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Impact of 719 Trp>Arg polymorfism of KIF 6 gene on coronary artery disease, contrast induced nephropathy and modulation of statin therapy effectiveness

Coronary artery disease (CAD) is a multifactorial and complex condition resulting from the interaction of genes and environmental factors. In the last decades statin treatment played a central role in the setting of cardiovascular prevention, in fact, in addition to their impact on cholesterol levels, statins have been shown to possess multiple non lipid-lowering pleiotropic effects such as anti-oxidant, anti-inflammatory and anti-thrombotic properties with enhancement of endothelial nitric oxide production and reducing of endothelin secretion (1-3). Therefore they are highly recommended both as chronic therapy in patients with CAD, but also with a loading dose in the early phase of acute coronary syndrome to stabilize the plaque and reduce the incidence of any complication expecially if a percutaneous coronary intervention (PCI) is performed. Genetics plays an important role in determining the inherent CAD vulnerability and in determining how a person responds to statin therapy.
Contrast-induced nephropathy (CIN) is a common complication of procedures that foresee the use of contrast media and is known as the third leading cause of hospital-acquired acute renal injury, accounting for 11% of all cases (4). In the last decades several therapies for the prevention of CIN, such as different hydration and alkalinization measures (5,6), N-acetylcysteine (NAC) (7), Fenoldopam, hemodyalisis and hemofiltration (8,9) have been explored in randomized clinical trial with conflicting results. Due to the important pleiotropics effect of statins, a large number of studies have assessed their role for the prevention of CIN. Recent studies (10,11) showed a protective effect of statin therapy in patients treated before coronary angiography/PCI, while other studies suggested that statin therapy is not useful for the prevention of CIN (12,13).
Kinesis-Like Protein 6 (KIF 6) is an omodimeric protein expressed in coronary arteries and other vascular tissue, that is involved in cellular microtubular transport (14).The impact of KIF 6 gene on cardiovascular risk modulation has been investigated since 2007 due to the presence of a single nucleotide polymorphism (non synonym replacement A>G) related with the replacement of Trp 719 with arginine (Arg). Several prospective trials and meta-analysis (15-17) assessed the association between this genetic variant (expressed in 60% of European population) and a significant increase of cardiovascular risk, anyway these results were not confirmed in a large study (Heart Protection study that involved more than 18000 patients (18). Several mechanism such as a modification in the particles binding capacity (19), a modulation in the endothelial cells progenitors growth (20) and an increased expression of KIF 6 in the population with the risk allele (21) has been proposed to explain this association. Particular attention in last years has been focused on the role of the Trp719ARg polymorphism in the modulation of response to statin treatment. Several clinical trials showed a significant association between anti-inflammatory, metabolics and vasoprotective effects of statin therapy and a reduction in cardiovascular events in the population with the risk allele (22,23). Moreover these protective effects with a significant reduction (about 13%) of LDL-cholesterol level and risk of cardiovascular events of statin therapy has been recently confirmed by two meta-analysis (24,25).

Study objectives

Our study have three main objectives to assess:

1. Assess the impact of Trp719Arg polymorphism on coronary artery disease in patients undergoing coronary angiography and/or PCI and on the modulation of the protective effects of statin treatment in this subgroup of patients.

2. Assess the impact of Trp719Arg polymorphism on the development of contrast induced nephropathy in patients undergoing coronary angiography and/or PCI and on the modulation of the protective effects of statin treatment in this subgroup of patients.

3. Assess the impact of Trp719Arg polymorphism on the modulation of a short term high dose statin therapy in naïve patients undergoing coronary angiography because of an acute coronary syndrome.

