SOCIETÀ ITALIANA PER LO STUDIO DELLA ATEROSCLEROSI (SISA)

XX Congresso della Sezione Toscana

Pisa, 7-8 Giugno 2019

Il **Congresso Regionale SISA della Sezione Toscana***, che si svolgerà il 7 e 8 giugno 2019 a Pisa, presso l'Area della Ricerca del CNR, sarà occasione per approfondire il ruolo delle dislipidemie nelle patologie d'organo e di sistema, valutando in particolare la relazione tra le dislipidemie ereditarie e le complicanze in ambito cardiovascolare e nefrologico. Saranno presi in esame gli aspetti fisiopatologici e clinici, discutendone le implicazioni per gli operatori sanitari ed i decisori pubblici.*

Il Congresso sarà occasione per un aggiornamento sulla disponibilità di nuovi percorsi terapeutici il cui impiego richiede non solo competenza farmacologica e diagnostica, ma anche consapevolezza della ricaduta sulla spesa sanitaria, indirizzando così verso scelte appropriate e socialmente sostenibili.

Una sezione del programma sarà dedicata al ruolo dell'aferesi delle lipoproteine come approccio terapeutico per la prevenzione e la cura di dislipidemie genetiche ancora orfane di un'efficace terapia farmacologica.

Fiduciosa che il Congresso sarà un appuntamento di interesse e di confronto scientifico,

Dott.ssa Tiziana Sampietro

COMUNICAZIONI ORALI

PCSK9: A ROLE IN THE PROGNOSTIC STRATIFICATION OF SEPTIC PATIENTS?

E. Camilleri, F. Innocenti[1], A. Sereni, A. Murri, E. Sticchi, B. Giusti, R. Marcucci, A.M. Gori, R. Pini[1]
Dep. of Experimental and Clinical Medicine, University of Florence-Atherothrombotic Diseases Center, Careggi Hospital Firenze; [1]High-Dependency Unit, Department of Clinical and Experimental Medicine, Azienda Ospedaliero-Universitaria Careggi, Firenze

Background. PCSK9 (proprotein convertase subtilisin/kexin type 9) plays a critical role in regulating circulating cholesterol levels, through the reduction of the membrane-associated low-density lipoprotein (LDL) receptor. In experimental models plasma PCSK9 levels were increased in sepsis. At normal levels, PCSK9 has no influence upon hepatocyte bacterial endotoxin clearance, but as levels rise, there is a progressive inhibition of clearance. Recently it has been shown that decreased function of PCSK9 increases survival of septic patients. Aim of this study was to assess prognostic value of an early assessment of PCSK9 levels in septic patients.
Methods. Between November 2011 and December 2016, 263 patients were enrolled in a prospective analysis aiming to find reliable biomarkers for an early sepsis diagnosis. Patients admitted to our High- Dependency Unit from the Emergency Department (ED) with a diagnosis of severe sepsis/septic shock were eligible. We evaluated vital signs and laboratory data at ED admission (T0), at 6 hours (T6) and after 24 hours (T24); Sequential Organ Failure Assessment (SOFA score) was calculated at every evaluation point. Primary end-points were 7-day and 28-day mortality.
Results. Mean age of the study population was 74±14 years, 58% male gender; mean SOFA score at admission was 5.3±2.7. The most frequent infection source was respiratory (45%). Day-28 mortality was 25%. PCSK9 normal values are lower than 250 ng/ml; in septic patients, at every evaluation point, PCSK9 level was significantly higher than normal range reported in previous studies (T0 661±405 ng/mL, T6 687±417 ng/mL, T24 718±430 ng/mL). There was no significant difference between patients with Gram+ or Gram- pathogen infection (T0: 641±493 ng/mL vs 701±406 ng/mL; T6: 652±433 ng/mL vs 769±389 ng/mL; T24: 690±397 ng/mL vs 811±501 ng/mL, all p=NS). No correlation between SOFA score values and PCSK9 levels at all evaluation were found. We divided the study populations in two subgroups according to the level of T0 SOFA score (≤ and >5): PCSK9 levels were comparable regardless the severity of sepsis-induced organ damage (T0: 696±381 vs 614±444; T6: 716±417 vs 631±421; T24: 709±413 vs 716±470 ng/mL, all p=NS). Finally we compared PCSK9 levels in patients with T0 lactate level ≤ and >2: even in this analysis we did not find any significant difference (T0: 672±485 vs 642±365; T6: 689±449 vs 664±373; T24: 711±392 vs 698±452 ng/mL, all p=NS). Only at T0 non-survivors by day-28 showed a significantly lower level than survivors (549±437 ng/mL vs 696±390 ng/mL, p=0.016); all the other evaluations were comparable regardless of the outcome, both considering day-7 and day-28 mortality rate. An Analysis for Repeated Measures between T0 and T24 levels did not show any significantly different trend between day-7 (T0: 668±389 vs 623±492; T24: 702±388 vs 843±669 ng/mL) and day-28 (T0: 696±390 vs 549±437; T24: 718±392 vs 719±553 ng/mL) survivors and non-survivors (all p=NS).
Conclusions. PCSK9 levels were increased in septic patients, but they did not show any significant association with indexes of hypoperfusion and with the severity of organ damage; patients with an above-the-median level showed a significantly reduced mortality rate.

