versus without iron supplement use at baseline are summarized in Table 1. Participants who used iron supplements were older (mean ± SD, 80.2 ± 6.9) than participants who did not use iron (79.0 ± 7.6) ($P = 0.04$), more likely taking AREDS supplement than non–iron users (82.9% vs. 59.6%, $P < 0.0001$), and more likely using antiplatelet or anticoagulant medications (59.1% vs. 50.9%, $P = 0.04$). A history of cardiovascular diseases was more prevalent in iron users than non–iron users (32.6% vs. 22.1%, $P = 0.002$). Of the 984 patients without iron use, 834 (90.9%) were not anemic, 42 (4.3%) had anemia in the past, and 48 (4.9%) had ongoing anemia. Among 181 participants with iron use, 127 (70.2%) did not have anemia, 25 (13.8%) had previous anemia, and 29 (16.0%) had ongoing anemia ($P < 0.0001$).

Table 1. Comparison of Baseline Characteristics Between Participants With Versus Without Use of Iron Supplements (N = 1,165*)

Baseline Characteristics	Iron Use at Baseline		*P*
	No (N = 984)	Yes (N = 181)	
Age (years): Mean (SD)	79.0 (7.6)	80.2 (6.9)	0.04
Female (%)	609 (61.9%)	112 (61.9%)	1.00
Former or current cigarette smoker (%)	559 (56.8%)	106 (58.6%)	0.66
Taking AREDS supplement (%)	586 (59.6%)	150 (82.9%)	<0.0001
Presence of diabetes (%)	166 (16.9%)	37 (20.4%)	0.24
Presence of hypertension (%)	674 (68.5%)	128 (70.7%)	0.55
Systolic BP (mmHg): Mean (SD)	135 (17.9)	133 (16.7)	0.09
Diastolic BP (mmHg): Mean (SD)	76 (10.1)	75 (9.7)	0.59
History of cardiovascular diseases	217 (22.1%)	59 (32.6%)	0.002
Osteoarthritis	452 (45.9%)	95 (52.5%)	0.11
Rheumatoid arthritis	56 (5.7%)	13 (7.2%)	0.43
Anemia			<0.0001
None	834 (90.9%)	127 (70.2%)	
Past	42 (4.3%)	25 (13.8%)	
Ongoing	48 (4.9%)	29 (16.0%)	
Baseline use of antiplatelet or anticoagulant drug (%)	501 (50.9%)	107 (59.1%)	0.04
CNV in the fellow eye	296 (30.1%)	9 (27.1%)	0.42
Visual acuity in the study eye: Mean (SD) in letters	60.4 (13.5)	60.9 (13.6)	0.61
Total area of CNV lesion (disk area): Mean (SD)	2.5 (2.5)	2.3 (2.6)	0.29
Total retinal thickness (μm): Mean (SD)	462 (189)	463 (178)	0.94

*Of 1,185 CATT participants, 20 patients did not have good image quality for determining retinal/subretinal hemorrhage, leaving 1,165 participants for analysis.

BP, blood pressure.

Table 2. Association of Iron Use With Retinal or Subretinal Hemorrhage in the Study Eye of CATT Participants at Baseline Among All Participants and Stratified by Hypertension Status

