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ORIGINAL RESEARCH

Fractional Flow Reserve Versus Instantaneous Wave-Free Ratio in Assessment of Lesion Hemodynamic Significance and Explanation of their Discrepancies. International, Multicenter and Prospective Trial: The FiGARO Study

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BACKGROUND: The FiGARO (FFR versus iFR in Assessment of Hemodynamic Lesion Significance, and an Explanation of Their Discrepancies) trial is a prospective registry searching for predictors of fractional flow reserve/instantaneous wave-free ratio (FFR/iFR) discrepancy.

METHODS AND RESULTS: FFR/iFR were analyzed using a Verrata wire, and coronary flow reserve was analyzed using a Combomap machine (both Philips-Volcano). The risk polymorphisms for endothelial nitric oxide synthase and for heme oxygenase-1 were analyzed. In total, 1884 FFR/iFR measurements from 1564 patients were included. The FFR/iFR discrepancy occurred in 393 measurements (20.9%): FFRp (positive)/iFRn (negative) type (264 lesions, 14.0%) and FFRn/iFRp (129 lesions, 6.8%) type. Coronary flow reserve was measured in 343 lesions, correlating better with iFR (R=0.56, P<0.0001) than FFR (R=0.36, P<0.0001). The coronary flow reserve value in FFRp/iFRn lesions (2.24±0.7) was significantly higher compared with both FFRp/iFRp (1.39±0.36), and FFRn/iFRn lesions (1.8±0.64, P<0.0001). Multivariable logistic regression analysis confirmed (1) sex, age, and lesion location in the right coronary artery as predictors for FFRp/iFRn discrepancy; and (2) hemoglobin level, smoking, and renal insufficiency as predictors for FFRn/iFRp discrepancy. The FFRn/iFRp type of discrepancy was significantly more frequent in patients with both risk types of polymorphisms (endothelial nitric oxide synthase$_r$+heme oxygenase-1$_r$): 8 patients (24.2%) compared with FFRp/iFRn type of discrepancy: 2 patients (5.9%), P=0.03.

CONCLUSIONS: Predictors for FFRp/iFRn discrepancy were sex, age, and location in the right coronary artery. Predictors for FFRn/iFRp were hemoglobin level, smoking, and renal insufficiency. The risk type of polymorphism in endothelial nitric oxide synthase and heme oxygenase-1 genes was more frequently found in patients with FFRn/iFRp type of discrepancy.

REGISTRATION: URL: https://clinicaltrials.gov; Unique identifier: NCT03033810.

Key Words: coronary flow reserve ■ fractional flow reserve ■ instantaneous wave-free ratio

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CLINICAL PERSPECTIVE

What Is New?

- Polymorphism in genes for endothelial synthase and hemoglobin oxygenase can play a role in fractional flow reserve negative/instantaneous wave-free ratio positive (FFRn/iFRp) discrepancy because of nonmaximal vasodilatation after adenosine administration.
- Further predictors for FFRn/iFRp discrepancy that are also related to submaximal vasodilatation capacity are smoking and chronic kidney disease; further predictors for FFRpositive/iFRnegative discrepancy are lesions located in the right coronary artery and coronary territory with a preserved coronary flow.
- The FFRpositive/iFRnegative can be found in situations with well-preserved endothelial function enabling a substantial increase of flow across a stenosis mainly in younger patients and more frequently in men than in women.

What Are the Clinical Implications?

- The FFR/iFR disagreement can be found in roughly 20% of examinations.
- This disagreement can be quite confusing during routine clinical practice.
- The aforementioned reasons for FFR/iFR discrepancies can help to underline either FFR or iFR results in these specific circumstances.

Nonstandard Abbreviations and Acronyms

CFR	coronary flow reserve
ENOS	endothelial nitric oxide synthase
FFR	fractional flow reserve
HO-1	heme oxygenase-1
iFR	instantaneous wave-free ratio
NO	nitric oxide

An essential part of an indication for coronary revascularization is recognition of the lesion causing the myocardial ischemia. Coronary angiography fails in the diagnosis of lesions causing ischemia, primarily in so-called "borderline" lesions (lesions with a 40%–70% reduction in the lumen diameter[1]). This problem is overcome by measuring the pressure gradient across the stenosis. In contemporary practice, we use 2 methods to measure such gradients: fractional flow reserve (FFR), which measures the pressure gradient during hyperemia and across the entire cardiac cycle; and the so-called resting indices (the most well known of which is instantaneous wave-free ratio—iFR), which measure the pressure gradient without drug-induced hyperemia during mid-diastole. Based on 2 recently published trials comparing FFR and iFR in routine practice,[2,3] both methods are considered equal.

Unfortunately, having both hyperemic and resting indices also opens new problems. Based on previous trials, it is known that the correlation between FFR and iFR is around 80% and that this correlation is much lower when we compare only measurements at or near cutoff points.[4] The FiGARO trial (FFR versus iFR in the Assessment of Lesions of Hemodynamic Significance, and an Explanation of Their Discrepancies) was designed to analyze lesions and patients with discordant FFR and iFR findings using clinical, angiographic, and laboratory examinations. Moreover, one of the potential, and, so far, untested reasons for the impaired reaction of endothelial cells to vasoactive drugs could be a genetic polymorphism in genes that play a role in endothelial-based vasodilatation. Endothelial nitric oxide synthase (ENOS) and heme oxygenase-1(HO-1) are enzymes that are crucial for vascular homeostasis, and alterations in their functions are implicated in endothelial dysfunction and development of atherosclerosis.[5,6] Also, common variants in both genes have been shown to alter enzyme function.[6,7] Because there are only limited data available concerning the genetic determinants of coronary artery response to hyperemic stimuli,[8] we sought to investigate whether the Glu298Asp polymorphism—in exon 7 of the ENOS gene—and the (GT)n polymorphism in the HO-1 gene promoter influence coronary pressure-derived indexes and whether these variants contribute to the occurrence of iFR/FFR discordance.

METHODS

Anonymized data and materials have been made publicly available at the web pages of Charles University Hospital in Prague and can be accessed at following addresses: https://int2.lf1.cuni.cz/1LFIK-26-version1-db_figaro_genes.xlsx; https://int2.lf1.cuni.cz/1LFIK-26-version1-db_figaro_patient_based.xlsx

Patients

We included patients indicated for coronary angiography for both chronic coronary syndromes and acute coronary syndromes (ACS), who underwent an assessment of the hemodynamic significance of coronary stenoses by FFR and iFR in 5 Czech centers (Charles University Hospital in Prague, Hospital Na Homolce in Prague, Masaryk University Hospital in Brno, University Hospital Ostrava, Hospital Trinec), 1 center from Japan (Gifu Heart Center), and 1 center in Argentina (Fundacion Favaloro, Buenos Aires). Patients with ACS underwent nonculprit artery examination during either

the primary or staged procedures. Lesions containing a thrombus, or stenoses supplying a myocardial wall with ECG signs of ischemia, were identified as culprit lesions.

