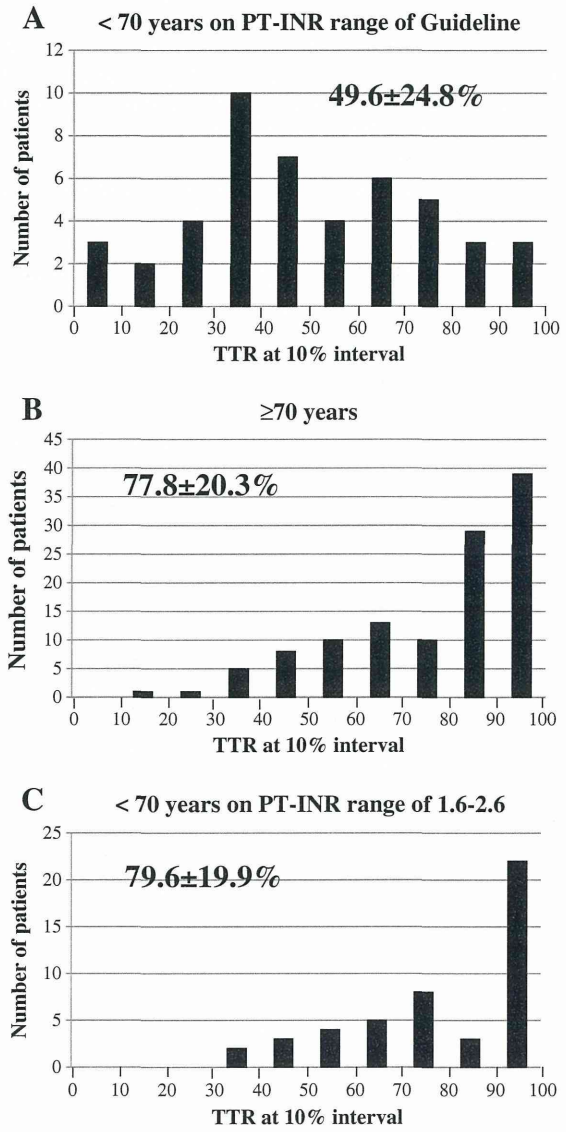

Fig. 1. TTR Distribution. Distribution of number of patients with time in the therapeutic range (TTR) from 0 to 100% at every 10% for patients aged <70 years on Japanese guideline (A), for patients aged ≥70 years (B), and for patients aged <70 years on INR range 1.6-2.6 (C). Data are expressed as means ± SD.

range of 1.6-2.6 even for these patients <70 years. Therefore, we judged that most of the participating cardiologists unconsciously tried to keep PT-INR values within 1.6-2.6 irrespective of the age of their patients. Hence, we reevaluated the association of TTR under this new range of PT-INR irrespective of age of the patients to more properly elucidate the patient's factor(s), which actually regulates the variation of TTR. TTR under 1.6-2.6 of PT-INR range in the patients <70 years was 79.5 ± 20.1% with this method. Univariate analysis using this new calculation revealed that lower height($R^2 = 0.026$, $p = 0.039$), female gender (male 82.1 ± 19.0 vs. female 75.2 ± 21.3, $p = 0.0275$), the higher serum creatinine ($R^2 = 0.03$, $p = 0.026$), the lower CCr($R^2 = 0.027$, $p = 0.036$), and presence of congestive heart failure ($p = 0.0018$), were significantly associated with the lower TTR (Table 2). However, factors such as age, body weight, serum albumin, dosage of warfarin, were not associated with TTR (Table 2). Further multivariate analysis revealed that female gender and presence of congestive heart failure were the independent predictors of poor control of TTR($p = 0.045$, $p = 0.0003$, respectively) (Table 3).