Iron Use at Baseline	n	Hemorrhage (%)	Unadjusted Analysis		Adjusted analysis†	
			OR (95% CI)	*P*	OR (95% CI)	*P*
Among all CATT participants (N = 1,165)						
Iron use				0.004		0.04
No	984	596 (60.6%)	1.00		1.00	
Yes	181	128 (70.7%)	1.57 (1.11–2.22)		1.47 (1.02–2.13)	
Iron dose				0.008*		0.048*
No iron use	984	596 (60.6%)	1.00		1.00	
<18 mg	40	26 (65.0%)	1.21 (0.62–2.34)	0.57	1.18 (0.60–2.35)	0.63
18–36 mg	87	53 (70.1%)	1.53 (0.95–2.46)	0.08	1.66 (1.01–2.73)	0.046
>36 mg	42	32 (76.2%)	2.08 (1.01–4.29)	0.046	1.38 (0.63–3.02)	0.42
Without hypertension at baseline (n = 363)						
Iron use				0.76		0.68
No	310	190 (61.3%)	1.00		1.00	
Yes	53	34 (64.2%)	1.13 (0.62–2.07)		0.87 (0.44–1.72)	
Iron dose				0.61*		0.56*
No iron use	310	190 (61.3%)	1.00		1.00	
<18 mg	12	8 (66.7%)	1.26 (0.37–4.29)	0.71	1.11 (0.29–4.28)	0.88
18–36 mg	19	12 (63.2%)	1.08 (0.42–2.83)	0.87	1.24 (0.45–3.47)	0.68
>36 mg	18	12 (66.7%)	1.26 (0.46–3.46)	0.65	0.45 (0.12–1.68)	0.23
With hypertension at baseline (n = 802)						
Iron use				0.005		0.006
No	674	406 (60.2%)	1.00			
Yes	128	94 (73.4%)	1.82 (1.20–2.78)		1.87 (1.19–2.92)	
Iron dose				0.005*		0.009*
No iron use	674	406 (60.2%)	1.00		1.00	
<18 mg	28	18 (64.3%)	1.19 (0.54–2.61)	0.67	1.32 (0.59–2.99)	0.50
18–36 mg	68	49 (72.1%)	1.70 (0.98–2.96)	0.06	1.84 (1.03–3.28)	0.04
>36 mg	24	20 (83.3%)	3.30 (1.12–9.76)	0.03	2.65 (0.85–8.25)	0.09

*From the test of linear trend. No dose information is available for 12 patients (4 without hypertension and 8 with hypertension); they were excluded from the analysis of dose association.

†Adjusted by age, sex, smoking status, dietary supplement use, hypertension, diabetes, anemia, CVD history, use of antiplatelet or anticoagulant, and CNV in the fellow eye.

Association Between Iron Supplement Use and Retinal or Subretinal Hemorrhage at Baseline

No differences were found in visual acuity, retinal thickness, and size of CNV between CATT participants with and without iron use (data not shown). Of interest, retinal/subretinal hemorrhage was present in 128 (70.7%) iron users and in 596 (60.6%) non–iron users ($P = 0.004$). This difference remained significant in a multivariate analysis (adjusted OR = 1.47, 95% CI, 1.02–2.13, $P = 0.04$, Table 2). The association of iron use with retinal/subretinal hemorrhage was dose dependent (65% for <18 mg, 70% for 18–36 mg, 76% for >36 mg, adjusted linear trend $P = 0.048$, Table 2). In particular, using 18 to 36 mg of iron was significantly associated with higher risk of retinal/subretinal hemorrhage (adjusted OR = 1.66, 95% CI: 1.01–2.73, $P = 0.046$) when compared with non–iron users.

Association of Iron Supplement Use With Retinal or Subretinal Hemorrhage by Baseline Hypertension Status

In participants with hypertension at baseline (n = 802), 94 of 128 (73.4%) participants with iron use and 406 of 674 (60.2%) without iron use had retinal/subretinal hemorrhage ($P = 0.005$). This association was significant in a multivariate analysis (adjusted OR = 1.87, 95% CI: 1.19–2.92, $P = 0.006$). Further analysis revealed a dose-dependent risk of hemorrhage (adjusted linear trend $P = 0.009$, Table 2). In particular, hypertensive participants with an iron dose of 18 to 36 mg had a significantly higher risk of hemorrhage (adjusted OR = 1.84, 95% CI: 1.03–3.28, $P = 0.04$, Table 2).

Among subjects without hypertension at baseline, iron use was not associated with retinal/subretinal hemorrhage (adjusted OR = 0.87, $P = 0.68$, Table 2). The interaction for association with hemorrhage between iron

use and hypertension was not statistically significant in the multivariate model ($P = 0.11$).

Association of Iron Supplement Use With Retinal or Subretinal Hemorrhage in Participants Without Anemia

Because anemia itself can cause retinal hemorrhage, we performed further analysis by excluding all participants with past or ongoing anemia (n = 144). Among 1,021 participants without anemia at baseline, 84 of 127 (66.1%) iron users and 532 of 894 (59.5%) non–iron users had retinal/subretinal hemorrhage (adjusted OR = 1.43; 95% CI: 0.95–2.14, $P = 0.09$, Table 3).