In some patients, we performed measurements in more than 1 coronary artery. We used all FFR/iFR measurements for *per-lesion analysis* to identify whether lesion-specific features (lesion location, coronary flow reserve [CFR] value) potentially influence iFR/FFR analyses.

The *per-patient* analysis was used for patient-related features (demography and type of polymorphism). For such analysis, we included the vessel with the most significant findings (in case all FFR and iFR measurements were concordant) or vessels with discrepancies in measurements (in case of discrepant and concordant FFR and iFR measurements in more than 1 examined vessel). In cases with 2 different discrepancies in 1 patient (this situation was found in only 3 patients), we chose the type of discrepancy with the more significant difference between FFR and iFR. Exclusion criteria were:hemodynamic instability; cardiopulmonary resuscitation in the same day, thrombosis in the target coronary artery, patients after coronary artery bypass grafting, severe bronchial asthma, significant valvular disease, or an atrioventricular block higher than the first degree.

FFR/iFR/CFR Measurements

The Philips-Volcano system with Verrata Plus wires was used for FFR/iFR measurements. The CFR value was analyzed by Doppler sensor-tipped wire using the Combo map console with Combo wire (both produced by Philips Volcano). CFR analysis was not obligatory and was left to the discretion of individual investigators.

Intracoronary nitroglycerin (200 µg) was routinely administered intracoronary before the FFR and iFR measurements to control vasomotor tone. Intracoronary adenosine, in a dose of 240 µg, was used in all cases for both pressure and flow measurements. The iFR was measured 3 times, FFR twice, and the mean measured value was used for analysis. An iFR cutoff value of ≤0.89 and FFR of ≤0.80 were used. The CFR value was measured 3 times, and for analysis the mean value was used, with a cutoff value <2.0.

Genetic Analysis

Patient DNA was isolated from peripheral blood leukocytes using standard techniques. Polymerase chain reaction was used for both the Glu298Asp polymorphism—in exon 7 of the ENOS gene—and the (GT)n polymorphism in the HO-1 gene promoter. The long alleles, with ≥ 25 GT repeats, lead to decreased HO-1 inducibility, whereas the short alleles demonstrate adequate HO-1 expression upon stimulation. A detailed description of the genetic analysis can be found in Data S1. A 3-dimensional picture of the ENOS gene is shown in Figure 1.

The FiGARO trial is registered in ClinicalTrials.gov with identifier NCT03033810. All patients signed an informed consent, and the study was approved by the local ethics committees.

Statistical Analysis

The FFR and iFR examinations were analyzed first as continuous variables and then as categorical variables (positive and negative) according to cutoff point. We identified 3 groups of examinations: FFR/iFR concordant, FFRp/iFRn discrepancy, and FFRn/iFRp discrepancy. We performed 3 types of comparisons: FFR/iFR agreement versus FFRp/iFRn discrepancy, FFR/iFR agreement versus FFRn/iFRp type of discrepancy, and FFRp/iFRn versus FFRn/iFRp types of discrepancy.

Mean values±SDs (or percentages) were calculated for all continuous variables. Differences between continuous variables were examined using the Student's *t*-test. For categorical variables, contingency tables were used to display frequency distributions. Statistical significance was calculated by Fisher's exact test. R environment was employed for statistical computation. A *P* value of 0.05 denoted the threshold of statistical significance.

Those predictors with a *P* value ≤0.2 were included in multivariable logistic regression analyses. These were performed to evaluate independent predictors of the discrepancy between FFR and iFR results on the basis of demographic and biomedical variables. We tried to find the best predictive models for both types of FFR/iFR discrepancy. All analyses used SPSS® software, version 24 (SPSS Inc., Chicago, IL).

RESULTS

From November 2015 to March 2020, we performed 1970 pairs of FFR and iFR measurements. We included 1884 pairs from 1564 patients (201 patients—12.9%—with ACS), with 86 pairs excluded owing to low quality of tracings. The coronary arteries examined were as follows: 1102 left anterior descending arteries—LAD (58.5%); 395 left circumflex arteries (21.0%); 362 right coronary arteries—RCA (19.2%); and, 25 left main coronary arteries (1.3%). Demography parameters for per-patient analysis are summarized in Table 1.

Per-Lesion Analysis

We analyzed 1884 lesions using FFR/iFR. The total number of FFR/iFR discrepancies was 393 (20.9%) from all FFR/iFR examinations. The mean value for FFR was 0.79±0.12, and for iFR 0.87±0.14. The overall correlation between FFR and iFR was high: R=0.81,

