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Short communication

CXCL5 gene polymorphisms and coronary collateralization

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ABSTRACT

Background: The presence of coronary collateralization is heterogenous, even amongst those with similar degrees of epicardial coronary artery stenoses. We hypothesized that genetic variation of CXCL5, a chemokine that mediates angiogenesis, is associated with coronary collateralization.
Methods: We genotyped subjects undergoing coronary angiography for single nucleotide polymorphisms of CXCL5 and determined the presence of spontaneously visible coronary collaterals.
Results: Subjects with collaterals had less angina (46 % vs 59 %, p = 0.006), and prior percutaneous coronary intervention (34 % vs 47 %, p = 0.010), and more hyperlipidemia (90 % vs 82 %, p = 0.018), peripheral arterial disease (25 % vs 17 %, p = 0.041), congestive heart failure (16 % vs 8 %, p = 0.007), and multi-vessel coronary artery disease (41 % vs 24 %, p = 0.0001) compared to those without collaterals. Multi-vessel disease and hyperlipidemia were positive predictors of angiographically visible collaterals while being a carrier of the CXCL5 polymorphism was a negative predictor.
Conclusions: Coronary collateralization may, at least in part, be genetically determined.

1. Introduction

The formation of coronary collaterals, a form of vascular remodeling that can be assessed angiographically, is a major compensatory mechanism in chronic ischemic heart disease (IHD). Subjects with IHD who have coronary collaterals have better outcomes, including a lower risk of myocardial infarction [1]. There is marked degree of heterogeneity in the presence of collaterals amongst patients with similar degrees of coronary artery stenoses, however. This heterogeneity is not explained by clinical factors alone and is speculated to be due to undiscovered genetic factors [2].

Chemokines are a superfamily of structurally homologous cytokine ligands that are important regulators of vascular remodeling [3]. They are classified structurally, based on conserved cysteine residues near their amino terminus, into CC, CXC, C, and CX_3C families. Within the CXC family, the presence of a 3 amino-acid sequence of glutamic acid-lysine-arginine (ELR) adjacent to the CXC motif determines the function of the ligands: ELR+ CXC chemokine ligands (which include CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, and CXCL12) are potent inducers of vascular remodeling and blood vessel formation, whereas the ELR− ligands (CXCL9, CXCL10, and CXCL11) inhibit the formation of blood vessels [3,4].

We previously reported that in patients with chronic IHD, higher blood concentrations of the angiogenic CXCL5 chemokine correlated with greater presence of coronary collaterals [5]. The gene for CXCL5 contains two single nucleotide polymorphisms (SNP) on chromosome 4: the rs352046 SNP in the promoter of the gene results in replacement of a guanine by a cytosine in position −156, and the rs425535 SNP causes replacement of a guanine with an adenine in position −398 in the protein-coding second exon [6]. It has previously been shown that there is a high degree of linkage between these two SNPs [7]. The aim of this study was to test the hypothesis that in patients with chronic IHD, SNPs of CXCL5 gene are associated with the presence of coronary collaterals.

2. Methods

We prospectively collected demographic and angiographic data from consecutive patients referred for an elective, clinically-indicated diagnostic coronary angiogram at the University of Virginia. All patients > 21 years old able to provide informed consent were eligible for