ASSOCIATION BETWEEN GLOBAL CARDIAC CALCIFICATION (GCCS) AND OSTEOPOROSIS

P. Carrai, T. Picchioni, M. Alessandri, A. Incisore, C. Caffarelli, S. Gonnelli
Department of Medicine and Neurosciences, University of Siena, Policlinico Le Scotte, Siena

Background. Epidemiologic and clinical data have suggested the existence of a biologic linkage between bone and vascular system. Osteoporosis and atherosclerosis are two prevalent major healthcare concerns that frequently coexist. Several studies reported correlation between lower values of Bone Mineral Density (BMD) and cardiovascular events. Furthermore literature suggests that cardiac calcification (measured with Global Cardiac Calcium Score, GCCS) is associated with cardiovascular events and mortality.
Aim. This study aimed to evaluate if cardiac calcium deposit was correlated with BMD.
Methods. In 36 subjectsassessed for bone fracture risk (mean age 72±5,7 years) we measured Bone Mineral Density (BMD) at lumbar spine (BMD-LS) at femur (Neck: BMD-FN; Total: BMD-FT) and we assessed with echocardiography a global cardiac calcium score (GCCS).
GCCS is a semi-quantitative score, that was applied assigning points for calcification in the aortic root and valve, mitral annulus and valve and sub-mitral apparatus, and points for restricted leaflets mobility (1).
Results. The results show that there is a significant inverse correlation between BMD-FN and BMD-FT with GCCS (r=-0,285, p<0,05 and r=-0,376, p<0,05 respectively). No significant correlation was found between BMD-LS and GCCS.
Dividing patients into two groups based on presence of bone fragility fractures (13 patients with fragility fractures and 23patientswithout) we observed that the value of GCCS was higher in patients with bone fragility fractures (2,54±1,3 Vs 2,30±1,5) but not statistically significant.
Moreover, dividing patients on the basis of presence of sarcopenia (9 patients with sarcopenia and 27 patients without sarcopenia) we found that the values of GCCS was higher in patients with sarcopenia (3,0±1,4 vs 2,1±1,4) even though the difference didn't reach statistical significance.
Conclusion. Our data suggest link between osteoporosis and cardiac calcification. The burden of cardiac calcium seems to be higher in patients with fragility fractures. These findings confirm that osteoporotic patients have an higher risk of cardiac and vascular calcification and this confirm the greater risk of cardiovascular events in osteoporotic patients.

Reference
1. Lu M.L., et al. J Am Soc Echocardiogr. 2016.

GENETIC SUSCEPTIBILITY FOR ANEURYSMAL DISEASE: ROLE OF LRP1 RS1466535, ZNF335 RS3827066 AND LDLR RS6511720 GENE POLYMORPHISMS