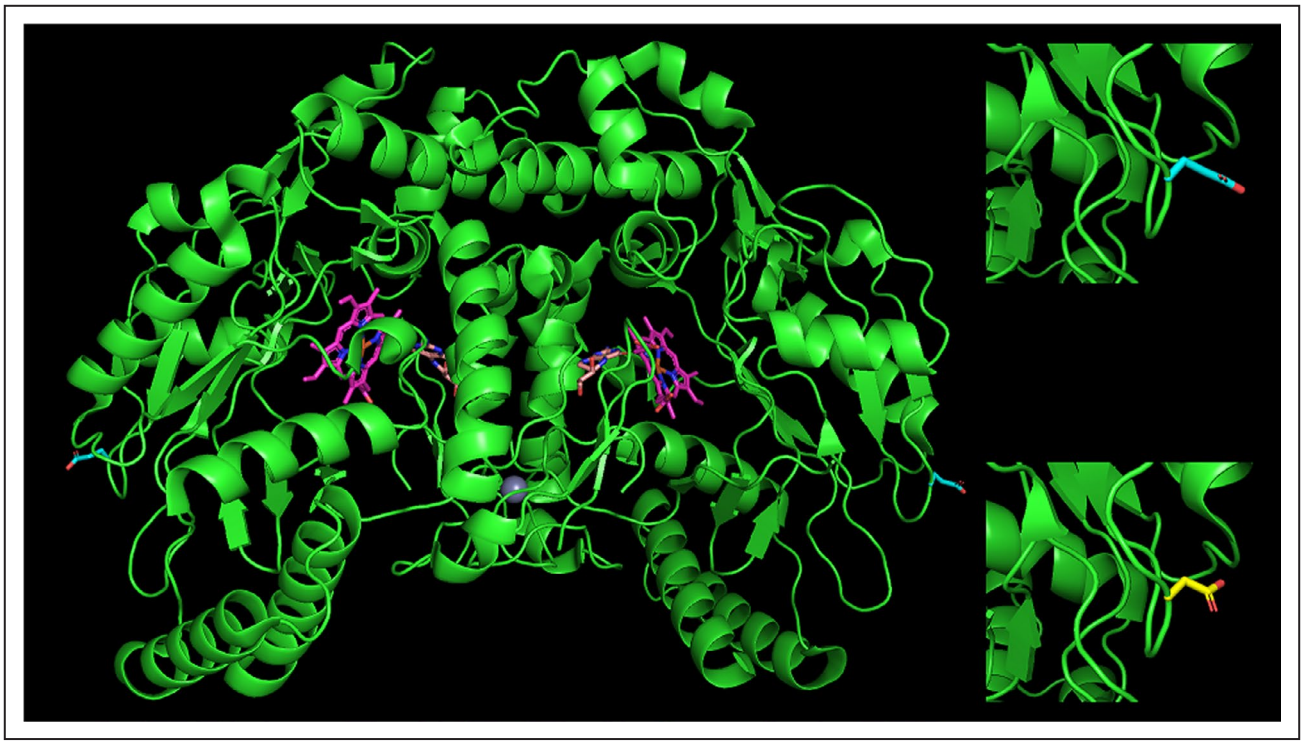

Figure 1. A 3-dimensional image of the ENOS heme domains.
Left panel: the homodimeric structure, alongside heme (pink) and the structural zinc atom (grey). Right panel: the Glu298 (blue) and Asp298 (yellow) represent amino acid residues corresponding to the polymorphic change. ENOS indicates endothelial nitric oxide synthase.

P<0.0001. However, in the area surrounding the cut-off point for iFR (0.85–0.95), the correlation with FFR was worse (R=0.45, *P*<0.0001). In the area close to the cutoff point for FFR (0.75–0.85), the correlation with iFR was even worse (R=0.33, *P*<0.0001). The correlation between FFR and iFR values, difference between them, and histograms for FFR and iFR are shown in Figures 2 and 3.

Taking FFR and iFR dichotomously, the FFRp/iFRn discrepancy was found in 264 lesions (14.0%), and the FFRn/iFRp discrepancy in 129 (6.8%). Matched positive results were found in 683 lesions (36.3%) and matched negative in 808 lesions (42.9%).

Correlation Between Coronary Flow Measurement and Pressure-Based Indices

Coronary flow was measured in 343 lesions with a mean CFR value of 1.66±0.61. CFR correlated more closely with iFR (R=0.56, *P*<0.0001) than with FFR (R=0.36, *P*<0.0001) (Figure 4). The CFR values are summarized in Table 2. The CFR value in FFRp/iFRn lesions was significantly higher compared with both FFRp/iFRp and FFRn/iFRn lesions. The CFR values in FFRn/iFRp lesions did not differ from FFRp/iFRp lesions, but it was lower compared with FFRn/iFRn lesions.

Correlations Between FFR/iFR Discrepancies from Angiographic Patterns

During the analysis of angiographic features, we found that discrepancies were more frequently located in RCA than left coronary artery—95 (26.5%) versus 293 (19.4%), *P*=0.003. This difference was caused by the FFRp/iFRn type of discrepancy, which was located in RCA in 81 lesions (23.4%) versus 184 lesions in left coronary artery (13.1%), *P*<0.0001. On the contrary, the FFRn/iFRp type of discrepancy was less frequent in RCA (18 lesions, 5.0%) versus left coronary artery (115 lesions, 8.6%), *P*=0.043, and this type of discrepancy was found more often in LAD than in non-LAD territory—97 lesions, (9.9%) versus 32 lesions (5.1%), *P*=0.0006.

We did not find any significant difference—in terms of the proximal, mid, or distal part of the coronary artery—in the occurrence of FFR/iFR discrepancies in lesion location. The lesion location in RCA was the only predictor for the FFRp/iFRn type of discrepancy found in multivariable logistic regression analysis (Table 3).

Per-Patient Analysis

Out of 1564 patients included in per-patient analysis, we found 379 discrepant FFR/iFR pairs (24.2%). They were FFRp/iFRn in 259 patients (16.6%), and FFRn/iFRp in 120 patients (7.7%).

Table 1. Demography Parameters in Patients With FFR/iFR Agreement and FFR/iFR Discrepancy

	FFR/iFR agreement 1185 pts	FFRp/iFRn 259 pts	FFRn/FFRp 120 pts	*P* value[1]	*P* value[2]	*P* value[3]
Age, y	69.0±9.7	66.8±9.9	70.9±10.5	0.001†	0.05†	0.0003†
Female sex	346 (29.3%)	40 (15.4%)	44 (36.7%)	<0.0001*†	0.08	<0.0001*†
Body mass index, kg/m^2	29.6±5.2	29.5±5.0	28.4±4.9	0.63	0.05	0.24
Myocardial infarction in past	157 (27.7%)	30 (11.6%)	20 (16.7%)	0.28	0.93	0.48
Diabetes	451 (38.5%)	86 (33.2%)	54 (45%)	0.12	0.09	0.015†
Arterial hypertension	895 (76.5%)	197 (76.1%)	91 (75.8%)	0.88	0.63	0.75
Hyperlipidemia	716 (61.3%)	155 (59.8%)	67 (55.8%)	0.83	0.46	0.61
Chronic kidney disease	43 (8.7%)	4 (1.5%)	15 (12.5%)	0.44	0.0005†	0.006†
Active smoking	265 (22.7%)	75 (29%)	36 (30%)	0.028†	0.04†	0.7
Beta blockers	513 (43.9%)	97 (37.5%)	58 (48.3%)	0.08	0.2	0.03†
Calcium channel blockers	490 (42%)	112 (43.2%)	46 (38.3%)	0.55	0.62	0.42
Nitrates	220 (18.9%)	46 (17.8%)	15 (12.5%)	0.78	0.12	0.2
Diuretics	2175 (38.3%)	29 (11.2%)	42 (35%)	0.38	0.0007†	0.001†
Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers	684 (58.6%)	153 (59.1%)	72 (60%)	0.63	0.47	0.74
Statins	644 (62.3%)	142 (54.8%)	59 (49.2%)	0.31	0.93	0.58
Acute coronary syndromes	150 (13.1%)	36 (13.9%)	15 (12.5%)	0.61	0.95	0.71
Ejection fraction, %	57.2%±12.2	58.4±11.8	57.2±11.9	0.19	0.97	0.41
Hemoglobin, g/L	136.7±21.8	137.0±19.7	131.3±17.7	0.82	0.01†	0.009†
Creatinine, µmol/L	107.2±113.2	99.2±112.2	140.6±169.6	0.31	0.005†	0.006†
Estimated glomerular filtration rate, mL/min	64.7±21.4	67.6±19.2	60.1±25.0	0.04†	0.04†	0.002†

We used Bonferroni corrections for multiple comparison, which decreased the level of significance from 0.05 to 0.00013. The significant results after this corrections are marked using a "*" symbol.

FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

P value[1]: FFR/iFR agreement vs FFRp/iFRn discrepancy.

P value[2]: FFR/iFR agreement vs FFRn/iFRp discrepancy.

