SOCIETA' ITALIANA
PER LO STUDIO
DELL'ATEROSCLEROSI

33° CONGRESSO NAZIONALE

S.I.S.A.

ROMA, 24/26 NOVEMBRE 2019

RIASSUNTO DELLE COMUNICAZIONI PRESENTATE AL 33° CONGRESSO NAZIONALE S.I.S.A.

SEPARATION AND CHARACTERIZATION OF DIFFERENT POPULATIONS OF EXTRACELLULAR VESICLES AND MEMBRANE-LESS NANOPARTICLS SECRETED BY A HUMAN LYMPH-NODE METASTATIC CELL LINE

F.M. Accattatis[1], A. Granata[1], S. Mazza[1], E. Vergani[2], M. Romano[3], A. Ridolfi[4], P. Bergese[5], A. Corsini[6], L. Arnaboldi[7]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari; [2]IRCCS Istituto Nazionale dei Tumori Milano; [3]Dipartimento di Medicina Molecolare e Traslazionale, Università degli Studi di Brescia e Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), Firenze; [4]Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), Firenze and CNR, Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), Bologna and Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze; [5]Dipartimento di Medicina Molecolare e Traslazionale, Università degli Studi di Brescia and Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), Firenze; [6]Dipartimento di Scienze Farmacologiche e Biomolecolari DiSFeB, Università degli Studi di Milano, IRCCS Multimedica, Milano; [7]Dipartimento di Scienze Farmacologiche e Biomolecolari DiSFeB, Università degli Studi di Milano
E-mail: lorenzo.arnaboldi@unimi.it

Introduction. Extracellular vesicles (EVs) are structures physiologically secreted by cells. Lack of separation and characterization methods impairs the comprehension of their biology. EVs are classified in small - (50-80 nm), large - (80-120 nm) exosomes and microvesicles (120-1000 nm). Nevertheless, a new population of non-membranous nanoparticles (exomeres; <50 nm) emerged.
Aims. To separate, quantify and characterize (dimensions, lipid/protein content, distribution) different EVs subpopulations. To pharmacologically modulate lipid-dependent pathways involved in EVs synthesis/secretion.
Materials and Methods. Lymph-node melanoma metastatic cell line (LM-16) are cultivated in 150 mm dishes and isolated by differential ultracentrifugation according to Livshits. Obtained EVs are analyzed by Transmission Electron Microscopy, Colorimetric Nanoplasmonic assay (CONAN), Atomic Force Microscopy (AFM), while lipids are detected by colorimetric assays and GLC.
Results. We show a relative and continuous increase in saturated fatty acids, ranging from microvesicles (35.89%) to exomeres (65.27%), counterbalanced by a decrease in unsaturated ones from 51.07% (microvesicles) to 29.38% (exomeres) and by an increase in plasmalogens from 5.33% to 13.02%, respectively. The mass of Phospholipid (PL) and of Free Cholesterol (FC) increases vs parental cells up to 4 and 14 folds respectively, together with the increased FC/PL, with specific changes.To assess purity, dimensional distribution and the real number of particles, preliminary data obtained by CONAN and AFM suggest a correspondence between calculated and experimental EVs radius, together with a relative sample purity, but adjustment will be necessary. EV populations will be characterized also by lipidomics and proteomics.
Future aims. The final goal will be to administer different EVs populations harvested from naïve - or pharmacologically - treated with drug affecting lipids metabolism (which has pivotal roles in their biogenesis and structural functions) parental cells to prove their effects in functional tests (e.g. proliferation, apoptosis, migration, invasion), to shed light on their pathophysiological roles, for a possible new pharmacological approach or the discovery of novel tumor biomarkers.

