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CLINICAL RESEARCH
Coronary heart disease

Replication study of 10 genetic polymorphisms associated with coronary heart disease in a specific high-risk population with familial hypercholesterolemia

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Aims Recent large association studies have revealed associations between genetic polymorphisms and myocardial infarction and coronary heart disease (CHD). We performed a replication study of 10 polymorphisms and CHD in a population with familial hypercholesterolemia (FH), individuals at extreme risk of CHD.

Methods and results We genotyped 10 polymorphisms in 2145 FH patients and studied the association between these polymorphisms and CHD in Cox proportional hazards models. We confirmed the associations between four polymorphisms and CHD, the rs1151640 polymorphism in the olfactory receptor family 13 subfamily G member 1 (OR13G1) gene (HR 1.14, 95% CI 1.01–1.28, $P = 0.03$), the rs11881940 polymorphism in the heterogeneous nuclear ribonucleoprotein U-like 1 (HNRPUL1) gene (HR 1.27, 95% CI 1.07–1.51, $P = 0.007$), the rs3746731 polymorphism in the complement component 1 q subcomponent receptor 1 (CD93) gene (HR 1.26, 95% CI 1.06–1.49, $P = 0.01$), and the rs10757274 polymorphism near the cyclin-dependent kinase N2A and N2B (CDKN2A and CDKN2B) genes (HR 1.39, 95% CI 1.15–1.69, $P < 0.001$).

Conclusion We confirmed previously found associations between four polymorphisms and CHD, but refuted associations for six other polymorphisms in our large FH population. These findings stress the importance of replication before genetic information can be implemented in the prediction of CHD.

Keywords Coronary heart disease • Familial hypercholesterolemia • Genetics • Polymorphism • Replication

Introduction

Coronary heart disease (CHD), and especially myocardial infarction (MI), is one of the most common causes of morbidity and mortality and has a strong genetic component.[1] The complexity of CHD and MI is illustrated by the many cell types that are involved in the atherosclerotic plaque and by the multiple processes that determine CHD risk, such as inflammation and thrombosis. Given this complexity, it is not clear which genes harbour the variation responsible for the genetic component of CHD. Recently, we conducted three large association studies to identify novel genetic variants associated with MI and early-onset MI.[2–4]

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A total of eight polymorphisms were found to be associated with MI or early-onset MI in three independent populations. Collaborators in these studies also found another polymorphism, which showed an association with MI in two independent populations.[5] More recently, two genome-wide association (GWA) studies found an additional association between polymorphisms nearby the cyclin-dependent kinase N2A and N2B (CDKN2A/B) genes and CHD. These latter polymorphisms were consistently associated with MI and CHD in independent populations.[6,7]

Replication of genetic associations in independent populations is essential to reduce the number of false-positive results and to further define the role of these variants in the susceptibility to complex disease. We therefore performed a replication study in a specific population of patients with familial hypercholesterolemia (FH), who have an extremely high risk of CHD and MI, to test whether these previous findings can be generalized to these high-risk patients. FH is an autosomal dominant disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene and results in severely premature CHD.[8–11] The incidence and age of onset of CHD varies considerably among individuals with FH.[12–14] Classical risk factors explain this variability to only a minor degree.[15] Probably, a substantial part of the variation in the incidence of CHD in this disorder is due to genetic factors outside the LDL receptor gene.[16,17]

The aim of this study was to replicate the associations between CHD and the eight polymorphisms discovered by our association studies, the polymorphism found by our collaborators, and the polymorphism near CDKN2A/B genes, which showed the strongest association with CHD in previous GWA studies, in a specific population with extreme CHD risk.

Methods

Study population

We studied a cohort of heterozygous FH patients, recruited from 27 lipid clinics in the Netherlands between 1989 and 2002. More detailed information on the study design and the study population was published previously.[15,18] In brief, the DNA of suspected FH individuals from Dutch lipid clinics is routinely submitted to a central laboratory for LDL-receptor-mutation analysis. We randomly selected 2400 unrelated FH individuals who fulfilled the internationally established FH diagnostic criteria.[15] The DNA of 2145 patients was available for the present analysis. The majority of the study population is of Caucasian descent (99%). All patients gave informed consent, and the ethics Institutional Review Board of each participating hospital approved the protocol.