S. Galora, E. Sticchi, R. De Cario, G. Pepe, A. Kura, A. Volta, B. Giusti
z-nm-bnm-bnm

Background. A genome wide association study (GWAS) evaluated the possible correlation of 535,296 polymorphisms with the abdominal aortic aneurism (AAA); the rs1466535 polymorphism in the low density lipoprotein receptor-related protein gene (LRP1) was associated as a risk factor, both in the internal replication study, and in a replication study conducted by our group. Further analysis showed other genetic variants associated with AAA including the rs3827066 polymorphism of the ZNF335 gene (zinc finger protein 335) and rs6511720 of the LDLR gene (low density lipoprotein receptor). Previous studies carried out on murine models have shown that the LRP1 knock-out in cells of the smooth musculature leads to vascular pathologies very similar to Marfan syndrome (MFS), triggering the development of aneurysmal disease. The aim of the study was to evaluate whether these three variants rs1466535, rs3827066 and rs6511720 are specific AAA susceptibility factors or if they are related to thoracic aneurysmal disease in MFS patients.
Methods. We used Gene Catcher (Invitrogen) on Freedom Evo platform (Tecan) for genomic DNA extraction from peripheral blood and subsequent genotyping analysis by real time PCR technology with TaqMan probes specific for the three polymorphisms (Life Technologies). We enrolled 225 patients with MFS referred to the Regional Reference Center for Marfan Syndrome and Related Diseases and 536 control subjects. Statistical analyzes were performed using the SPSS v.19 software (SPSS Inc, Chicago, IL, USA).
Results. Polymorphisms are in Hardy-Weinberg equilibrium both in patients and controls. Traditional cardiovascular risk factors analysis highlights an higher prevalence of dyslipidemia, diabetes, smoking and high blood pressure in controls than in patients with MFS (34.9% vs 13.2%, p<0.0001; 5.6% vs 0%, p=0.001; 44.6% vs 17.9%, p<0.0001; 24.4% vs 17.7%, p=0.052). The genotype distribution analysis highlights a prevalence of homozygous TT for the polymorphism rs1466535 LRP1 in controls than in patients (8.1% vs 4%, p=0.042), suggesting a possible protective role. About rs3827066 ZNF335, the T allele carriers (CT + TT) had an higher prevalence in MFS patients compared to controls (28.9% vs 20.6%, p=0.025), suggesting a possible role as a risk factor. The genotype distribution of the LDLR rs6511720 polymorphism did not differ statistically between cases and controls (p=0.407). At multivariate logistic regression (adjusted for traditional cardiovascular risk factors), the rs3827066 and the did not remain statistically significant [rs3827066: OR=0.70 (95% CI 0.39 to 1.22), p=0.215 and rs1466535: OR =0.72 (95% CI 0.26 to 2.01), p=0.536]. Furthermore these three polymorphisms were not significant determinants of the diameters of the thoracic aortic root in MFS patients.
Conclusions. Rs14665365 polymorphisms of the LRP1 gene, the rs3827066 polymorphism of the ZNF335 gene and rs6511720 of the LDLR gene previously associated with AAA do not represent significant and independent determinants of thoracic aneurysmal disease in MFS patients.

DIRECT ORAL ANTICOAGULANTS ADMINISTRATION IS ASSOCIATED WITH VARIATIONS IN INFLAMMATORY AND METALLOPROTEINASE LEVELS

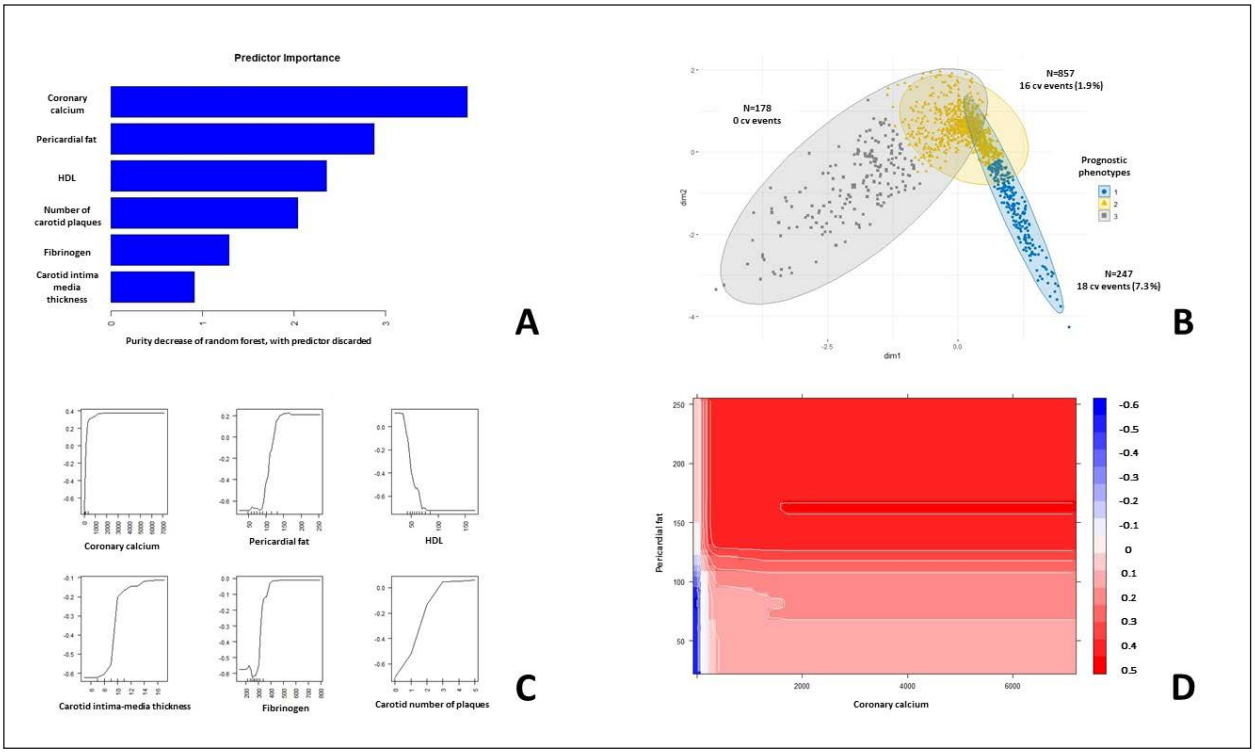
R. Marcucci[1], E. Camilleri[2], D. Poli[3], L. Mannini[3], B. Giusti[2], A.P. Cellai[3], A. Alessandrello Liotta[3], A. Sereni[2], R. De Cario[2], A.Kura[2], A. Volta[2], E. Sticchi[2], A.M. Gori[2]
[1]Center for Atherothrombotic Disease, Department of Experimental and Clinical Medicine, University of Florence; [2]University of Florence; [3]AOU Careggi, Florence