The Genotype Analysis of Metabolizing Enzymes

The genotype frequency of CYP2C9 was 147 of 1*/1* (95.5%), 7 (4.5%) of 1*/3* and none of 3*/3*. The genotype frequency of VKORC1 was 130 (84.4%) of AA, 23 (14.9%) of GA, and 1 (0.6%) of GG. The genotype frequency of CYP4F2 was 85 95 (55.0%) of CC, 52 (33.8%) of CT, and 7 (11.0%) of TT. The genotype frequencies of CYP2C19 2* was 76 (49.4%) of CC, 50 (32.5%) of CT, and 28 (18.2%) of TT. The genotype frequencies of CYP2C19 3* was 117 (76.0%) of CC, 35 (22.7%) of CT, and 2 (1.3%) of TT (Fig. 3). Because the genetic variability in the patients under warfarin therapy plays an important role in determining the dosage of warfarin, we also evaluated the association between the genetic variability and warfarin dosage at the enrollment. About CYP2C9, compared with patients with 1*/1*, dosage of warfarin of the patients with 1*/3* were significantly lower (2.9 ± 1.2 vs. 2.1 ± 0.43 mg; $p < 0.05$). As for VKORC1, compared with patients with AA, dosage of warfarin of the patients with GA were significantly higher (4.0 ± 1.6 vs. 2.9 ± 1.0 mg; $p < 0.01$) (Fig. 3). The mean dosage of warfarin in CYP4F2 CC, CT, and TT are (2.7 ± 1.3, 2.9 ± 1.0, 3.4 ± 1.2, respectively, P = ns). The mean dosage of warfarin in CYP2C19*2 CC, CT, and TT are (2.9 ± 1.3, 2.8 ± 1.0, 2.9 ± 1.1, respectively, P = ns) and in CYP2C19*3 CC, CT, and TT are (2.8 ± 1.2, 2.9 ± 1.0, 3.0, respectively, P = ns).

We investigated the relationship between TTR and polymorphism of metabolic enzymes, CYP2C9 and VKORC1 because those might have influenced dosage of warfarin. However there is no significant association between TTR and those polymorphism of metabolic enzymes, CYP2C9 and VKORC1 (Table 2).

Discussion

Major Findings

The major findings in this study are 1) female gender and presence of congestive heart failure were the independent predictor of lower TTR when their relationship was analyzed under the assumption that participating cardiologists tried to keep PT-INR of 1.6-2.6 even if the patient was younger than 70 years. 2) Genetic variability of metabolizing enzymes plays an important role in determining the dosage of warfarin but variability of PT-INR (TTR) was not affected by the variability. Recently two articles reported the results of analysis about the factors that influenced TTR variation [5,17]. Okumura and co-workers reported that age and warfarin dosage were two independent predictors of TTR in Japanese NVAF patients [5]. In their study, TTR in patients <70 and ≥70 years was 46 ± 23% and 77 ± 17% [5], which are almost identical to our results. They concluded that TTR is strongly influenced by age presumably because Japanese physicians administered warfarin so as to attain the lower PT-INR value than what is recommended in the guideline due to an anxiety against the hemorrhage accident even knowing the recommendation in Japanese guideline: PT-INR control of 2.0-3.0 in patients <70 years and 1.6-2.6 ≥ 70 years. Because TTR value mainly depends on which PT-INR range was used for the calculation by definition, TTR in those <70 years becomes inevitably lower when actual PT-INR range is dissociated from the recommended PT-INR range. We found that PT-INR values in patients <70 years distributed in the same range in patient age ≥70 years in this study, which is the same result as in a recent large Japanese cohort, J-RHYTM study, about warfarin control for NVAF [13]. Furthermore, recently a report from Kotani et al. suggested that PT-INR level between 1.6 and 2.6 was permitted as a proper range irrespective of the patients' ages in Japanese population [16]. After confirming these points, we reevaluated the association of TTR using this actual range of PT-INR of 1.6-2.6 irrespective of age of the patients. Interestingly enough, this new calculation could elucidate that height, gender, serum creatinine, CCr, and presence of congestive heart failure were significantly associated with TTR, but age, body weight, hypertension, diabetes mellitus, prior stroke/transient ischemic attack, serum albumin, dosage