When 1,021 participants without anemia were stratified by the baseline hypertension status, iron use was significantly associated with hemorrhage among those with hypertension (adjusted OR = 1.85, 95% CI: 1.12–3.05, $P = 0.02$), but was not significant among those without hypertension ($P = 0.81$). A dose of 18 to 36 mg iron was significantly associated with higher risk of hemorrhage among those with hypertension (adjusted OR = 2.05, 95% CI, 1.07–3.92, $P = 0.03$) (Table 3).

Association of Iron Supplement Use With Retinal or Subretinal Hemorrhage in Participants With Various Age-Related Macular Degeneration SNPs

To investigate if the association of iron with hemorrhage was affected by SNP variations, we analyzed the SNP-associated AMDs including *CFH* Y402H (rs1061170), *ARMS2* (rs10490924), *HTRA1* (rs11200638), C3 (rs2230199), LIPC (rs10468017), *CFB* (rs4151667), and *C2* (rs547154).[13,14] In the 835 CATT participants who were genotyped, we did not find that risk alleles in *ARMS2*, *HTRA1*, *C3, LIPC, CFB, and*

Table 3. Association of Iron Use With Retinal or Subretinal Hemorrhage in the Study Eye of CATT Participants at Baseline Among Those Without Past History or Ongoing Anemia (n = 1,021)

			Unadjusted Analysis		Adjusted analysis†	
Iron Use at Baseline	n	Hemorrhage (%)	OR (95% CI)	*P*	OR (95% CI)	*P*
All subjects without anemia at baseline (n = 1,021)						
Iron use				0.15		0.09
No	894	532 (59.5%)	1.00		1.00	
Yes	127	84 (66.1%)	1.33 (0.90–1.97)		1.43 (0.95–2.14)	
Iron dose				0.12*		0.07*
No iron use	894	532 (59.5%)	1.00		1.00	
<18 mg	35	22 (62.9%)	1.15 (0.57–2.32)	0.69	1.16 (0.57–2.39)	0.68
18–36 mg	70	49 (70.0%)	1.59 (0.94–2.69)	0.09	1.89 (1.09–3.27)	0.02
>36 mg	13	7 (53.8%)	0.79 (0.27–2.38)	0.68	0.65 (0.21–2.00)	0.45
Without hypertension at baseline (n = 323)						
Iron use				0.61		0.81
No	287	172 (59.9%)	1.00		1.00	
Yes	36	20 (55.6%)	0.83 (0.42–1.68)		0.86 (0.52–1.43)	
Iron dose				0.76*		0.91*
No iron use	287	172 (59.9%)	1.00		1.00	
<18 mg	10	6 (60.0%)	1.00 (0.28–3.63)	1.00	1.15 (0.29–4.60)	0.85
18–36 mg	18	12 (66.7%)	1.34 (0.49–3.66)	0.57	1.50 (0.52–4.38)	0.46
>36 mg	5	1 (20.0%)	0.17 (0.02–1.52)	0.11	0.16 (0.02–1.56)	0.12
With hypertension at baseline (n = 698)						
Iron use				0.04		0.02
No	607	360 (59.3%)	1.00		1.00	
Yes	91	64 (70.3%)	1.63 (1.01–2.62)		1.85 (1.12–3.05)	
Iron dose				0.06*		0.02*
No iron use	607	360 (59.3%)	1.00		1.00	
<18 mg	25	16 (64.0%)	1.22 (0.53–2.80)	0.64	1.36 (0.58–3.22)	0.48
18–36 mg	52	37 (71.2%)	1.69 (0.91–3.15)	0.10	2.05 (1.07–3.92)	0.03
>36 mg	8	6 (75.0%)	2.06 (0.41–10.3)	0.38	1.86 (0.36–9.73)	0.46

*From the test of linear trend. No dose information is available for 9 patients (3 without hypertension and 6 with hypertension); they were excluded from the analysis of dose association.

†Adjusted by age, sex, smoking status, dietary supplement use, hypertension, diabetes, CVD history, use of antiplatelet or anticoagulant, and CNV in the fellow eye.