Background. Although using drugs directly targeting factor Xa or thrombin (DOACs) for effective anticoagulation is now well established, evidence has emerged suggesting that factor Xa and thrombin are involved in other pathophysiological cellular processes, including inflammation and atherosclerotic plaque stabilisation. DOACs clinical efficacy and safety has been widely demonstrated in various clinical studies, but very few studies have instead evaluated their possible pleiotropic effects, especially anti-inflammatory and anti-proliferative ones. These effects, demonstrated for other cardiovascular drugs (such as statins and parenteral anticoagulants), underline the role of inflammation as a common pathophysiological substrate in diseases for which direct oral anticoagulants are used.
Purpose. To evaluate the effect of DOACs on inflammatory and metalloproteinase molecules.
Methods. Fifty-four patients with non-valvular atrial fibrillation and venous thromboembolism were included in the study. Sixteen patients were prescribed dabigatran, twenty rivaroxaban and eighteen apixaban. Inflammatory markers (IL-6, IL-8, IL-1RA, IL-10, TNF-alpha, VEGF-alpha, ICAM-1 and VCAM-1) and metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-10) were measured 1 month and 6 months after the initiation of DOACs (T1 and T2, respectively). Biomarkers were assessed by using the Bio-plex suspension array system. In order to evaluate the grade of inflammation, patients treated with DOACs were divided into three categories on the basis of the grade of inflammation (low, moderate and elevated grade of inflammation). At this purpose the levels of cytokines IL-1Ra, IL-6, IL-8, IL-10 and TNF-α were divided into tertiles and were used to calculate a global grading of inflammation.
Results. Mean age was 73±15 years, 40.7% (22) were women and the mean CHADS2 score was 2.65±1.2. Among the inflammatory markers, IL-10, TNF-alpha, VCAM-1 and VEGF levels were significantly (p<0.05) higher at T2 than at T1. As concerns pro-/anti-inflammatory ratios, 6 months after the initiation of DOACs (T2) IL-6/IL-10, IL-8/IL-10 ratios were significantly lower (p<0.01) than those found at T1. At T2 we found significantly (p<0.05) higher levels of MMP-7 and MMP-10. As concerns the others MMPs, no significant differences were observed between T2 and T1 levels. No significant correlations inflammatory, metalloproteinases molecules and DOACs plasma concentrations were also found. By using the global inflammatory score, we identify at T1 30 patients (55.5%) in which the global inflammation level was low, 10 patients (18.5%) with moderate grade of inflammation and 14 (30%) with high level of inflammation. Treatment for six months with DOACs is associated with a significant reduction of patients classified in the group with high level of inflammation.
Conclusions. Treatment with new anticoagulants is associated with variations in systemic inflammation markers, and in metalloproteinases, suggesting that, beyond the well known anticoagulant activities, DOACs may modulate also the inflammatory and remodeling processes and that thrombin and factor Xa appear to be involved in the pathophysiological mechanisms of non- valvular atrial fibrillation.