During the observation period, with a mean duration of 5.0 ($\pm$4.7) years, phenotypic data (including CHD events) were acquired by review of medical records by a trained team of 13 data collectors. For this data collection, we used a pre-defined protocol.[18] Medical records were used to acquire information on age, sex, smoking, body mass index (BMI), the presence of diabetes mellitus (patients using anti-diabetic medication or fasting plasma glucose $>$6.9 mmol/L) and the presence of hypertension (patients with a documented diagnosis using anti-hypertensive medication or a systolic blood pressure $>$140 mmHg and/or diastolic blood pressure $>$90 mmHg at three consecutive office visits).

Plasma total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and triglycerides were measured by standard methods in fasting patients withdrawn from lipid-lowering medication at least 6 weeks prior to blood collection. LDL cholesterol was calculated with the Friedewald formula.[19]

Coronary heart disease definition

CHD was defined as the presence of (i) MI, proved by at least two of the following: (a) classical symptoms ($>$15 min), (b) specific ECG abnormalities, or (c) elevated cardiac enzymes ($>2\times$ upper limit of normal); (ii) percutaneous coronary intervention or other invasive procedures; (iii) coronary artery bypass grafting; (iv) angina pectoris, diagnosed as classical symptoms in combination with at least one unequivocal result of (a) exercise test, (b) nuclear scintigram, (c) dobutamine stress ultrasound, or (d) $>$70% stenosis on a coronary angiogram. In case of doubt about the diagnosis CHD, it was presented to an independent cardiologist, using anonymous copies of the necessary documents from the medical records.

Genetic analyses

We selected 10 polymorphisms of which we expected to have enough statistical power ($>$80%) based on effect sizes and genotype frequencies in literature.[2–7] These a priori power calculations were based on a person-years approach as applied in the present study. The following polymorphisms were investigated: rs12510359 in the palladin (PALLD) gene,[3] rs619203 in the v-ros UR2 sarcoma virus oncogene homologue 1 (ROS1) gene,[3] rs1376251 in the taste receptor type 2 member 50 (TAS2R50) gene,[3] rs1151640 in the olfactory receptor family 13 subfamily G member 1 (OR13G1) gene,[3] rs4804611 in the zinc finger protein 627 (ZNF627) gene,[3] rs1010 in the vesicle-associated membrane protein 8 (VAMP8) gene,[4] rs11881940 in the heterogeneous nuclear ribonucleoprotein U-like 1 (HNRPUL1) gene,[4] rs3746731 in the complement component 1 q subcomponent receptor 1 (C1QR1 or CD93) gene,[2] rs11666735 in the Fc fragment of IgA receptor (FCAR) gene,[5] and rs10757274 $\sim$100 kb upstream of the CDKN2A and CDKN2B genes.[7] All genotypes were determined using fluorescence-based TaqMan allelic discrimination assays and analysed on an ABI Prism 7900 Sequence Detection System (Applied Biosystems). The rs619203 polymorphism in the ROS1 gene was not in Hardy–Weinberg equilibrium ($P = 0.01$ in the whole group, and $P = 0.01$ in the patients without CHD). To ensure that this was not due to technical reasons, we genotyped the rs529038 polymorphism that was in almost complete linkage disequilibrium with the rs619203 polymorphism in our original study with only four discordant calls.[3] In our population, these polymorphisms were concordant in $>$99%. The further analyses were therefore conducted with the rs619203 polymorphism. Primer and probe sequences are presented in the Supplementary material online, *Table S1*. Reaction components and amplification parameters were based on the manufacturer's instructions using an annealing temperature of 60°C. Results were scored blinded to CHD status. The genotyping of all polymorphisms had success rates between 92 and 94%. A total of 204 random duplicate samples showed highly concordant results ($>$99%).