Methods

1. KIF 6, coronary artery disease (CAD) and contrast induced nephropathy (CIN)

Clinical, demographic and angiographic data of patients undergoing coronary angiography and/or PCI in chronic therapy with statins or "naïve"will be collected in our dedicated database protected by password. Patients with impaired renal function at baseline (creatinine clearance <60ml/min) will be treated with standard hydration (1ml/kg/h of saline solution 0.9% 12h before and after the procedure or with saline solution 0,5ml/kg/h, if ejection fraction ≤40% or with sodium bicarbonate received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure). The association between KIF 6 variant and the extent of coronary artery disease will be assessed after the coronary angiography by quantitative coronary angiography (QCA) analysis with "edge-detection system" (Siemens Acom Quantor QCA, Erlangen, Germany) by two experienced cardiologist who have not information about the patients' genetic profile. In all patients creatinine and creatinine clearance will be assessed at baseline, 24 and 48h after contrast exposure. Among these patients we will assess the incidence of CIN (defined as an absolute increase of 0.5mg/dL or a relative increase >25% in serum creatinine levels at 24 and 48h after the procedure) and the impact of statin treatment on contrast induced kidney injury both in patients with or without the risk allele. Then we will divide the population in two groups according to the presence of statin treatment at admission and we will assess the modulation of the polymorphism on the protective effect of statin both on coronary artery disease or on contrast induced nephropathy.

2. KIF 6 and modulation on the effect of a short term high dose statin treatment

A total population of 200 statin free patients with acute coronary syndrome treated within 24h with PCI will be treated with high dose statin treatment (Athorvastatin-80mg or Rosuvastatin40mg). All patients will undergo coronary angiography and subsequent PCI. All most important inflammatory biomarkers (PCR, fibrinogen and phospholipase A2) and lipid profile will be assessed at admission, before discharge and 3 months after discharge and will be evaluated their association with Trp719Arg polymorphism.

Genetic analysis

A blood sample for the determination of Trp719Arg polymorphism will be collected for all patients. DNA extraction, amplification of the region of interest with polymerase chain reaction and treatment with restriction enzyme Bst4CI will be performed for each sample. Primer 5'AGA CAT CGT ACT CCC AGC ATG GA 3' will be used for amplification. The PCR product (139bp long) will be divided into two fragments (105+34bp) in case of "wild type" variant; otherwise the polymorphic variant (719Arg) will not have a restriction site, showing a 139bp fragment at the subsequently electrophoretic run on agarose gel.

Coronary angiography and QCA

Coronary angiography will be performed, preferring a radial approach, using 6-French right and left heart catheters. Quantitative coronary angiography will be performed by experienced interventional cardiologists by automatic edge-detection systems (Siemens Acom Quantcor QCA, Erlangen, Germany). After the visual inspection of the coronary artery, the frame of optimal clarity was selected, showing lesion at maximal narrowing and arterial silhouette in sharpest focus. After the calibration of guiding catheter, analysed arterial segment with coronary lesion was defined by moving the cursor from the proximal to the distal part of coronary artery to ensure adequate determination of reference diameter. We have measured minimal luminal diameter, reference diameter, percent diameter stenosis, and length of the lesion.
Significant coronary artery disease was defined as a stenosis more than 70%, while borderline stenosis if between 30 and 70%.

Statistical analysis

Statistical analysis was performed with the SPSS 17.0 statistical package. Continuous data were expressed as mean $\pm$ SD (25-75 percentile) and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively.
Hypothesis 1. On the basis of a CAD prevalence of 70% an increased absolute risk of 7% (between 70% and 77%) will be considered clinically significant. Expecting a polymorphism prevalence of about 60% with a statistical power of 80% and alpha error of 0.05 we decided to analyze a total population of 1500 patients.
Hypothesis 2. On the basis of a CIN prevalence of 8% an increased absolute risk of 5% (between 10% and 15%) will be considered clinically significant. Expecting a polymorphism prevalence of about 60% with a statistical power of 80% and alpha error of 0.05 we decided to analyze a total population of 1500 patients.

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Pubblications

1. Verdoia M, **Barbieri L**, Schaffer A, Cassetti E, Di Giovine G, Bellomo G, Marino P, Sinigaglia F, De Luca G. Platelet distribution width and the risk of periprocedural myocardial infarction in patients undergoing percutaneous coronary intervention.
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Congress facts

1. Sulle Sponde del Ticino (emoclinic symposium) (May 2014)-**Partecipant**

2. EuroPCR 2014 **ORAL PRESENTATION** (May 2014): **Barbieri L**, Verdoia M, Schaffer A, Cassetti E, Di Giovine G, Marino P, De Luca G "Two catheters for one perforation"-Clinical case.