THE INTERPLAY OF PERICARDIAL FAT AND CORONARY CALCIUM ON CARDIOVASCULAR RISK, HIGHLIGHTED BY CT IMAGING WITH A MACHINE LEARNING APPROACH

A. Ripoli[1], D. Della Latta[1], N. Martini[1], D. Chiappino[1]
[1]Deep Health Unit, Fondazione Toscana "Gabriele Monasterio"

Purpose. Atherosclerosis is a complex disease, whose progression can be conveniently assessed by a plethora of biomarkers. Aim of this work is the assessment of their global impact on cardiovascular risk, taking into account their interwoven relationships, and capturing the underlying mechanisms of disease.
Material and Methods. We investigated a sample constituted by 1282 subjects from the asymptomatic general population of the MHELP screening study (age: 60.7+/-8.3 years; males =44.8%; cardiovascular events =2.6%). Every subject underwent an MSCT examination, an echo study and bio-humoral laboratory tests.
A quantification of heart and pericardial fat volumes was obtained from CT imaging. For this purpose, a fully convolutional U-Net architecture was trained on the dataset, extracting the mask related to the heart area. On the obtained mask, a threshold was applied (-190: -40 HU) to extract and quantify pericardial fat. Risk analysis was performed with a machine learning approach on a set of 60 potential predictors. Considering the small number of events, we built a weighted random forest (WRS) on the whole set of predictors and on a more compact set of six predictors, selected by univariate logistic regression.
Results. The best predictive performance was furnished by WRS grown on the subset of six predictors (coronary calcium, pericardial fat, HDL, fibrinogen, carotid intima-media thickness and number of carotid plaques), obtaining an out-of-bag sensitivity of 78%, a specificity of 76% and a global accuracy of 76%. The ranking of importance of the predictors was calculated, while their percentage impact on risk was computed by partial dependence plots (PDPs). PDPs were computed for the single predictors and for the interplay of calcium and fat. The fuzzy clustering of WRS proximity measure was used to identify three partially overlying prognostic phenotypes, with an average increase in the risk from 0% to 1.9% and 7.3%.
Conclusions. The major impact of coronary calcium on cardiovascular risk appears to be modulated by pericardial fat.
Clinical relevance. Our approach furnished a compact and accurate algorithm to predict cardiovascular events in the general population.



LIPOPROTEIN (A) LEVELS PREDICTS MORTALITY AND HAEMORRHAGIC TRANSFORMATION IN ISCHEMIC STROKE PATIENTS TREATED WITH THROMBOLYSIS: RESULTS FROM THE MAGIC STUDY

E. Sticchi, A. Sereni, B. Piccardi, A.M. Gori, M. Nesi, A. Murri, V. Palumbo, B. Giusti, F. Arba, P. Nencini, G. Pracucci, A. Kura, D. Inzitari
Vnb.<bv<

Background and purpose. Lipoprot ein(a) [Lp(a)] is endowed with both proatherosclerotic and prothrombotic properties: promotes smooth muscle cell proliferation, endothelial cell adhesion molecule expression, foam cell generation, and endothelial dysfunction. Elevated Lp(a) levels are significantly associated with higher incidence of ischemic stroke. Aim: We aimed to investigate whether Lp(a) levels taken before recombinant plasminogen activator (rtPA) administration predict adverse clinical outcomes in tPA t reated ischemic stroke patients of the MAGIC Study.
Methods. Blood sampl es were taken before and 24-h after rt-PA from 327 patients (mean age 68 years, median NIHSS 11). Pre-rt-PA values and delta median values [(24-h biomarker - pre-rt-PA biomarker)/(pre-rt-PA biomarker)] of Lp(a) were analyzed in relation to:
1) 3-month death;
2) symptomatic hemorrhagic transformation.
Results. Pre-tPA levels of Lp(a) were significantly different in patients who died with respect to survivors [141(92-258) vs 90(44-175) mg/L, p=0.010]. Similarly, Lp(a) values were significantly higher in patients with than without haemorrhagic transformation [138(72-263) vs 91(44-175) mg/L, p=0.049]. Adjusting for age, sex, glycemia, baseline NIHSS, history of atrial fibrillation, or congestive heart failure, history of inflammatory diseases or infections occurred within the last 7 days before stroke onset, pre-tPA Lp(a), in addition to pre-tPA A2M and deltaMMP-9 remained significantly and independently associated with 3 month-death [OR (95% CI): pre-tPA Lp(a): 2.63(1.26-5.48), p<0.010; A2M: 1.63(1.18-2.25), p=0.003; deltaMMP9:1.72(1.20-2.47), p=0.003]. At logistic regression analysis, after adjustment for major clinical determinants of outcomes, in addition to deltaMMP-9, pre-tPA Lp(a) levels remain a significant and independent determinant of hemorrhagic transformation [OR (95% CI) Lp(a): 1.78 (1.04-3.03), P=0.035; deltaMMP9: 1.48(1.08-2.102), p=0.014].
Conclusion. Our findings suggest that in addition to A2M and deltaMMP-9 also high levels of Lp(a) are significant and independent markers of mortality and hemorrhagic transformation. Lp(a) may be used to improve prediction of unfavourable outcomes in the clinical setting of ischaemic stroke patients treated with thrombolytic therapy.