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enrollment. This study was approved by the institutional review board of the University of Virginia and all patients provided written informed consent. Patients were fasting and at steady-state prior to coronary angiography. Following vascular access via the femoral, brachial, or radial artery and prior to coronary angiography or heparin administration, a 30 mL peripheral blood sample was drawn from the side-arm of the sheath, anticoagulated with sodium EDTA, immediately placed on ice, and processed within 30 min of retrieval. Platelet-free plasma was aliquoted and frozen at −80 °C for subsequent measurement of CXCL5 by multiplex immunoassay using the manufacturer's instruction (Luminex, Bio-Rad, Bio-plex 200 system, Hercules, California; Procarta Cytokine Assay kit, Panomics, Inc., Fremont, California). The CXCL5 SNPs (rs352046 and rs425535) were quantified using Illumina Inc.'s Custom Genotyping Service (San Diego, CA). Coronary angiography was performed using standard techniques. Patients whose angiograms were read as having at least one epicardial artery lesion ≥90 % (prerequisite for spontaneous collateral recruitment [8]) were assessed for the presence or absence of spontaneously visible angiographic collaterals (L.C.L. and E.C.K.). The Fisher's Exact test was used to compare categorical values between patients with and without collaterals and the Wilcoxon rank-sum test was used for continuous variables. All continuous variables are reported as medians with interquartile ranges (IQR). Homozygosity was expected to be infrequent [7], therefore, the -156C/C and 398AA/homozygotes were included with heterozygotes and labeled as -156C carriers and 398A carriers. Plasma CXCL5 concentrations were analyzed by comparing carriers of the variant allele to homozygotes. A logistic regression model was used to generate a multivariable model to determine the factors associated with the presence or absence of collaterals. Candidate variables included sex, age, race, extent of coronary artery disease, hypertension, diabetes, hyperlipidemia, prior coronary artery revascularization, statin therapy, and the CXCL5 polymorphisms. A stepwise backward selection was performed using a threshold of $p < 0.10$. All analyses were performed with SAS 9.1 (SAS Institute, Cary, NC) or GraphPad PRISM (version 9). A two-sided p value of <0.05 was considered statistically significant.

3. Results

Of the 897 consecutive subjects who were enrolled, 449 (50 %) had angiographic evidence of at least one epicardial coronary artery with ≥90 % diameter stenosis. These 449 subjects (251 with and 198 without collaterals) comprised the study cohort. Subjects with collaterals had less angina (45 % vs 59 %, p = 0.006), and prior percutaneous coronary intervention (34 % vs 47 %, p = 0.010), and more hyperlipidemia (90 % vs 82 %, p = 0.018), peripheral vascular disease (25 % vs 17 %, p = 0.041), congestive heart failure (16 % vs 8 %, p = 0.007), and three-vessel coronary artery disease (41 % vs 24 %, p = 0.0001) compared to those without collaterals (Table 1). In the multivariate analysis, factors associated with the presence of collaterals were hyperlipidemia and multi-vessel coronary artery disease, and factors associated with the absence of collaterals were the CXCL5 polymorphisms (Table 2). CXCL5 plasma levels were measured in a total of 180 patients (the first 90 consecutively enrolled patients with an angiographically visible collateral, and the first 90 consecutively enrolled patients without a collateral). Compared to patients without angiographically visible coronary collaterals, those with collaterals had significantly higher plasma levels of CXCL5 (8517 pg/mL [5052–14,452] vs. 5354 pg/mL [1425–8878], p=0.0001). However, there was no significant difference in CXCL5 levels according to genotype: CXCL5 levels for the rs352046 G/G vs. C carrier were 7124 pg/mL [4322–14,452] vs. 9617 pg/mL [5280–15,261], p = 0.384; CXCL5 levels for the rs425535 G/G vs. A carrier were 7985 pg/mL [4465–14,639] vs. 9714 [5166–11,549], p = 0.654.

Table 1
Baseline characteristics of subjects with and without collaterals.