P value[3]: FFRp/iFRn vs FFRn/iFRp discrepancies.

† $P < 0.0001$.

The FFR value was higher in female (0.8±0.12) than in male patients (0.78±0.12), *P*<0.0001. The iFR, on the contrary, did not differ according to sex: female (0.87±0.14), male (0.87±0.13), *P*=0.48. Using dichotomous analysis, female patients had positive FFR less frequently than male patients (42.4% versus 54.8%, *P*<0.0001), but the number of positive iFR findings was the same for both men and women (42.6% versus 43.3, *P*=0.74). There was no difference between women and men in CFR value (1.6±0.59 versus 1.71±0.63, *P*=0.17).

Patients with the FFRp/iFRn type of discrepancy were younger, taller (174.8 cm±7.7 versus 171.54 cm±9.5, *P*=0.007), less often female than male, and had higher estimated glomerular filtration rate

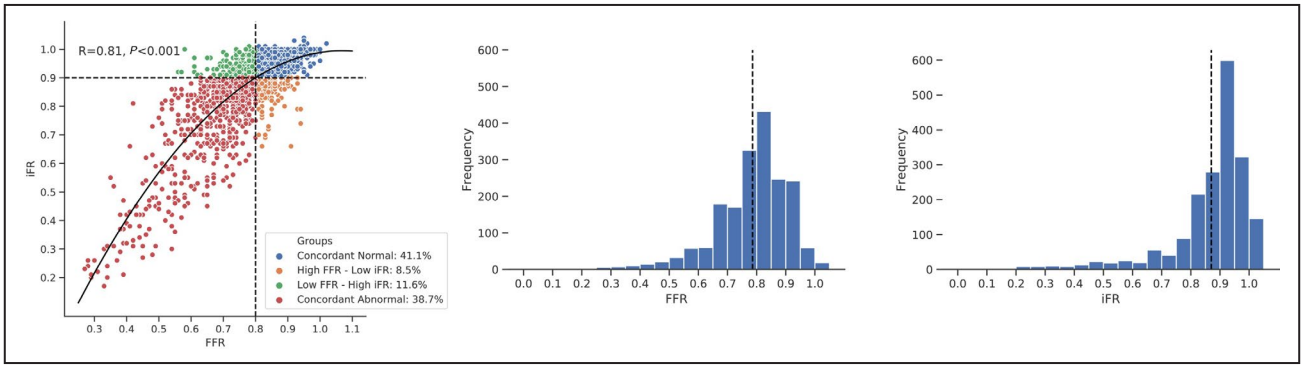

Figure 2. Correlation between FFR and iFR values and histograms for FFR and iFR values.
FFR indicates fractional flow reserve; and iFR, instantaneous wave-free ratio.

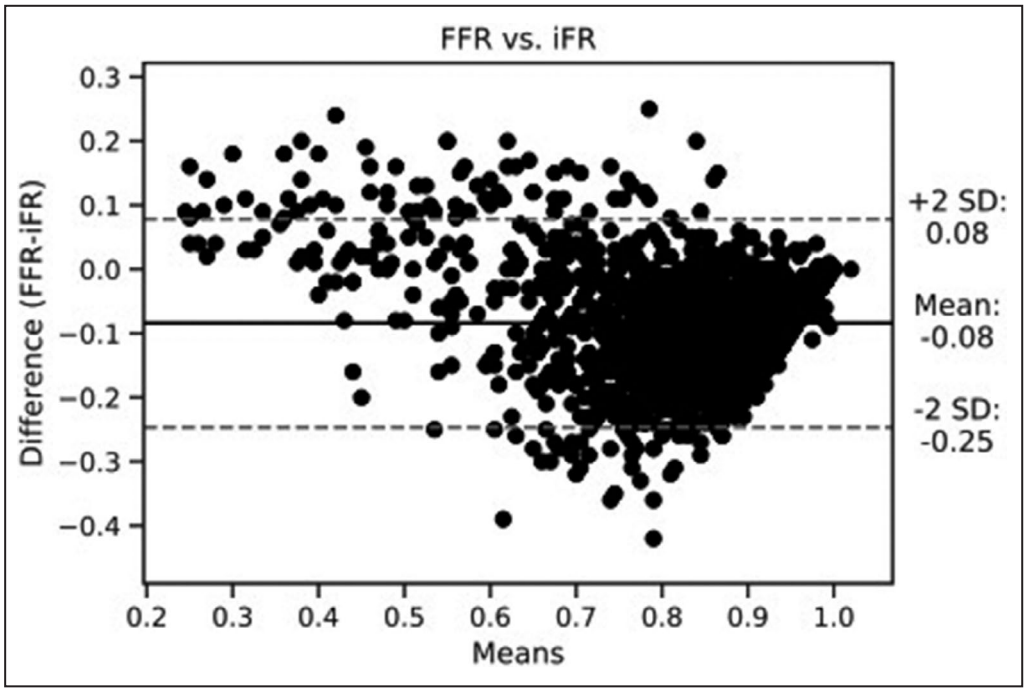

Figure 3. Bland-Altman plot of difference between FFR and iFR.
FFR indicates fractional flow reserve; and iFR, instantaneous wave-free ratio.

compared with patients without an FFR/iFR discrepancy. Multivariable logistic regression analysis confirmed sex and age as predictors for the FFRp/iFRn type of discrepancy (Table 4).

Patients with the FFRn/iFRp type of discrepancy were older; had lower body mass index; more frequently suffered from chronic kidney disease (CKD); were more often smokers; were more frequently treated by diuretics; and had lower levels of hemoglobin, higher levels of creatinine, and lower estimated glomerular filtration rate.

Multivariable logistic regression analysis confirmed hemoglobin level, smoking, and CKD as predictors for the FFRn/iFRp type of discrepancy (Table 5).