LARGE GENES PANEL TARGETED HIGH-THROUGHPUT SEQUENCING IN DETERMINING MONOGENIC AND POLYGENIC NATURE OF FAMILIAL HYPERCHOLESTEROLEMIA IN RESEARCH AND DIAGNOSIS

E. Sticchi[1], A. Volta[1], A. Magi[1], R. De Cario[1], A. Kura[1], A. Murri[1], E. Grifoni[2], A.M. Gori[2], R. Marcucci[2], B. Giusti[2]
[1]Dept. Ex p. and Clinical Medicine, Univ. of Firenze;
[2]Dept, Exp. and Clinical Medicine, Univ. of Florence, Atherothrombotic Diseases Centre, Careggi Hospital, Florence

Background. Familial hypercholesterolaemia (FH) is an autosomal dominant genetic disorder characterized by elevated plasma levels of low density lipoprotein-cholesterol (LDL-C), which can lead to premature cardiovascular disease. FH is caused mainly by mutations in *LDLR*, *APOB* and *PCSK9* genes. However, many subjects with primary hypercholesterolemia did not demonstrate functional mutations in any of these genes. Aim of this work is to sequence, through a targeted high-throughput sequencing (HTS) strategy, 55 genes, involved in FH or other alterations of lipid profile, in patients with suspected FH. The design also allow to genotyped all variants included in the Talmud et al. (2013) low-density lipoprotein cholesterol gene score and in statins pharmacogenetics. We would evaluate whether this approach allows an improvement in diagnosis of genetic dyslipidemias and in research of novel associated genes, polygenic profiles impact and pharmacogenetic approaches.
Methods. Subjects with suspected FH are identified using the most common diagnostic algorithm, the Dutch Lipid Clinic Network Score. We analyzed 30 clinically probable or definite FH patients. DNA libraries were prepared using Agilent Haloplex HS Enrichment System and the sequencing was performed using Illumina MiSeq Reagent Kit v3. Sequencing results were analyzed using both Agilent SureCall software and a pipeline developed by the bioinformatics group of our Department. The possible pathogenicity of variants was evaluated using six different *in silico* tools. Results: In 16 out of 30 patients, we found major mutations that can explain their lipid profile. The remaining patients showed a burden of polymorphisms at high and low/very low allele frequency in different genes that in part represent, based on some studies, known risk factors for or atherosclerotic disease that could confirm a polygenic predisposition to the disease. We started the segregation analysis in families of two patients. In one family the role of mutations in ABCG5 gene in lipid profile alteration was demonstrated. Furthermore, our data suggested that the simultaneous evaluation of genes involved in lipid-lowering drugs pharmacogenetics could improve a personalized management of patients with an earlier achievement of the therapeutic target also avoiding adverse effects.
Conclusions. Our results showed the significant advantages of using an HTS approach for the diagnosis and study of FH. Indeed, with our specific 55 genes panel we can not only screen the 3 major genes involved in FH, but we can also define the role of other major genes (e.g, ABCG5) and have a larger view about the patient's polygenic predisposition to the lipid disorders. The expansion of the case study will allow to confirm the hypothesis that familial hypercholesterolaemia can also be caused by an accumulation of common small-effect LDL-C-raising alleles. Finally, the screening of the genes involved in lipid-lowering drugs pharmacogenetics could allow to apply an early personalized therapy.