THE EFFECT OF WEIGHT LOSS ON CIRCULATING PCSK9 LEVELS IN OBESE PATIENTS

A. Altomari[1], M.G. Zenti[1], S. Galvan[1], C. Maneschi[1], M. Aventaggiato[1], D. Sandri[1], E. Paiola[1], M. Battistoni[1], M.G. Lupo[2], G. Targher[1], N. Ferri
[1]UOC Endocrinologia, Diabetologia e Metabolismo, AOUI Verona e Università di Verona; [2]Dipartimento di Scienze del Farmaco, Università di Padova
E-mail: anna.altomari@hotmail.it

Background. The main role of PCSK9 is the regulation of low-density lipoprotein-cholesterol, however, several evidence indicate that PCSK9 may play a role on visceral adipose tissue deposition and hypertriglyceridemia, disorders observed in obese and insulin resistant patients AIM: to investigate the effects of weight-loss on PCSK9 plasma concentration in obese patients.
Methods. Preliminary data of a non-randomized-observational-study on 10 patients (3M/7F), BMI 43.7±7.9 kg/m^2; age 42.7±11 years, who underwent bariatric surgery (4 Sleeve gastrectomy and 6 Gastric Bypass). We performed an OGTT at baseline (T0) and 6 months after surgery (T6), to assess glucose, insulin and PCSK9 plasma levels at 0-60-120-180 minutes. PCSK9 levels were compared with a group of 12 healthy subjects (4M/8F), BMI 20.7±2,1 kg/m^2; age 34±11 years, with normal glucose-tolerance.
Results. Body-Weight and fat-mass significantly decreased after surgery (Δweight: -33.8±14.9 kg and Δfat-mass: -27.3±8.1 kg; p<0.01 for both). At T6, we observed a significant decrease in glucose (T0: 6.4±1.8 mmol/L; T6: 4.9±0.4 mmol/L p<0.01), insulin (T0: 16.4±9.9 mU/L; T6: 6.1±1.7 mU/L; p<0.01) and PCSK9 plasma-levels (T0: 299±55 ng/mL; T6: 256±22 ng/mL; p<0.05).At baseline, PCSK9 showed a progressive reduction after oral glucose-loading and concomitant hyperinsulinemia (insulin-peak at 60 min of 75.1±40.0 mU/L, PCSK9 nadir at 120 minute, 256±62 ng/mL), which was not observed at T6 as in the control group.
Discussion. In our study weight-loss determines a significant reduction of PCSK9 levels. In addition, we observed a progressive reduction of PCSK9 plasma-levels in response to glucose loading. PCSK9 clearance occurs very rapidly (within 90-120 minutes) and the mass of adipose tissues appears to play a major role. Indeed, PCSK9 remains constant during the OGTT test in healthy subjects with normal glucose tolerance and BMI of 20.7, and, more importantly, in obese patients that change BMI from 43.7 to 31.9 kg/m^2.
Conclusions. We observed a previously unknown phenomena characterized by a very rapid clearance of PCSK9 from circulation in response to glucose overload in insulin resistant obese patients. This effect suggests a role of adipose tissue on PCSK9 uptake and clearance, potentially through the very low-density lipoprotein receptor.

TELOMERES ALTERATIONS WITH LIFELONG EXPOSURE TO GENETICALLY DETERMINED HYPERCHOLESTEROLEMIA

A. Baragetti[1], F. Pellegatta[2,3], L. Grigore[2,3], L. Da Dalt[1], F. Bonacina[1], A. Moregola[1], V. Zampoleri[1,2], A. Pirillo[2], G.D. Norata[1,2], A.L. Catapano[1,3]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Milano; [3]IRCCS Multimedica Hospital, Sesto San Giovanni, Milano;
E-mail: andrea.baragetti@unimi.it