3. EuroPCR 2014-**ORAL PRESENTATION** (May 2014): Verdoia M, Schaffer A, Barbieri L, Cassetti E, Marino P, De Luca G "Benefits from new adenosine-diphosphate antagonists as compared to clopidogrel in patients with stable angina or acute coronary syndrome: a meta-analysis of randomised trials ".

4. Congresso GISE regionale (June 2014)-**Partecipant**

5. ESC congress 2014-**MODERATED POSTER SECTION** (August 2014) **Barbieri L**, Verdoia M, Schaffer A, Marino P, De Luca G. "Do statins prevent contrast induced nephropathy in patients undergoing coronary angiography or percutaneous intervention? A meta-analysis of 7 randomized trials"

6. ESC congress 2014-**MODERATED POSTER SECTION** (August 2014) **Barbieri L**, Verdoia M, Schaffer A, Cassetti E, Marino P, Suryapranata H, De Luca G." Uric acid levels and the risk of contrast induced nephropathy in patients with impaired renal function undergoing coronary angiography or percutaneous intervention"

7. V International ACS cross road - **POSTER SECTION** (October 2014) **Barbieri L**, Verdoia M, Schaffer A, Cassetti E, Marino P, Suryapranata H, De Luca G." Uric acid levels and the risk of contrast induced nephropathy in patients with impaired renal function undergoing coronary angiography or percutaneous intervention".

8. V International ACS cross road - **POSTER SECTION** (October 2014) **Barbieri L**, Verdoia M, Schaffer A, Marino P, Suryapranata H, De Luca G. "Impact of gender on uric acid levels and its relationship with the extent of coronary artery disease: a single-centre cohort study"

9. V International ACS cross road (October 2014)– **Winner** of Young Interventionalist Competition: Clinical case "2 catheters for 1 perforation"

10. 35° Congresso nazionale Società Italiana di cardiologia Invasiva (GISE 2014**)-BEST POSTER SECTION** (October 2014): **Barbieri L**, Verdoia M, Schaffer A, Cassetti E, Marino P, Suryapranata H, De Luca G." Uric acid levels and the risk of contrast induced nephropathy in patients with impaired renal function undergoing coronary angiography or percutaneous intervention".

11. 35° Congresso nazionale Società Italiana di cardiologia Invasiva (GISE 2014**)- POSTER SECTION** (October 2014): Verdoia M, **Barbieri L**, Schaffer A, Di Giovine G, Cassetti E, Marino

P, De Luca G “Impact of diabetes mellitus on periprocedural myocardial infarction in patients undergoing coronary stent implantation”

12. 35° Congresso nazionale Società Italiana di cardiologia Invasiva (GISE 2014**)- ORAL PRESENTATION** (October 2014) **Barbieri L**, Verdoia M, Schaffer A, Marino P, Suryapranata H, De Luca G. “Impact of gender on uric acid levels and its relationship with the extent of coronary artery disease: a single-centre cohort study”

13. 35° Congresso nazionale Società Italiana di cardiologia Invasiva (GISE 2014**)- ORAL PRESENTATION** (October 2014): **Barbieri L** “2 Catheters for 1 Perforation”

14. 35° Congresso nazionale Società Italiana di cardiologia Invasiva (GISE 2014**)- ORAL PRESENTATION** (October 2014) Verdoia M, **Barbieri L**, Schaffer A, Marino P, De Luca G. “Bivalirudin as compared to unfractionated heparin in patients undergoing percutaneous coronary revascularization: a meta-analysis of 16 randomized trials

Courses and Seminars

- July 14, 2014 **Gene Therapy application** (Prof Follenzi)

- **The Borghese Sessions** (Prof Steven R Ellis)
  September 8, 2014 Skin as an organ; Layers of skin, cell types, developmental origins
  September 9, 2014 Cell-Cell Interactions – anchoring junctions and occluding junctions, tight junctions
  September 10, 2014 Cell Matrix Interactions – basal lamina; Epithelial-mesenchymal transition
  September 11, 2014 Angiogenesis; Innervation
  September 15, 2014 Basal layer stem cells, symmetric versus asymmetric divisions, transient amplifying cells; Solar radiation, nucleotide excision repair
  September 16, 2014 Basal and squamous cell carcinomas; Melanoma – biology
  September 17, 2014 Melanoma – treatment; Contact dermatitis
  September 22, 2014 Other skin disorders, Other components of skin