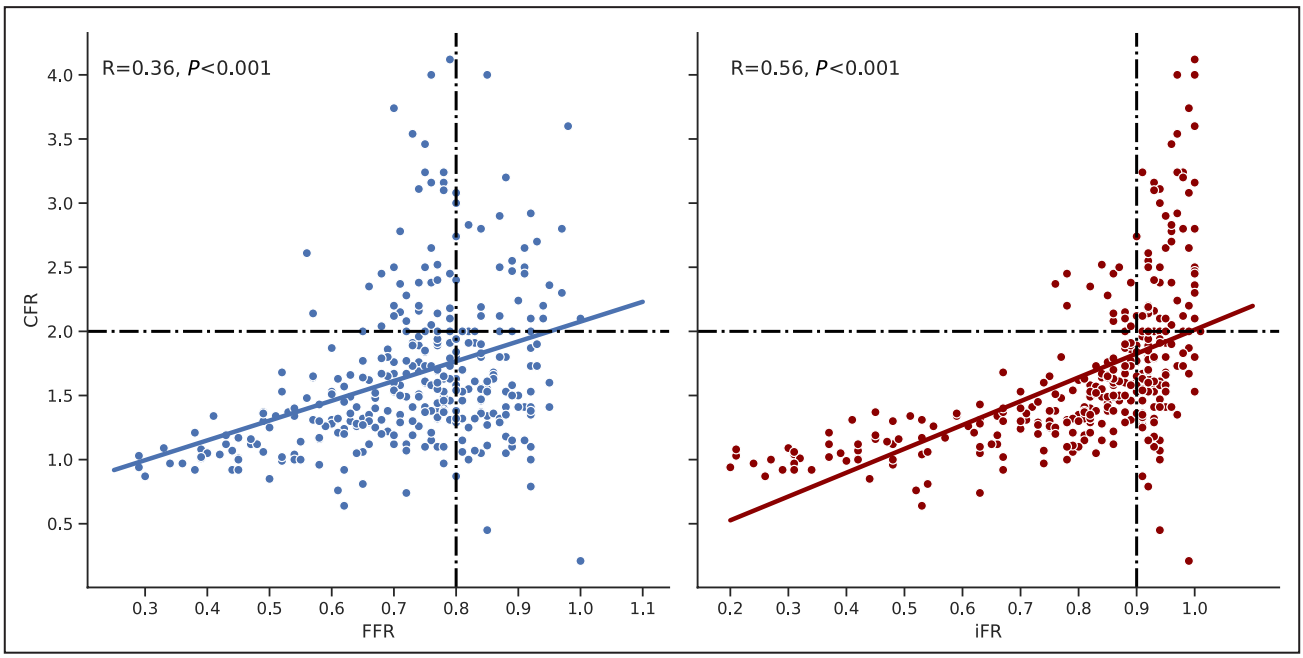

Figure 4. Correlation between CFR and FFR, and between CFR and iFR.
CFR indicates coronary flow reserve; FFR, fractional flow reserve; and iFR, instantaneous wave-free ratio.

Table 2. CFR Values in Lesions With and Without FFR/iFR Discrepancy

Type of lesions	CFR1	CFR2	*P* value
1/FFRp/iFRn vs 2/FFRp/iFRp	2.24±0.70	1.39±0.36	<0.0001
1/FFRp/iFRn vs 2/FFRn/iFRn	2.24±0.70	1.8±0.64	<0.0001
1/FFRn/iFRp vs 2/FFRp/iFRp	1.41±0.37	1.39±0.36	0.85
1/FFRn/iFRp vs 2/FFRn/iFRn	1.41±0.37	1.8±0.64	0.011

CFR indicates coronary flow reserve; FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

When comparing patients with the FFRp/iFRn discrepancy to patients with the FFRn/iFRp types of discrepancy, those with FFRp/iFRn were younger and less frequently were women, diabetics, patients with CKD, users of beta blockers, or users of diuretics. Also, they had a higher level of hemoglobin, lower level of creatinine, and higher glomerular filtration. Diuretics and CKD were identified as predictors for the FFRn/iFRp type of discrepancy using multivariable logistic regression analysis (Table 6).

Genetic Analysis

Genetic analysis was performed for a total of 224 patients, originating only from Czech centers. Out of these, 5 patients were not included in the analysis for low quality of FFR/iFR tracings. There were no significant differences in the demographic parameters between ENOS risk ($ENOS_r$)and protective carriers ($ENOS_p$). Patients with risk type of polymorphism in gene for HO-1 ($HO\text{-}1_r$) were more frequently women and suffered less frequently from CKD compared with those with protective phenotype ($HO\text{-}1_p$). Demographic data of patients with genetic analyses are listed in Table S1.

The types of polymorphism associated with risk were found in 112 patients (51.1%) in the gene for ENOS, and in 60 patients (27.4%) in the gene for HO-1. The number of patients with both types of risk polymorphism was 28 (12.8%), and the number of patients without any risk polymorphism was 75 (34.2%). The occurrence of risk polymorphism in the genes for ENOS and HO-1 in patients with the FFR/iFR discrepancy is summarized in Table 7.

Out of 219 FFR/iFR examination pairs, 67 (30.6%) discrepancies were found: FFRp/iFRn in 34 patients (15.5%) and FFRn/iFRp in 33 patients (15.1%). Risk types of polymorphisms were found nonsignificantly more frequent in patients with FFRn/iFRp compared with those with FFRp/iFRn: for $ENOS_r$ 19 (57.6%) versus 18 (52.9%), P=0.7 and for $HO\text{-}1_r$ 12 (36.4%) versus 9 (26.5%), P=0.38. The FFRn/iFRp type of discrepancy was significantly more frequent in patients with both risk type of polymorphisms ($ENOS_r$+$HO\text{-}1_r$): 8 patients (24.2%) compared with FFRp/iFRn type of discrepancy: 2 patients (5.9%), P=0.03.

Patients with $ENOS_r$ and $HO\text{-}1_r$ did not differ in FFR or iFR values compared with those with $ENOS_p$ and $HO\text{-}1_p$: for ENOS, FFR was 0.81±0.09 versus 0.82±0.09, P=0.23, and iFR was 0.89±0.1 versus 0.89±0.09, P=0.83; and, for HO-1 the FFR value was 0.82±0.09 versus 0.81±0.09, P=0.56, and iFR was 0.89±0.08 versus 0.89±0.1, P=0.98. However, there was a trend for higher FFR values in patients with risk type of polymorphism in both gene types compared with patients with no/or one type of risk polymorphism (0.84±0.06 versus 0.81±0.09, P=0.066). Unlike the FFR values, the iFR values did not differ between those 2 types of patients (0.90±0.06 versus 0.89±0.1, P=0.58).

Furthermore we analyzed a numerical difference between iFR and FFR in patients with FFR/iFR discrepancy. The iFR/FFR difference was 0.085±0.07 in $ENOS_r$ and 0.072±0.07 in $ENOS_p$, P=0.19, in patients with $HO\text{-}1_r$ 0.074±0.07 and in those with $HO\text{-}1_p$0.08±0.07, P=0.51. There was a trend for lower iFR/FFR difference in patients with risk types of polymorphisms in both genes compared with patients with either both protective polymorphisms or with only 1 type of risk polymorphism (0.059±0.05 versus 0.082±0.07, P=0.09).