C2 exacerbate the retinal/subretinal hemorrhage among the iron using CATT participants. However, CATT participants with risk allele of *CFH* tend to have a higher risk of retinal/subretinal hemorrhage. Among iron users, retinal/subretinal hemorrhage occurred in 21 (67.7%) of 31 participants with no risk allele of *CFH*, in 38 (66.7%) of 57 participants with one risk allele, and in 31 (77.5%) of 40 participants with two risk alleles (linear trend P = 0.34). Among the participants with two risk alleles of *CFH* (n = 265), iron use was significantly associated with a higher risk of hemorrhage in univariate analysis (OR = 2.25, P = 0.04) and was borderline significant in multivariate analysis (adjusted OR = 2.17; 95% CI: 0.94–5.01, P = 0.07, Table 4), but association was not significant among participants with one (P = 0.38) or zero *CFH* risk alleles (P = 0.67). The interaction between iron use and *CFH* for the association with retinal or subretinal hemorrhage was not statistically significant in the multivariate analysis (P = 0.21).

Association of Iron Supplement Use With the Size of Retinal or Subretinal Hemorrhage

Iron use was associated with a larger size of retinal/subretinal hemorrhage (linear trend P = 0.01, Table 5). A higher dose of iron use was associated with a larger hemorrhage (linear trend P = 0.007). The percentage of hemorrhage greater than 1 DA was 9.2% among non–iron users, 10% among iron users with dose less than 18 mg, 13.8% among those with a dose of 18 to 36 mg, and 14.3% among those with a dose greater than 36 mg.

Discussion

The potential contribution of iron to the development of AMD has been recognized for over a decade.[7,8] Several case reports have described patients who developed retinal degeneration after intramuscular or intravenous iron therapy.[3,15,16] However, no study has investigated whether oral iron supplements can affect the retina. Our analysis shows for the first time that oral iron supplement use is associated with a higher risk of retinal/subretinal hemorrhage in eyes with neovascular AMD, and the association was dose dependent, particularly among those with hypertension.

Because anemia can cause retinal hemorrhage, we performed additional analysis by excluding all participants with a history of or ongoing anemia and still found a significant association between iron supplement use and retinal/subretinal hemorrhage. This finding is clinically important and indicates that nonanemic patients with neovascular AMD who take oral iron supplements may be at risk of retinal/subretinal hemorrhage. This risk is increased in participants with hypertension (OR = 1.85, P = 0.02), but not so in participants without hypertension (OR = 0.86, P = 0.81). This significant association of iron use with retinal/subretinal hemorrhage was strongest among those taking an iron dose of 18 to 36 mg (OR = 2.05, P = 0.03). However, these results should be interpreted with caution because it is unclear why nonanemic CATT participants (127 of 1,021) also used iron supplements. It is possible that some comorbidities in CATT participants, such as chronic kidney disease and heart failure, could potentially confound our findings.

Table 4. Association of Iron Use With Retinal or Subretinal Hemorrhage in the Study Eye of CATT Participants at Baseline

Stratified by *CFH* Genotype							
				Unadjusted Analysis		Adjusted analysis*	
Iron Use at Baseline	n	Hemorrhage (%)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
CFH = CC (C is the risk allele for *CFH*)							
Iron use			0.04		0.04		0.07
No	225	136 (60.4%)		1.00		1.00	
Yes	40	31 (77.5%)		2.25 (1.02–4.96)		2.17 (0.94–5.01)	
CFH = TC							
Iron use			0.22		0.22		0.38
No	329	191 (58.1%)		1.00		1.00	
Yes	57	38 (66.7%)		1.45 (0.80–2.61)		1.34 (0.69–2.61)	
CFH = TT							
Iron use			0.93		0.93		0.67
No	139	93 (66.9%)		1.00		1.00	
Yes	31	21 (67.7%)		1.04 (0.45–2.39)		1.28 (0.47–3.18)	

*Adjusted by age, sex, smoking status, dietary supplement use, hypertension, diabetes, anemia, CVD history, use of antiplatelet or anticoagulant, and CNV in the fellow eye.