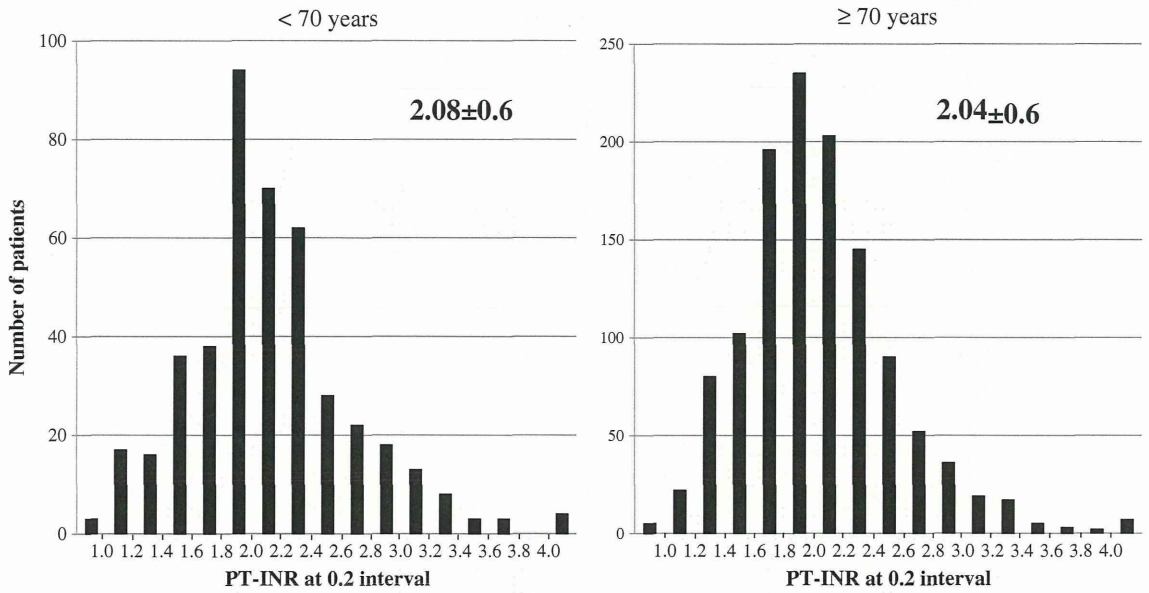

Fig. 2. PT-INR distribution in patients with < 70 and ≥ 70 years. Distribution of number of patients with PT-INR from < 1.0 to ≥ 4.0 at every 0.2 for patients aged < 70 and ≥ 70 years. Data are expressed as means ± SD.

of warfarin, genetic factors, and antiplatelet drug administration were not. Further multivariate analysis confirmed that gender and presence of congestive heart failure were the independent predictor of TTR. We consider that it was only possible to uncover the hidden relationships between the factors and TTR control by changing range of PT-INR control from that in guideline to actual one.

Sub-analysis of RE-LY trial recently reported that the degree of adherence, regardless whether it was intentional or not, to a simple warfarin dosing algorithm predicted improved TTR and accounted for considerable part of TTR variation between centers and countries rather than patient's factors [17]. This result might come from the nature of RE-LY trial that it is a large scale multi-country and multicenter trial and the enrolled patients might have been controlled more strictly than usual from the initiation period of anti-coagulation because it was well supervised study. In the article, the patient's factors such as higher age, female gender, smoking, congestive heart failure, diabetes mellitus, co-administration of amiodarone, and usage of insulin were associated with the lower TTR. These results are partially consistent with our results where multivariate analysis revealed female gender and presence of congestive heart failure, as independent predictors of TTR.