DISCUSSION

Using data from 1884 FFR/iFR pairs of examinations from 1564 patients, we found 79.1% agreement in diagnostic classification between FFR and iFR, which correlates well with similar trials.[4,9]

Table 3. Predictors for FFRp/iFRn According to Angiographic Parameters

Parameters included in model	Unstandardized coefficient B	SE	Wald	*P* value for significance
Proximal location	−0.09	0.183	0.23	0.63
Diameter stenosis	0.005	0.008	0.36	0.55
Lesion location in Right coronary artery	0.75	0.195	14.7	0.0001
Tandem lesion	0.153	0.21	0.51	0.48

Model for prediction of FFRp/iFRn type of discrepancy. Nagelkerke R Square 0.029. Hosmer-Lemeshow test: chi-square 9673, *P* value 0.289.
CFR indicates coronary flow reserve; FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

Table 4. Predictors for the FFRp/iFRn Type of Discrepancy

Parameters included in model	Unstandardized coefficient B	SE	Wald	Significance
Sex	0.66	0.22	9.4	0.002
Age	-0.02	0.009	7.1	0.008
Using beta blockers	-0.21	0.17	1.43	0.23
Ejection fraction of left ventricle	0.01	0.008	2.27	0.13

Model for prediction of FFRp/iFRn type of discrepancy. Nagelkerke R Square 0.037. Hosmer-Lemeshow test: chi-square 14 923, *P* value 0.061.
FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

The FFRp/iFRn Type of Discrepancy

The CFR value in FFRp/iFRn lesions was significantly higher compared with both FFRp/iFRp and FFRn/iFRn lesions. CFR correlated more closely with iFR than with FFR. Very similar results were found in a study done by Cook et al, who analyzed a similar group of patients (301 patients, mean age 60 years, 4% of patients with ACS).[10] They found a higher CFR value in FFRp/iFRn when compared with CFR in FFRp/iFRp lesions. Unlike our results, they found the same CFR values in FFRp/iFRn and FFRn/iFRn. This difference can be caused by lower CFR values in FFRn/iFRn lesions in our data set as compared with Cook et al (1.8 versus 2.41). Reasons for lower CFR in our population can be explained by the greater age of patients (68 versus 60 years), the higher occurrence of diabetes (38.9% versus 22%), and the higher proportion of patients with ACS (13.3% versus 4%) compared with the population in the study done by Cook et al. When we compared patients with negative results for both FFR and iFR with the FFRp/iFRn type of discrepancy, we found a lower age (70.6±9.7 versus 66.9±9.6, $P<0.001$), a higher proportion of ACS (127, 19.8% versus 5, 1.9%, $P<0.001$), and a trend toward a higher occurrence of diabetes (254, 39.6% versus 86, 33.2%, $P=0.07$) in the discrepancy group. All of these factors increase microvascular resistance and, as a result, decrease CFR.

Higher coronary flow in the FFRp/iFRn type of discrepancy was also found in the JUSTIFY-CFR (Joined Coronary Pressure and Flow Analysis to Determine Diagnostic Characteristics of Basal and Hyperemic Indices of Functional Lesion Severity-Coronary Flow Reserve) study,[11] where Petraco et al demonstrated a closer correlation between iFR and CFR compared with FFR and CFR, using data from an analysis of 216 stenoses. Hwang et al,[12] found higher sensitivity and specificity of iFR over FFR when compared with the "gold standard" of ^{13}N-ammonia positron emission tomography CFR.

The higher CFR values found in FFRp/iFRn lesions can be explained by the known fact that coronary flow can be substantially increased—during adenosine administration—only in nonflow limiting lesions.[13,14] CFR reflects both epicardial stenosis severity and microvascular functions. Well-preserved microvascular functions are essential for an adequate response to drugs causing vasodilatation. Pressure gradient across stenosis is related to the level of coronary flow. Even a nonsignificant pressure gradient can become significant in a case of high flow. The higher the level of flow achieved, the higher the pressure gradient found.

Age, sex (male), and location of a lesion in the RCA were found to be associated with the FFRp/iFRn discrepancy in models predicting this type of discrepancy. A higher occurrence of the FFR/iFR discrepancy in the RCA was also found in other studies.[15,16] Explanation could be a different type of coronary flow in RCA, where maximum coronary flow can occur during late systole or early diastole, instead of mid-diastole where iFR is measured. However, this problem has not been solved yet. Kobayashi et al published a study showing a higher rate of FFR/iFR discrepancy in the left main and proximal left anterior descending artery.[17]

A younger age—compared with the agreement group—in patients with the FFRp/iFRn type of discrepancy was also found in studies by Lee[18] and Derimay,[19] which is probably related to a higher CFR in younger people. Derimay[19] also revealed the FFRp/iFRn discrepancy to be more frequent in proximal lesions, which we did not confirm.

Table 5. Predictors for FFRn/iFRp Type of Discrepancy

Parameters included in model	Unstandardized coefficient B	SE	Wald	Significance
Weight	–0.01	0.008	3.16	0.08
Using diuretics	0.5	0.29	2.9	0.09
Hemoglobin, g/L	–0.01	0.007	3.9	0.05
Smoking	0.67	0.29	5.4	0.02
Chronic kidney disease	0.89	0.37	5.8	0.02

Model for prediction of FFRn/iFRp type of discrepancy. Nagelkerke R Square 0.098. Hosmer-Lemeshow test: chi-square 3549, *P* value 0.895. FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

Table 6. Predictors for FFRp/iFRn Type Discrepancy Among Patients With any Type of Discrepancy

Parameters included in model	Unstandardized coefficient B	SE	Wald	Significance
Sex	1.033	0.44	5.4	0.07
Beta blockers	−0.15	0.43	0.13	0.73
Diuretics	−0.87	0.39	4.9	0.03
Chronic kidney disease	−1.38	0.63	4.8	0.03

Model for prediction of FFRn/iFRp type of discrepancy. Nagelkerke *R* Square 0.18. Hosmer-Lemeshow test: chi-square 2517, *P* value 0.867. FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; n, negative; and p, positive.

The FFRp/iFRn type of discrepancy was found to be more frequent in men. Higher FFR values in women, along with similar iFR and CFR values in both sexes, were also found in a study recently published by Yonetsu et al.[20] They found the FFRp/diastolic pressure ratio with negative finding discrepancy to be less frequent in women than in men (diastolic pressure ratio is equivalent to iFR[21]). Our finding of a higher occurrence of the FFRp/iFRn type of disagreement in men also correlates with a study done by Lee et al.[18] Moreover, higher FFR values in women, and similar iFR values in women and men, have also been found in other trials.[22–24]

The FFRn/iFRp Type of Discrepancy

The FFRn/iFRp lesions exhibited lower CFR values compared with FFRn/iFRn lesions, and the same CFR values as lesions with both positive FFR and iFR examinations. Similar results were found by Cook et al.[10]

Increased microvascular resistance, which can be identified by a lower CFR value, caused a relatively higher FFR than iFR for lower reaction to administered adenosine. This fact leads to more frequent finding of FFRn/iFRp discrepancy in such patients.[25]

Unlike the FFRp/iFRn type of discrepancy, the FFRn/iFRp type was less frequent in RCA (18 lesions, 5.0%) versus left coronary artery (115 lesions, 8.6%), *P*=0.043, and this type of discrepancy was found more often in LAD territory, compared with other coronary arteries (97 lesions, 9.9% versus 32 lesions 5.1%, *P*=0.0006). LAD dominance in the FFRn/iFRp type of discrepancy was also found in a study done by Derimay.[19]

Table 7. FFR/iFR Discrepancy and Type of Polymorphism in Genes for ENOS and HO-1

	FFR/iFR discrepancy	FFR/iFR agreement	*P* value
$ENOS_r$	37 (55.2%)	75 (49.3%)	0.42
$HO\text{-}1_r$	21 (31.3%)	39 (25.6%)	0.39
$ENOS_r$ and $HO\text{-}1_r$	10 (14.9%)	18 (11.8%)	0.53
$ENOS_p$ and $HO\text{-}1_p$	19 (28.4%)	56 (36.8%)	0.22

ENOS indicates endothelial nitric oxide synthase; FFR, fractional flow reserve; HO-1, heme oxygenase-1; and iFR, instantaneous wave-free ratio. Indexes "r" and "p" represents risk and protective type of gene polymorphisms.