Aim. Telomere Length Shortening (TLS) reflects cellular senescence and associates with cardiovascular disease (CVD). Moreover, effects on TLS by CVD risk factors have been demonstrated. Among them, however, despite elevated cholesterol levels were correlated with shorter Leukocytes Telomere Length (LTL), it is still underexplored if this relation mirrors exposure over time to elevated LDL-C, causal for enhanced CVD risk.
Methods. To study if TLS associates with lifelong exposure to hypercholesterolemia, we measured LTL of genomic DNA from 206 hypercholesterolemic patients referring to the Lipid clinic of Milano (within the LIPIGEN Registry). Exome sequence screening for Familial Hypercholesterolemia (FH)-related mutations on LDL gene confirmed a total of 135 heterozygous FH (HeFH) probands. LTL was available in 320 subjects from a large population-based cohort (the PLIC Study), without positive genetic diagnoses and with 136.37±35.58 mg/dL as mean LDL-C).
Results. LTL of HeFH was shorter vs that of hypercholesterolemic patients without genetic positivity for FH (non-FH HC, n=71) and controls (1.33+/-0.03 vs 1.44+/-0.07 vs 1.51+/-0.03 p<0.001). Pairing for LDL-C, LTL was still shorter in HeFH vs non-FH HC (1.33+/-0.05 vs 1.55+/-0.08, p=0.019), independently from previous CVD and statins. LTL was shorter only in HeFH below 35 years-old without statins, but no longer between HeFH and nonFH-HC over fifties. Blood LTL was reduced in one-years-old LDLR KO rodents vs wild-types peers (0.83+/-0.14 vs 1.11+/-0.27, p<0.001), confirming TLS as a conserved trait of genetically determined hypercholesterolemia in mammals. Cholesterol levels correlated with expansion of multipotent proliferative stem cells niche in bone marrow where TL was enhanced in LDLRKO mice vs wild-type (3.42+/-1.19 vs 0.85+/-0.14, p=0.002).
Conclusions. By using genetic models and translations in mice, we found significant TLS in circulating cells with lifelong exposure to elevated LDL-C. Taken together these findings entice further investigations to study relations between hypercholesterolemia and commitment of hematopoiesis over evolution.

LIVER FIBROSIS AND METABOLIC SYNDROME PREDICT CARDIOVASCULAR EVENTS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

F. Baratta[1], D. Pastori[1], F. Angelico[2], A. Balla[3], A.M. Paganini[3], N. Cocomello[4], D. Ferro[4], F. Violi[4], A.J. Sanyal[5], M. Del Ben[4]
[1]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome; [3]Department of General Surgery and Surgical Specialties "Paride Stefanini", Sapienza University of Rome; [4]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [5]Division of Gastroenterology, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA
E-mail: francesco.baratta@uniroma1.it

Background and Aims. In addition to liver-related complications, people with NAFLD have also an increased risk of developing cardiovascular diseases (CVD), such as myocardial infarction and stroke, which represent the major causes of death in this setting. Liver fibrosis has been shown to be most important factor affecting the prognosis of patients with NAFLD3, but the relationship between liver fibrosis and cardiovascular events (CVEs) has been poorly investigated.
Methods. Secondary pre-specified analysis of the prospective observational PLINIO study (Progression of LIver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver dIsease: an Observational Cohort study), study including 898 consecutive outpatients screened for liver steatosis by ultrasound. Liver fibrosis was assessed by FIB-4 >2.67 and by NAFLD Fibrosis Score (NFS) >0.676. CVEs were prospectively collected including fatal/nonfatal ischemic stroke and myocardial infarction, cardiac/peripheral revascularization, new-onset arterial fibrillation and cardiovascular death.
Results. Mean age was 56.4±12.7 years and 37.5% of patients were women. Most patients were overweight and obese, type 2 diabetes mellitus in 25.7% and metabolic syndrome in 48.6%. NAFLD was diagnosed in 660 (76.4%) patients. In a median follow-up time of 41.4 months (3044,4 patient-years), 58 CVEs (1,9%/year) were registered. The rate of CVEs was higher in patients with (n=643, 2,1%/year) compared to those without NAFLD (n=255, 1,0%/year, p=0.066). At multivariable Cox proportional regression analysis, NAFLD predicted CVEs (Hazard Ratio [HR]:2.41, p=0.036), after adjustment for metabolic syndrome. In NAFLD patients, male sex (HR:2.10, p=0.039), previous CVEs (HR:3.52, p=0.010), metabolic syndrome (MetS) (HR:2.89, p=0.006) and FIB-4>2.67 (HR:4.02, p=0.023) were independent predictors of incident CVEs. Similar results were obtained using NFS >0.676 (HR:2.35, p=0.038).
Conclusions. We confirm that patients with NAFLD are characterized by a high risk for CVEs. In NAFLD patients, liver fibrosis indexes, together with the presence of MetS, were independent predictors of incident CVEs.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 PREDICTS THE EXTENSION OF ATHEROSCLEROTIC DISEASE IN THE CORONARY ARTERIES OF PATIENTS UNDERGOING CORONARY ANGIOGRAPHY

V. Bianconi[1], M.R. Mannarino[1], R. Curcio[1], J. Fusaro[1], A. Sahebkar[2], M. Pirro[1]
[1]Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Medicine, University of Perugia; [2]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad Universityof Medical Sciences, Mashhad, Iran; School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
E-mail: v.bianconi.vb@gmail.com