Table 5. Association Between Iron Use and the Size of Retinal/Subretinal Hemorrhage

		Size of Retinal/Subretinal Hemorrhage at Baseline				
Iron Use at Baseline	N	No Hemorrhage (n = 441)	≤1 DA (n = 611)	>1, ≤2 DA (n = 59)	>2 DA (n = 54)	Linear Trend *P*
Iron use						0.01
No	984	388 (39.4%)	506 (51.4%)	47 (4.8%)	43 (4.4%)	
Yes	181	53 (29.3%)	105 (58.0%)	12 (6.6%)	11 (6.1%)	
Iron dose*						0.007
No iron use	984	388 (39.4%)	506 (51.4%)	47 (4.8%)	43 (4.4%)	
<18 mg	40	14 (35.0%)	22 (55.0%)	0 (0.0%)	4 (10.0%)	
18–36 mg	87	26 (29.9%)	49 (56.3%)	6 (6.9%)	6 (6.9%)	
>36 mg	42	10 (23.8%)	26 (61.9%)	5 (11.9%)	1 (2.4%)	

*No dose information is available for 12 patients; they were excluded from the analysis of dose association.
DA, disk area.

Over half of the US adults aged 20 years or older take at least one dietary supplement,[17,18] despite nutrients from fortified food. Dietary supplement use is most common among older people, women, whites, and highly educated individuals. Over-the-counter supplements are often self-prescribed or recommended by nurses and dietitian without clear medical indications.[19,20] Of note, the iron-containing multivitamin/multimineral is the most common dietary supplement.[18] Thus, it is important to increase awareness of the potential side effects of nonindicated oral iron supplements.

In a previous study, we found that iron levels were most increased in the retinal pigment epithelium and choroid in mice treated with intravenous iron.[3] Before our study, Cibis et al[16] had reported, in dogs with repeated intravenous iron injection, that granular iron deposits form in the endothelial cells and choroid stromal histiocytes. More specifically, the terminal branches of the short and long posterior ciliary arteries were a preferential site of granular iron deposits. Vessel lumens in the choriocapillaris appeared obstructed because of swelling of the iron-laden endothelial cells.[16] In a patient who had received about 150 blood transfusions for the treatment of severe aplastic anemia, complete obstruction of capillaries in the retina and the choriocapillaris was detected. The main site of granular iron deposits included endothelial cells or macrophages and perivascular tissue in the choroid.[16] Together, these findings suggest a potential mechanism of retinal/subretinal hemorrhage related to oral iron supplements; it is possible that in patients with neovascular AMD taking iron supplements, within neovascular vessels, iron toxicity in vascular endothelial cells predisposed to hemorrhage.

Single nucleotide polymorphism in the human *CFH* gene (Y402H) is significantly associated with an increased risk of AMD.[21–24] Our previous analysis showed that CATT patients with a higher number of risk alleles for *CFH* had decreased total thickness of the retina.[25] Of interest, the present analysis shows among patients with two risk alleles of *CFH*, taking iron supplements was borderline significantly associated an increased risk of retinal/subretinal hemorrhage (adjusted OR = 2.17, $P = 0.07$), but the association was not significant among patients with one risk allele (adjusted OR = 1.34, $P = 0.38$) or no risk allele (adjusted OR = 1.28, $P = 0.67$). The pathophysiology could involve a combination of complement dysregulation and iron supplementation. A recent study by Ueda et al[26] showed that dysfunction of *CFH* can lead to thrombotic microangiopathy in multiple organs including the retina. In addition, a study by Li et al[27] showed that iron treatment increases both complement C3 mRNA and protein levels in retinal pigment epithelium cells in cell culture and in mice. Thus, in patients with AMD, the environment (iron supplements) may interact with genetics (*CFH* risk) to damage vascular endothelial cells within the retina. Further investigations are warranted to elucidate the mechanisms of iron and complement dysregulation in retinal pigment epithelium and retinal vascular endothelial cells.

In summary, in this post hoc secondary analysis of CATT data, we found that among all CATT participants with neovascular AMD, use of oral iron supplements was significantly associated with retinal/subretinal hemorrhage at baseline in a dose-dependent manner, and particularly among patients with hypertension, iron supplementation was associated with nearly twice the risk of retinal/subretinal hemorrhage. These results argue that, in addition to considering the well-known side effects of iron use, such as gastrointestinal discomfort, clinicians should be aware of the potential risk of retinal/subretinal hemorrhage among patients with neovascular AMD taking oral iron supplements. The results of this secondary analysis of CATT data indicate a need for validating the findings with future investigations of the risk of retinal/subretinal

hemorrhage with iron supplementation in AMD populations and in other populations.

Key words: AMD, oral iron supplements, retinal/subretinal hemorrhage.

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