Multivariable logistic regression analysis confirmed associations between hemoglobin level, smoking, and the presence of CKD and the FFRn/iFRp type of discrepancy.

One possible explanation for the lower hemoglobin level in patients with the FFRn/iFRp type of discrepancy may be increased blood flow in patients with chronic anemia. This increased blood flow causes a greater loss of translesional pressure, (especially in diastole) and leads to more positive iFR.[26] In a study done by Östlund-Papadogeorgos et al.,[27] lower intramyocardial resistance was found in patients with lower hemoglobin levels, and a higher occurence of the FFRn/RFRp discrepancy in patients with lower hemoglobin level was found in studies published by Kato et al.[28] and Muroya et al.[26]

We found a higher occurrence of the FFRn/iFRp type of polymorphism in patients with CKD. This type of patient is known to have a higher index of microcirculatory resistance, worse endothelial function, and lower FFR values compared with patients with preserved renal function.[29,30] A worse correlation between FFR and myocardial perfusion scintigraphy[31] was found in patients with CKD. Basal coronary flow is probably not affected as much as hyperemic, which could be one of the reasons for the FFRn/iFRp type of discrepancy in patients with CKD. This finding has also been found in other trials.[27,32]

It is very well known that smoking is a factor that strongly contributes to endothelial dysfunction, mainly through a decrease in the availability of nitric oxide (NO).[33] So, it is not surprising to find that smoking is associated with the FFRn/iFRp discrepancy, which is primarily caused by endothelial dysfunction. However, the number of smokers did not significantly differ between the FFR/iFR agreement and the FFR/iFR discrepancy groups in studies by Lee[18] and Derimay.[19] Therefore, the relationship between smoking and the FFRn/iFRp discrepancy remains unclear.

The rate of FFR/iFR discrepancy did not differ in patients with either risk or protective type of polymorphisms in ENOS/HO-1 genes. However, we found significantly more frequent FFRn/iFRp type of discrepancy, compared with FFRp/iFRn one, in patients with risk type of polymorphism in both genes. This difference was caused by higher FFR values and subsequently lower iFR/FFR difference in patients with $ENOS_r$ and $HO\text{-}1_r$ compared with those with both protective polymorphisms or with risk

polymorphism in only 1 gene. Lower iFR/FFR difference in patients with risk types of polymorphisms was probably caused by worse endothelial functions in such patients.

The ENOS G894T polymorphism has been shown to be associated with decreased NO production in endothelial cells, under basal conditions and in response to shear stress.[34] We hypothesize that endothelial dysfunction, caused by impaired ENOS function, can lead to a decreased reaction to adenosine administration.

Adenosine-induced vasodilation is, at least partly, NO dependent.[35] Adenosine enhances the release of NO from endothelial cells via the adenosine 2A receptor.[36] Naber et al.[8] found nonsignificant lower basal and hyperemic flow in patients with the risk type of polymorphism in the ENOS gene, in a study assessing coronary flow by Doppler measurement in 97 patients. Lower hyperemic flow in patients with a lower level of ENOS gene activity could decrease endothelial reaction to administered adenosine. HO-1 enhances ENOS activation and prevents ENOS uncoupling, thereby acting to preserve adequate NO production, and thus endothelial function.[37,38] Under these conditions, HO-1 enhances eNOS activation and prevents eNOS uncoupling, thereby acting to preserve adequate NO production and thus endothelial function.[37] This can be a possible explanation for the higher occurrence of the FFRn/iFRp discrepancy among patients with both genetic polymorphisms.

Before any clinical implications are assumed, these results must first be confirmed in other trials. In the event of confirmation, the possibility to prefer iFR measurement over FFR, in patients with the risk type of polymorphism in the gene for ENOS, could be opened. But, more than implications for clinical practice, these findings show the complexity of coronary circulation and contributing factors.

The Comparison of FFRp/iFR and FFRn/iFRp Type of Discrepancies

In a comparison of patients with FFRp/iFRn and patients with FFRn/iFRp, we found differences similar to those in comparisons between patients with FFRn/iFRp and the agreement group. Additional differences were found: a higher occurrence of diabetes and a higher number of patients treated with beta blockers. However, they were not confirmed in multivariable regression analysis.

It is not surprising that diabetes—a known factor for impaired microvascular function—was found more frequently in patients with the FFRn/iFRp type of discrepancy. Both Derimay[19] and his group and Arashi et al.[32] found this difference, too.

A higher occurrence of treatment with beta blockers in patients with the FFRn/iFRp discrepancy, compared with patients with the FFRp/iFRn discrepancy is interesting. Ebihara et al.[39] showed a negative correlation between rate pressure product (calculated as systolic blood pressure multiplied by heart rate) and iFR, but not with FFR. This means that patients with a high rate pressure product had relatively lower iFR compared with FFR value. This can be an indirect marker of the FFRn/iFRp type of discrepancy. A higher heart rate in patients with FFRn/iFRp compared with patients with FFRp/iFRn was confirmed in a study by Arashi et al. Patients usually do not take peroral medication on the day of the procedure, so patients who have not taken their beta blockers might have a higher relative blood pressure and heart rate (as a result of small rebound phenomenon), compared with patients without this kind of medication. Unfortunately, we did not analyze systolic blood pressure and heart rate in our study, so we cannot prove this hypothesis.

CONCLUSIONS

The main finding of the FiGARO trial is a 21% disagreement between FFR and iFR measurements. This was caused by well-preserved endothelial function enabling a substantial increase of flow across a stenosis in the FFRp/iFRn discrepancy and inadequate vasodilation after the administration of hyperemic drugs, leading to the FFRn/iFRp discrepancy. The FFRp/iFRn discrepancy can be found in lesions located in the RCA, coronary territory with a preserved CFR, in younger patients, and more frequently in men than in women. The FFRn/iFRp discrepancy type is probably caused by nonadequate endothelial reaction to vasodilatation drugs and can be found in carriers of the risk type of polymorphism in the gene for ENOS and HO-1, in smokers, and in patients with CKD. These results should be taken into consideration during the assessment of coronary physiology, especially in cases with FFR/iFR discrepant measurements.