Statistical analyses

For differences in cumulative CHD risk between groups, we used Kaplan–Meier curves and the log-rank test. We tested for normality by drawing normal Q–Q plots for the untransformed and log-transformed continuous variables. Plasma triglycerides were tested after logarithmic transformation. Hardy–Weinberg equilibrium of the polymorphisms was tested with an exact test.[20]

Since there is little literature about the studied polymorphisms, we chose the mode of inheritance on the basis of the genotypic test

(2-df). This resulted in the use of a dominant genetic model for the PALLD, TAS2R50, and FCAR polymorphisms, the recessive genetic model for the ROS1, VAMP8, and CD93 polymorphisms and the polymorphism near the CDKN2A/B genes. The additive model was chosen for the OR13G1, ZNF627, and HNRPUL1 polymorphisms.

To determine the association between the polymorphisms and CHD, we used Cox proportional hazards models.[21] Patients without CHD were censored at the date of the last lipid clinic visit or at the date of death attributable to causes other than CHD. The proportional hazards assumption was tested by drawing log minus log plots of the survival function and was met for all Cox proportional hazard models. In the primary model, we adjusted for year of birth, sex, and smoking. For smoking, we implemented a linearly decreasing risk effect for the 6 years after cessation.[22] A secondary model was constructed to investigate whether potential associations could be explained by possible intermediary variables, such as hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides. Postmenopausal women are at increased risk of developing CHD compared with premenopausal women.[23] Unfortunately, we do not have information about the age of menopause in our cohort. Alternatively, we studied the presence of an age effect among women by additionally adjusting the Cox proportional hazards models for age tertiles,[24] which were defined by cut-off values of 42.7 and 56.6 years. This adjustment did not change the results (data not shown).

The following co-variables had missing values: smoking (9.4%), hypertension (1.0%), BMI (14.0%), plasma HDL cholesterol (18.6%), and plasma triglycerides (15.9%). Therefore, we applied the multiple imputation method of the areglmpute function of the *R* statistical package to impute these missing values.[25] Imputation methods substitute the missing values with plausible values on the basis of the relationship between the variable with missing values and the available information. With multiple imputation, 10 completed data sets were created, and subsequently 10 analyses were performed by treating each completed data set as a real complete data set. Finally, the results from these analyses were combined to obtain the effect estimates, while properly taking into account the uncertainty in the imputed values. It has been shown that imputation is beneficial for handling missing data in epidemiologic methods.[26]

Since testing multiple polymorphisms could have led to false-positive associations due to multiple testing, we estimated the false-discovery rate (FDR) and considered an FDR $<5\%$ acceptable.[27] An exact description of the calculation of the FDR has been published previously.[3]

We further investigated the associations between the polymorphisms and cardiovascular risk factors (age, sex, smoking, hypertension, diabetes mellitus, BMI, plasma LDL and HDL cholesterol, and plasma triglycerides), by using the χ^2-test, *t*-test, and ANOVA.

All data are provided as mean $\pm$ standard deviation, unless stated otherwise, and all reported *P*-values are based on two-sided tests of significance. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with the SPSS for Windows 12.0.1 statistics programme and the *R* statistical package.[25]

Results

Patient characteristics

Table 1 shows the cumulative lifetime risks of CHD till the age of 40, 50, and 60 years, whereas the clinical characteristics of the 2145 patients are presented in the Supplementary material online, *Table S2*. During a total of 106 772 person years, 607 (28%) patients had at least one CHD event. The mean age of onset of the first CHD event was 48.8 $\pm$ 10.7 years. The following variables were associated with a higher cumulative CHD risk: sex, smoking, plasma total, HDL and LDL cholesterol levels below the median, and plasma triglyceride levels above the median (*Table 1*).