Variable	Collaterals N = 251	No collaterals N = 198	p value
Age	63 ± 11	63 ± 11	0.299
Male gender	193 (77 %)	151 (76 %)	1.000
Race			0.068
White	214 (85 %)	182 (92 %)	
Black	35 (14 %)	14 (7 %)	
Hispanic	3 (1 %)	2 (1 %)	
Hypertension	205 (82 %)	165 (83 %)	0.559
Diabetes mellitus	96 (38 %)	71 (36 %)	0.581
Tobacco use	71 (28 %)	49 (25 %)	0.387
Angina	114 (45 %)	117 (59 %)	0.006
Family history of premature CAD	115 (46 %)	79 (40 %)	0.196
Hyperlipidemia	226 (90 %)	163 (82 %)	0.018
Congestive heart failure	40 (16 %)	15 (8 %)	0.007
Prior stroke	23 (9 %)	12 (6 %)	0.219
Peripheral arterial disease	63 (25 %)	34(17 %)	0.041
History of arrhythmia	27 (11 %)	15 (8 %)	0.246
Prior myocardial infarction	94 (37 %)	65 (33 %)	0.350
Prior percutaneous coronary intervention	86 (34 %)	93 (47 %)	0.010
Prior coronary artery bypass graft surgery	61 (24 %)	43 (22 %)	0.505
Beta-blocker	193 (77 %)	158 (80 %)	0.366
Angiotensin converting-enzyme inhibitor	128 (51 %)	99 (50 %)	0.967
Aspirin	237 (94 %)	186 (94 %)	0.996
Insulin	53 (21 %)	35 (18 %)	0.321
Oral hypoglycemic	40 (16 %)	32 (16 %)	0.993
Calcium channel blocker	44 (18 %)	23 (12 %)	0.055
P2Y12 inhibitor	78 (31 %)	66 (33 %)	0.694
Statin	212 (84 %)	160 (81 %)	0.989
Extent of CAD			<0.0001
1-vessel	62 (25 %)	93 (47 %)	
2-vessel	86 (34 %)	58 (29 %)	
3-vessel	103 (41 %)	47 (24 %)	
Total cholesterol (mg/dL)	167 [139–225]	161 [136–194]	0.123
Low density lipoprotein (mg/dL)	102 [76–142]	96 [72–122]	0.128
High density lipoprotein (mg/dL)	37 [30–44]	38 [30–45]	0.809
Triglycerides (mg/dL)	147 [103–214]	148 [89–223]	0.827

CAD, coronary artery disease. Data are expressed as mean ± standard deviation, median [25–75 % interquartile range], or as number (percentage).

Table 2
Predictors of the presence of coronary artery collaterals.

Variable	Univariate analysis		Multivariate analysis	
	Estimate ($\times 10^4$)	p value	Estimate ($\times 10^4$)	p value
Sex	−0.725	0.468	–	–
Age	−0.769	0.442	–	–
Race				
White	−0.364	0.716	–	–
Black	0.262	0.794	–	–
Hypertension	−1.374	0.169	–	–
Diabetes mellitus	+0.069	0.945	–	–
Tobacco use	+1.021	0.307	–	–
Hyperlipidemia	+2.348	0.019	+2.845	0.004
Prior revascularization	−1.484	0.138	–	–
Statin use	−1.270	0.204	–	–
Multi-vessel disease	+4.397	0.001	+5.054	<0.01
rs352046 variant allele	−1.510	0.034	−2.048	0.001
rs425535 variant allele	−1.663	0.041	−2.121	0.034

4. Discussion

In patients with chronic IHD, coronary collateralization maintains myocardial viability in the collateral-fed distribution and is associated

with fewer and smaller myocardial infarctions, less ventricular aneurysm formation, better left ventricular function, less arrhythmias and better survival compared to those who do not recruit collaterals [9]. Patients with similar extent and severity of coronary artery disease exhibit marked heterogeneity in the presence of angiographically detectable spontaneous coronary collaterals, but the biological basis of this variability is not known- in particular, this heterogeneity is not fully explained by traditional cardiac risk factors. Our data suggest that coronary collateralization may, at least in part, be genetically determined.

The CXC chemokines are potent mediators of vascular remodeling and we previously showed that the blood concentration of ELR+ ligands (including CXCL5), which mediate angiogenesis, were positive predictors of coronary collaterals, whereas ELR− ligands, which are anti-angiogenic, were negative predictors of coronary collaterals [5]. CXCL5 is expressed by epithelial and vascular smooth muscle cells after stimulation by pro-inflammatory cytokines such as tumor necrosis factor and interleukin-1β and its functions include attracting and activating neutrophils and endothelial cells [4]. The CXCL5 gene is on chromosome 4q13-q21, in the same region as other CXC family genes, and consists of 4 exons and 3 introns. There are two SNPs in this gene, rs352046 and rs425535 [6]. They have a high degree of linkage ($D' = 1, r^2 = 0.94$), and high variant allele frequencies (16–17 %) have been reported for both in the European and United States populations [7]. In one study of 114 healthy adults, carriers of the -156C allele had significantly higher median plasma levels of CXCL5 compared to those with the -156GG genotype [7]. In our cohort of patients with coronary artery disease, we did not find significant differences in CXCL5 levels according to genotype. The high rate of statin treatment (>80 %), which has been associated with attenuation of CXCL5 production [10], may have influenced circulating CXCL5 levels in our cohort.