Although clinically available warfarin is a racemic mixture, (S)-warfarin is five times more potent as an anticoagulant than (R)-warfarin. (S)-warfarin is primarily metabolized into 7-hydroxylated form in humans, principally by cytochrome P450, subfamily IIC, polypeptide 9 (CYP2C9). So far, it is reported that CYP2C9 has two types of variants (CYP2C9*2 (R144C) and CYP2C9*3 (I359L)), though there is no report of CYP2C9*2 allele in the Asian people. Previous studies have demonstrated that the warfarin 7-hydroxylase activity of the CYP2C9*3 variant allele is approximately 1/10 of wild type of CYP2C9 (CPY2C9*1) and such carriers of the variant allele required a lower maintenance dose of warfarin due to the decreased metabolism [6]. In the present study, we also showed that the dosage of warfarin in the patients with CYP2C9 1*/3* were significantly lower compared with patients with 1*/1*, which coincided with previous reports [6–8]. However, we did not find any significant association between TTR and CYP2C9 genotypes. Warfarin exerts its anticoagulant effects by interfering with regeneration of vitamin K by reduction of its 2, 3-epoxide in the vitamin K cycle, leading to inhibition of gamma-carboxylation of vitamin K-dependent clotting factor II (prothrombin), VII, IX and X. This vitamin K epoxide reductase is encoded by vitamin K epoxide reductase complex subunit 1 (VKORC1). In this study we showed that patients with VKORC1 GA were administrated with significantly higher dosage of warfarin compared with patients with VKORC1 AA. These results also coincided with previous reports [6–8]. However, again, there is no significant association between TTR and VKORC1 genotypes. We concluded that genetic variability, such as CYP2C9 and VKORC1, among patients plays an important role in determining the dosage of warfarin but not variation of TTR after once it comes to a maintenance phase.

Table 2
Candidate factors related to the better TTR value (as of PT-INR range 1.6-2.6 irrespective of age).

	R square	P-value	number of patients
Height	0.026	0.039*	163
Body Weight		0.09	163
Age		0.39	163
Gender		0.0275*	163
Creatinine	0.03	0.026*	163
Creatinine clearance	0.027	0.0355*	163
Serum albumin		1.07	163
Component of CHADS2 score			
Congestive heart failure		0.0018*	163
Hypertension		0.12	163
Age		0.41	163
Diabetes Mellitus		0.41	163
Stroke		0.22	163
Warfarin dosage		0.51	163
CYP2C9 genotype variants		0.12	154
VKORC 1 genotype variants		0.12	154
Antiplatelet co-administration		0.67	163

Significant p value expressed * symbol.

Table 3
Multiple stepwise regression analysis of the variables that were related to TTR (as of PT-INR range 1.6-2.6 irrespective of age).

	β	SEM	t	P value
Congestive heart failure	10.17	2.77	3.66	0.0003*
Gender (female)	3.13	1.54	2.02	0.045*

Significant p values are expressed with asterisk.