Polymorphisms and coronary heart disease

Table 2 shows the genotype frequencies of the 10 polymorphisms. All polymorphisms were in Hardy–Weinberg equilibrium, except for the rs619203 polymorphism in the ROS1 gene ($P = 0.01$). The associations between the polymorphisms and CHD are presented in *Table 3*. Carriers of one G-allele of the OR13G1 polymorphism had a 14% higher risk of CHD, whereas carriers of two G-alleles had a 30% higher risk of CHD, compared with carriers of two A-alleles of this polymorphism ($P = 0.03$, primary model, *Table 3*). Carriers of one A-allele of the HNRPUL1 polymorphism had a 27% higher risk of CHD, whereas carriers of two A-alleles had a 61% higher risk of CHD, compared with carriers of two T-alleles of that polymorphism ($P = 0.007$, primary model, *Table 3*). Patients homozygous for the T-allele of the CD93 polymorphism had a 26% increased risk of CHD compared with patients with at least one C-allele of that polymorphism ($P = 0.01$, primary model, *Table 3*). Patients homozygous for the G-allele of the polymorphism near the CDKN2A/B genes had a 39% higher risk of CHD than patients with at least one A-allele of that polymorphism ($P < 0.001$, primary model, *Table 3*). The other polymorphisms were not significantly associated with CHD (*Table 3*). Additional adjustment for hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides yielded similar results (*Table 3*).

Polymorphisms and cardiovascular risk factors

The TAS2R50 polymorphism was associated with a slightly increased BMI (25.2 $\pm$ 3.6 kg/m^2 for the TC+CC genotypes vs. 24.6 $\pm$ 3.2 kg/m^2 for the TT genotype, $P = 0.04$). The ZNF627 polymorphism showed an association with increased TC levels (9.0 $\pm$ 1.7/9.5 $\pm$ 1.9/9.6 $\pm$ 2.0 mmol/L for the GG/GA/AA genotypes, respectively, $P = 0.01$). The VAMP8 was associated with an increased BMI (25.6 $\pm$ 3.8 kg/m^2 for the GG genotype vs. 25.0 $\pm$ 3.5 kg/m^2 for the AA+AG genotypes, $P = 0.01$). The CD93 polymorphism was associated with the presence of hypertension (11.0% for the TT genotype vs. 7.8% for the CC+CT genotypes, $P = 0.02$). Finally, the polymorphism near CDKN2A/B was associated with the presence of diabetes mellitus (6.6% for the GG genotype vs. 4.1% for the AA+AG genotypes, $P = 0.02$).

Discussion

We confirmed associations between four polymorphisms and CHD in this study of FH patients. These four polymorphisms were among a set of 10 that were recently found associated with MI or CHD in genome-wide or gene-centric association studies. The replicated polymorphisms are in the OR13G1 gene,[3]

Table 1 Clinical characteristics and outcome of 2145 patients with FH

Clinical characteristic	*N*	Total events	Cumulative CHD risk (%)			
			40 years	50 years	60 years	*P*-value*
Sex						
Female	1105	220	2.6	10.3	26.0	<0.001
Male	1040	387	11.8	35.1	62.7	
Smoking						
Never	522	101	4.2	11.2	26.6	<0.001
Ever	1422	456	8.2	27.1	50.1	
Hypertension						
No	1933	505	7.2	22.9	42.1	0.3
Yes	190	96	7.4	20.0	44.9	
Diabetes mellitus						
No	2021	539	7.3	22.4	42.1	0.1
Yes	124	68	6.6	21.3	46.0	
BMI						
≤25	981	217	7.0	21.4	38.5	0.1
25<BMI≤30	701	212	8.2	21.8	39.8	
>30	163	57	9.3	27.3	56.0	
Total cholesterol						
≤9.20 mmol/L	988	266	6.9	25.3	45.5	0.01
>9.20 mmol/L	934	254	6.0	18.6	38.5	
LDL cholesterol						
≤6.99 mmol/L	861	230	6.7	23.7	43.9	0.01
>6.99 mmol/L	856	194	5.6	17.0	35.4	
HDL cholesterol						
>1.16 mmol/L	867	180	3.7	13.9	31.2	<0.001
≤1.16 mmol/L	879	257	8.8	27.1	50.2	
Triglycerides						
≤1.57 mmol/L	907	176	4.7	18.4	35.6	0.01
>1.57 mmol/L	898	292	7.6	23.4	44.6	
Total	2145	607	7.2	22.3	42.4	