Limitations

One of the main study limitations is the low number of patients included in genetic subanalysis. Therefore, the relationship between the FFRn/iFRp discrepancy and genetic polymorphisms in the genes for ENOS and HO-1 is only an interesting association, which must be confirmed in a larger trial. The relatively low value of flow measurements (compared with total FFR/iFR examinations) is comparable to other, similar trials. The CFR value was surprisingly low in the FFRn/iFRn group, which shows quite frequent microvascular dysfunction in such patients, but this finding probably reflects the clinical reality in patients with coronary artery disease.

Another limitation is the possible influence of pressure drift. Although testing of pressure drift (and a repeat of the measurement if the pressure drift exceeds 0.05) was strongly recommended, this step was not reported. Analysis of both patients with chronic coronary syndromes and ACS in 1 cohort was used to

analyze possible contributors for the FFR/iFR discrepancy in a whole spectrum of clinical scenarios. It is one of the study limitations. However, this approach was also used in other trials comparing FFR/iFR.[2,3,18,19]

Another limitation is the presence of 2 different types of FFR/iFR discrepancy in 2 different vessels. Such lesions were analyzed separately in *per-lesion* analysis. For *per-patient* analysis, we chose the more significant of the discrepancies. However, this situation occurred in only 3 patients out of the whole cohort.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1
Table S1

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SUPPLEMENTAL MATERIAL

Data S1. Description of Genetic Analysis

Patient DNA was isolated from peripheral blood leukocytes using standard techniques. Polymerase chain reaction (PCR) was performed using oligonucleotide primers designed to amplify exon 7 of the ENOS gene. Sample amplification was performed in an MJ Research DYAD 220 Peltier Thermal Cycler (Conquer Scientific, San Diego, CA). The following primers were used: forward primer ENOS7-sense, 5'-GAG ATG AAG GCA GGA GAC AGT-3' and reverse primer ENOS7-anti, 5'-TCC ATC CCA CCC AGT CAA T-3'. The mixture (final volume 25 µl) was incubated at 94 °C for 3 min, followed by 30 cycles (each 25 s at 94 °C, 40 s at 59 °C, and 40 s at 72 °C) at 72 °C for 10 min. Restriction analysis was performed by incubating three units of MboI restriction enzyme (Promega, Madison, WI) with the amplified DNA for 12 h overnight at 37 °C. The restriction products were separated by electrophoresis in a 3.8% agarose gel. The analysis of restriction products was performed after the addition of ethidium bromide. The GG (Glu/Glu) variant was classified as protective, whereas the GT (Glu/Asp) and TT (Asp/Asp) variants were classified as the risk variant.

The region of the HO1 gene promoter containing a poly (GT)n repeat was amplified by PCR that included a fluorescently labelled sense primer (HMOX1_S 5-AGAGCCTGCAGCTTCTCAGA-3) and an antisense primer (HMOX1_AS 5-ACAAAGTCTGGCCATAGG AC-3). All PCR products were generated in 25 µl volumes containing Plain Combi PP Master Mix (Top-Bio, Prague, Czech Republic), 1.6 pmol forward primer, 1.6 pmol reverse primer and 25 ng of template DNA. All amplifications were performed in a Dyad thermocycler (BIORAD, Hercules, CA) with the following protocol: a 5-minute denaturation at 95 °C was followed by 30 cycles of 30 s at 95 °C, 30 s at 66 °C, 30 s at 72 °C and then a final extension at 72 °C for 5 minutes. The PCR product sizes were determined using Li-cor 4200 (LI-COR Biosciences, Lincoln, NE) and ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA) DNA sequencers. We used

IR700 labelled primers for Li-cor analysis and 6-FAM labelled primers for ABI analysis. The determination of fragment length was accomplished using SagaGT (LI-COR Biosciences) and Peak Scanner™ Software (Applied Biosystems). Selected samples were sequenced in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) automated DNA sequencer and then included as size markers in every electrophoresis run. We divided alleles according to the number of GT repeats in two categories: class S (short) alleles with less than 25 (GT)n, and class L (long) alleles, with 25 or more (GT)n. Homozygous class S and heterozygous class S carriers were grouped together (protective variant) and compared to homozygous class L carriers (risk variant).

Table S1: Demographic features in patients with genetic analyses

Type of gene	ENOS			HO-1		
Polymorphism	risk	protective	p value	risk	protective	p value
Age	67.0 ± 9.3	67.3 ± 10.4	0.81	67.9 ± 8.8	66.8 ± 10.2	0.48
Female	26 (24.4%)	31 (27.0%)	0.33	23 (38.3)	34 (21.4%)	**0.01**
ACS	6 (5.7%)	10 (9.7%)	0.28	4 (7.0%)	12 (8.0%)	0.82
MI in past	37 (33.6%)	29 (27.4%)	0.32	15 (25.4%)	51 (32.5%)	0.31
DM	48 (43.6%)	43 (40.6%)	0.65	20 (33.9%)	71 (45.2%)	0.13
Arterial hypertension	82 (74.6%)	83 (78.3%)	0.52	44 (74.6%)	121 (77.1%)	0.70
HLP	73 (66.4%)	64 (60.4%)	0.36	33 (55.9%)	104 (66.2%)	0.16
CKD	14 (12.7%)	13 (12.4%)	0.94	3 (5.1%)	24 (15.4%)	**0.03**
Active smoking	44 (40.4%)	35 (33.0%)	0.26	23 (39.0%)	56 (35.9%)	0.68
Beta blockers	79 (72.5%)	69 (65.7%)	0.28	41 (71.3%)	107 (68.2%)	0.59
CCB	32 (29.6%)	31 (29.5%)	0.97	15 (26.3%)	48 (30.8%)	0.53
RAAS blockers	75 (68.8%)	72 (68.6%)	0.97	35 (61.4%)	112 (71.3%)	0.17
Nitrates	7 (6.4%)	7 (6.7%)	0.94	5 (8.8%)	9 (5.7%)	0.44
Diuretics	46 (42.2%)	38 (36.2%)	0.37	22 (38.6%)	62 (39.5%)	0.91
Statins	46 (76.7%)	41 (70.7%)	0.46	28 (77.8%)	59 (72.0%)	0.5
EF LV	54.8 ± 12.9	54.5 ± 11.3	0.83	53.4 ± 13.0	55.1 ± 11.7	0.35

ENOS: endothelial nitric oxide synthase, HO-1: hem-oxygenase 1, ACS: acute coronary syndrome, MI: myocardial infarction, DM: diabetes mellitus, HLP: hyperlipidemia, CKD: chronic kidney disease, CCB: calcium channel blockers, RAAS: renin-angiotensin-aldosterone system, EF LV: ejection fraction of left ventricle