Our study has several limitations. First, we may have underestimated the presence of collaterals by measuring only spontaneously visible coronary collaterals. Second, it is possible that there were differences in clinical factors we did not collect. Third, we did not find a difference in CXCL5 levels amongst the variant alleles which may be associated with a high rate of statin treatment. Fourth, and most importantly, ours is a single center study that requires validation in a larger cohort.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.J. Regieli, J.W. Jukema, H.M. Nathoe, A.H. Zwinderman, S. Ng, D.E. Grobbee, Y. van der Graaf, P.A. Doevendans, Coronary collaterals improve prognosis in patients with ischemic heart disease, Int. J. Cardiol. 132 (2009) 257–262.
[2] J. Koerselman, P.P. de Jaegere, M.C. Verhaar, D.E. Grobbee, Y. van der Graaf, Prognostic significance of coronary collaterals in patients with coronary heart disease having percutaneous transluminal coronary angioplasty, Am. J. Cardiol. 96 (2005) 390–394.
[3] E.C. Keeley, B. Mehrad, R.M. Strieter, Chemokines as mediators of neovascularization, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 1928–1936.
[4] R.M. Strieter, P.J. Polverini, S.L. Kunkel, D.A. Arenberg, M.D. Burdick, J. Kasper, J. Dzuiba, J. Van Damme, A. Walz, D. Marriott, S.Y. Chan, S. Roczniak, A. B. Shanafelt, The functional role of the ELR motif in CXC chemokine-mediated angiogenesis, J. Biol. Chem. 270 (1995) 27348–27357.
[5] E.C. Keeley, J.R. Moorman, L. Liu, L.W. Gimple, L.C. Lipson, M. Ragosta, A. M. Taylor, D.E. Lake, M.D. Burdick, B. Mehrad, R.M. Strieter, Plasma chemokine levels are associated with the presence and extent of angiographic coronary collaterals in chronic ischemic heart disease, PLoS ONE 6 (2011), e21174.
[6] M.M. Amoli, B. Larijani, W. Thomson, W.E.R. Ollier, M.A. Gonzalez-Gay, Two polymorphisms in the epithelial cell-derived neutrophil-activating peptide (ENA-78) gene, Dis. Markers 21 (2005) 75–77.
[7] I. Zineh, C.L. Aquilante, T.Y. Langaee, A.L. Beitelshees, C.B. Arant, T.R. Wessel, R. S. Schofield, CXCL5 gene polymorphisms are related to systemic concentrations and leukocyte production of epithelial neutrophil-activating peptide (ENA-78), Cytokine 33 (2006) 258–263.
[8] D.C. Levin, Pathways and functional significance of the coronary collateral circulation, Circulation 50 (1974) 831–837.
[9] P. Meier, S. Gloekler, R. Zbinden, S. Beckh, S.F. de Marchi, S. Zbinden, K. Wustmann, M. Billinger, R. Vogel, S. Cook, P. Wenaweser, M. Togni, S. Windecker, B. Meier, C. Seiler, Beneficial effect of recruitable collaterals : a 10-year follow-up study in patients with stable coronary artery disease undergoing quantitative collateral measurements, Circulation 116 (2007) 975–983.
[10] I. Zineh, A.L. Beitelshees, G.J. Welder, W. Hou, N. Chegini, J. Wu, S. Cresci, M. A. Province, J. Spertus, Epithelial neutrophil-activating peptide (ENA-78), acute coronary syndrome prognosis, and modulatory effect of statins, PLoS ONE 3 (2008), e3117.