For triglycerides, total, LDL, and HDL cholesterol, we used the median to split the total population in two subpopulations. CHD, coronary heart disease; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *Log-rank test.

the HNRPUL1 gene,[4] the CD93 gene,[2] and near the CDKN2A/B genes.[7]

The rs10757274 polymorphism that is located ~100 kb upstream of the CDKN2A/B genes was discovered by a large GWA study, and the association with CHD was confirmed in four Caucasian populations.[7] The locus on chromosome 9p21 in which this polymorphism is located was also associated with MI and CHD in two other independent GWA studies,[6,28] and a recent prospective meta-analysis gave further evidence of the involvement of this locus in CHD.[29] The CDKN2A/B genes are tumour-suppressor genes involved in the regulation of cell proliferation, cell aging, and apoptosis,[30] which are all important in atherogenesis.[31] This locus might therefore play a role in cell cycle checkpoints which are important in repair of DNA that has been damaged by for example oxidative stress in atherosclerotic plaques. Future studies are required to elucidate the exact underlying mechanism by which this polymorphism or locus affects CHD risk.

The three other polymorphisms are located in genes that are relatively unknown in the field of cardiovascular disease and atherosclerosis. HNRPUL1 encodes a heterogeneous nuclear ribonucleoprotein and plays a role in RNA transport, processing, and transcriptional regulation. Furthermore, it has been speculated that this gene is involved in cell cycle regulation,[32,33] which might constitute a link with the proposed functionality of the polymorphism near the CDKN2A/B genes. In our original study, the HNRPUL1 polymorphism was associated with early-onset MI,[4] which might be the reason why we were able to replicate this polymorphism, as FH is an important cause of severely premature

CHD. It has been suggested that the CD93 gene is involved in intercellular adhesion, and leukocyte extravasation.[34] These are two important processes in the development of atherosclerosis[31] and could be the pathophysiological mechanisms underlying the association between variation within the CD93 gene and CHD. The mechanism through which the OR13G1 polymorphism influences CHD is unknown but might be related to dietary choices.

We did not find associations for the polymorphisms in the PALLD, ROS1, TAS2R50, ZNF627, VAMP8, and FCAR genes in our FH population. We could not find support for the hypothesis that hypercholesterolemia explains why these polymorphisms were not significant, whereas the four other polymorphisms were. The simplest explanation is that these associations were false-positive findings in the earlier studies, or false-negative findings in the present study. Lack of power is a well-known problem for small effects. Our a priori power calculations based on the effect sizes and genotype frequencies of the original studies showed sufficient statistical power for all polymorphisms (>80%). However, mostly we found smaller effect sizes for the polymorphisms than that in the original studies, which is in line with a study by Ioannidis *et al.*[35] If these lower effect sizes are true for FH populations, we might have had insufficient statistical power for the detection of these associations.

In contrast to the present study, one population-based replication study showed a significant association between the ROS1 polymorphism and MI, whereas the PALLD, TAS2R50, OR13G1, and ZNF627 polymorphisms were not associated with MI.[36] Yet another study found that none of these polymorphisms was significantly associated with MI in a case–control design.[37] The reason for these discrepancies could be found in the genetic heterogeneity or differences in functionality of this polymorphism across different populations. This could also be the reason for the fact that we did not find an association between CHD and the other non-significant polymorphisms in this study.

Two topics regarding the statistical analysis merit discussion. First, association studies of multiple polymorphisms could lead to false-positive findings due to multiple testing. We addressed this multiple-testing issue by calculating the FDR for all polymorphisms.[3,27] All four significant variants met the FDR criterion of 5%, indicating that the expected proportion of false-positives among all significant tests is below 5%. A Bonferroni correction would have been strongly over-punitive in case of low false-positive proportions.[38] Second, women who are menopausal are at increased risk of developing CHD.[23] Information on age of menopause was not available in our study, but we estimated that approximately half of the women had passed menopause at the end of follow-up. Among women, we adjusted for age tertiles in order to take this possible confounder into account, but this did not change our results (data not shown). Age did not confound our findings, but we are aware that our findings in women may only apply to populations with a similar distribution of age and menopause.