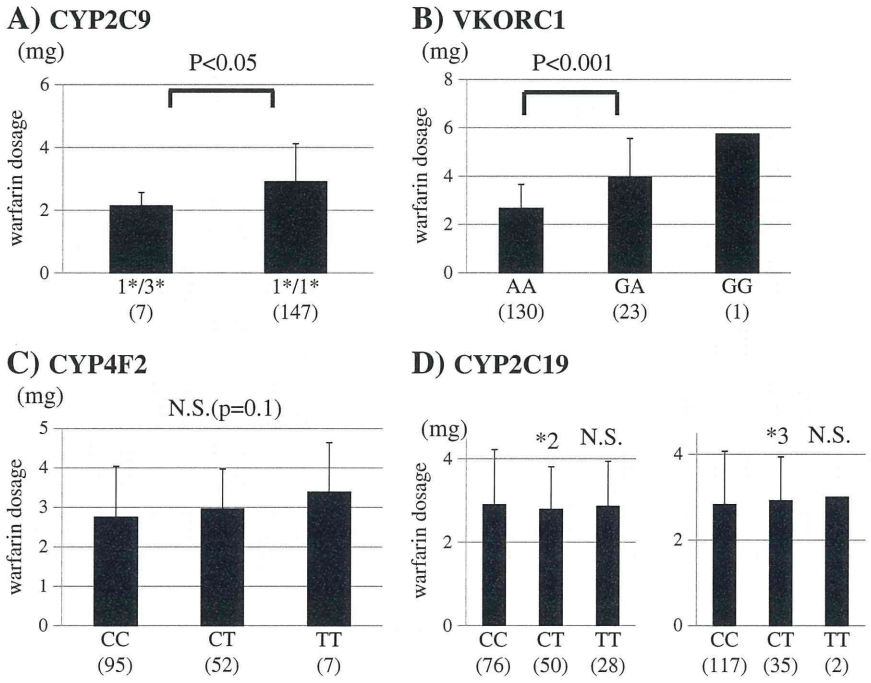

Fig. 3. Genotype variants and warfarin dose. Numbers in parenthesis represent the number of patients. Data are expressed as means ± SD.

As for the reason why the female patients and those with congestive heart failure had the lower TTR, one possibility is that PT-INR had been kept at relatively low values due to concern about the bleeding events in these patients. We, therefore, evaluated the relationship between these factors and HAS-BLED score [18]. Though we found that, compared without congestive heart failure patients, HAS-BLED score in the patients with congestive heart failure patients were significantly higher (1.66 ± 0.9 vs 2.8 ± 1.2 respectively, $p < 0.01$) but there was no significant relationship between female gender and HAS-BLED score. The reason why congestive heart failure had negative correlation with TTR is not clear but previous report from RE-LY trial was consistent with our results, though they did not also find proper explanation for this. We tried to analyze the relationship between TTR and HAS-BLED score because we considered that Japanese physicians might have intentionally administered warfarin so as to attain the lower PT-INR value than what is recommended in the guideline due to an anxiety against the hemorrhage accident. HAS-BLED score consists of hypertension, abnormal renal/liver function, stroke, bleeding history or predisposition, elderly, drugs/alcohol concomitantly and labile INR. However, it is obvious that labile INR defined as <60% of TTR is inappropriate to be included in the analysis. Also hypertension, abnormal renal function, stroke, elderly, and drugs concomitantly had already existed in our original calculation as analysing factors. Thus we adopted only abnormal liver function, bleeding history or predisposition, and alcohol concomitantly as independent candidates which affect TTR. As for alcohol, since only one patient had taken alcohol regularly, we considered that to include this factor has no mean and abandoned to include in this analysis. Thus, we analysed the relationship between TTR and two components of HAS-BLED score; abnormal liver function, bleeding history or predisposition. Though thirteen patients have abnormal liver function and 6 patients had bleeding history, these components were not significantly associated with TTR ($p = 0.82$ in abnormal liver function, $p = 0.55$ in bleeding history or predisposition).

Study Limitations

As we retrospectively analyzed the records of NVAF patients who were taking warfarin and who could come to each hospital on foot, this study might have excluded some immobile patients as the result of a major stroke or hemorrhage due to poor control of PT-INR, i.e. very low TTR. In another words, there remains a possibility that our average of TTR might have been skewed toward better side. To overcome this shortcoming, a larger prospective study is needed.

Though we found that the most participated cardiologist in our study controlled using 1.6-2.6 of PT-INR range irrespective of age and this tendency was confirmed by a recent Japanese large cohort, we have no data about the outcome of this lower control. Whether our analysis has clinical meaning partially or not depends on the outcome using this control range. We have to pay attention to the result of the cohort study.

Clinical Implications

As we found that female gender, and presence of congestive heart failure were associated to the lower TTR control, more attention should be paid to such high risk patients in daily practice.

Conflict of Interest Statement

All authors declare no conflicts of interest.

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