In the present population, higher levels of total and LDL cholesterol were associated with a lower cumulative CHD risk ($P = 0.01$, *Table 1*). An explanation for this paradoxical effect could be that FH patients with total and/or LDL cholesterol levels above the median received cholesterol-lowering therapy at a younger age

Table 2 Frequency distributions of polymorphisms

Gene	Polymorphism	Genotype	Frequency
PALLD	rs12510359	AA/AG/GG	12.0/44.5/43.5
ROS1	rs619203	GG/GC/CC	57.3/35.4/7.3
TAS2R50	rs1376251	TT/TC/CC	10.1/43.9/45.9
OR13G1	rs1151640	AA/AG/GG	19.8/48.7/31.4
ZNF627	rs4804611	GG/GA/AA	6.7/40.0/53.3
VAMP8	rs1010	AA/AG/GG	32.0/48.3/19.7
HNRPUL1	rs11881940	TT/TA/AA	2.3/26.0/71.7
CD93	rs3746731	CC/CT/TT	19.5/49.5/31.1
FCAR	rs11666735	GG/GA/AA	86.5/12.8/0.8
Near CDKN2A/B	rs10757274	AA/AG/GG	28.1/49.8/22.0

Table 3 Association between polymorphisms and CHD

Gene	Polymorphism	Genetic mode	Primary model		Secondary model		
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	FDR[b]
PALLD	rs12510359	Dominant	1.09 (0.85–1.41)	0.5	1.03 (0.80–1.34)	0.8	0.80
ROS1	rs619203	Recessive	0.88 (0.63–1.23)	0.5	0.84 (0.60–1.18)	0.3	0.38
TAS2R50	rs1376251	Dominant	1.31 (0.97–1.78)	0.08	1.25 (0.93–1.70)	0.1	0.20
OR13G1	rs1151640	Additive	1.14[a] (1.01–1.28)	0.03	1.15[a] (1.02–1.30)	0.02	0.04
ZNF627	rs4804611	Additive	0.98[a] (0.87–1.12)	0.8	0.97[a] (0.85–1.11)	0.7	0.78
VAMP8	rs1010	Recessive	0.87 (0.70–1.08)	0.2	0.87 (0.70–1.08)	0.2	0.29
HNRPUL1	rs11881940	Additive	1.27[a] (1.07–1.51)	0.007	1.28[a] (1.15–2.32)	0.006	0.03
CD93	rs3746731	Recessive	1.26 (1.06–1.49)	0.01	1.24 (1.05–1.48)	0.01	0.03
FCAR	rs11666735	Dominant	1.16 (0.91–1.46)	0.2	1.16 (0.92–1.47)	0.2	0.29
Near CDKN2A/B	rs10757274	Recessive	1.39 (1.15–1.69)	<0.001	1.39 (1.15–1.69)	<0.001	0.01

95% CI, 95% confidence interval; CHD, coronary heart disease; HR, hazard ratio. Primary model adjusted for sex, year of birth and smoking. Secondary model additionally adjusted for hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides. [a]Hazard ratio per risk allele. [b]FDR, false-discovery rate.

than patients with levels below the median (42.2 vs. 45.0 years, respectively, $P < 0.001$, data not shown).

In conclusion, we have confirmed the previously found associations between four polymorphisms and CHD in a large population of patients with FH. Further studies should elucidate the pathophysiological mechanisms underlying these associations. Genetic association studies will lead to further identification of potential modifier genes for CHD in FH patients or other high-risk populations. If replicated, these genetic risk factors can be incorporated into better tools for CHD risk prediction.

Supplementary material

Supplementary Material is available at *European Heart Journal* online.

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CLINICAL VIGNETTE

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In vivo molecular imaging of angiogenesis, targeting $\alpha_v\beta_3$ integrin expression, in a patient after acute myocardial infarction

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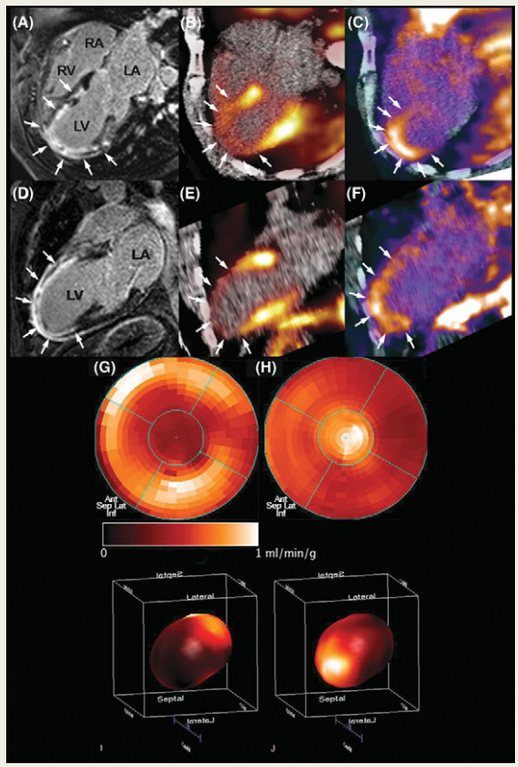
A 35-year-old Caucasian male presented with chest pain and nausea in the emergency room. The initial ECG displayed sinus-rhythm with ST elevation in I, aVL, V_{1-5}, ST-depression in III, and aVR and pathological Q waves in V_{1-5}. The laboratory results showed severely elevated cardiac enzymes.

Percutaneous coronary intervention (PCI) with stenting of the completely occluded proximal left anterior descending (LAD) was performed without complications.

Two weeks after the myocardial infarction and PCI, the patient underwent MRI and PET/CT evaluation based on a clinical research protocol (study approved by the ethics committee). Cine-MRI (steady-state-free-precession) displayed an impaired systolic function (left ventricular ejection fraction, 33%). Fifteen minutes after contrast injection (0.2 mmol gadolinium-diethlenetriamine pentaacetic acid), a nearly complete transmural delayed enhancement (>75%) was observed in the anterior-, anteriorseptal-, anteriorlateral-, and apical-wall (*Panels A, D*).

The positron emission tomography (PET) examination (Biograph 16 PET/CT, Siemens, Germany) with ^{13}N-ammonia revealed a severely reduced myocardial blood flow in the distal anterolateral, apical and inferoseptal region. (*Panels B, E*).

A novel $\alpha_v\beta_3$-targeting PET agent (^{18}F-Galakto-RGD) was used to assess integrin expression, which potentially represents angiogenesis involved in the regeneration process after myocardial infarction. The $\alpha_v\beta_3$ integrin is a key mediator of angiogenesis and thus may be an important diagnostic and therapeutic target associated with myocardial repair processes after ischaemic injury. Focal tracer retention was localized in the infarcted area defined by the extent of delayed enhancement MRI and severely reduced myocardial blood flow (MBF) (*Panels C, F*). This signal may indicate the myocardial healing taking place within the infarcted area as demonstrated in animal models.



Panel A and D. CMR with delayed enhancement (arrows) extending from the anterior wall to the apical region in the four- (A) and two-chamber (D) view. *Panel B and E*. Identically reproduced location and geometry with severely reduced myocardial blood flow using ^{13}N-ammonia, corresponding to the regions of delayed enhancement by CMR (arrows). *Panel C and F*. Focal ^{18}F-RGD signal co-localized to the infarcted area. This signal may reflect angiogenesis within the healing area (arrows). *Panel G and I*. Polar map (I: 3D) of myocardial blood flow assessed by ^{13}N-ammonia indicating severely reduced flow in the distal LAD-perfused region. *Panel H and J*. Co-localized ^{18}F-RGD signal corresponding to the regions of severely reduced ^{13}N-ammonia flow signal, reflecting the extent the of $\alpha_v\beta_3$ expression within the infarcted area.