Introduction. Among the multitude of traditional and emerging cardiovascular risk factors, hypercholesterolemia, particularly elevated plasma low-density lipoprotein cholesterol (LDL-C), has been established as an unequivocal causative factor for coronary heart disease (CHD). Albeit different functions and roles of proprotein convertase subtilisin/kexin type 9 (PCSK9) have been proposed, its role in regulating plasma LDL-C concentrations by promoting LDL receptor (LDLR) degradation is remarkably consistent. A prospective association between plasma PCSK9 concentrations and CHD risk has been found in several clinical studies. Importantly, some studies have remarked the dependence of this association from plasma lipids, whereas other studies a significant independence. Such a controversial scenario emerging from prospective studies performed in different clinical settings underlies the debate on whether PCSK9 may exert a proatherogenic effect that is independent of lipid pathways and, more specifically, of plasma LDL-C. Based on the avaiable studies, the association between PCSK9 and atherosclerotic burden at the coronary district remains still unclear and requires further clarification. The aim of this study was to investigate the association between plasma PCSK9 concentrations, current acute coronary syndrome (ACS), coronary artery disease (CAD) presence, severity and extension and the burden of coronary calcifications in patients undergoing coronary angiography for suspected ACS.
Materials and Methods. One hundred and one patients, with or without current ACS, were recruited for this cross-sectional study. CAD presence was defined based on either the presence or absence of at least one significant (≥50%) CAD lesion (SCAD). CAD severity was classified according to the absence of coronary lesions, the presence of non-significant (<50%) CAD (MCAD) or SCAD in at least one major coronary artery. Patients with one, two or three significantly diseased major coronary arteries were defined as 1-SCAD, 2-SCAD and 3-SCAD, respectively. The cumulative length of SCAD lesions and the amount of calcifications in coronary arteries were also estimated.
Results. Plasma PCSK9 concentrations were higher in patients with SCAD as compared to those without (p=0.012). A significant increase in plasma PCSK9 concentrations was observed with greater CAD severity (p=0.042). Higher plasma PCSK9 concentrations were found in 3-SCAD patients as compared to either 2-SCAD or 1-SCAD (p<0.001). PCSK9 increased with the cumulative length of SCAD lesions and the burden of calcifications (p<0.05 for both comparisons). Multivariable adjustment abolished the association between PCSK9 and either CAD presence or severity, but not the association between PCSK9 and the number of significantly diseased vessels, SCAD lesion length and the burden of coronary calcifications. ACS was associated with a borderline significant increase of plasma PCSK9 concentrations among patients not taking statins (p=0.05).
Conclusions. In this study circulating PCSK9 concentrations discriminated patients with greater coronary atherosclerotic lesion extension and calcification, and were increased in patients with current ACS.

DISCOVERY THE HYPOCHOLESTEROLEMIC EFFECT OF MENAQUINONE-7

N. Biancorosso[1], M.G. Lupo[1], E. Brilli[2], G. Tarantino[2], M.P. Adorni[3], G. Vivian[1], M. Salvalaio[1], S. Dall'Acqua[1], H. Chen[4], A. Bressan[5], E. Faggin[5], M. Rattazzi[5], N. Ferri[1]
[1]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova; [2]PharmaNutra S.p.A., Pisa; [3]Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma; [4]Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China, [5]Dipartimento di Medicina, Università degli Studi di Padova
E-mail: noemibiancorosso@gmail.com

Appropriate nutraceutical combinations may represent a valid approach to prevent vascular calcification associated to chronic kidney disease (CKD). In the present study, we tested the effect of diet supplementation of MK-7 (3.5 µg/g diet) in combination to magnesium carbonate and sucrosomial® iron on vascular calcification in uremic rats. Rats were randomly divided into three groups, control (normal diet), uremic (diet containing 0.5% adenine) and supplemented uremic diet (0.5% adenine, MK-7, magnesium carbonate and sucrosomial® iron). After 6 weeks, serum and vascular calcification were examined. Uremic diet increased creatinine and phosphate levels and extensive vascular calcification, as determined by von Kossa staining and biochemical determination of the Ca2+ content. Interestingly, the uremic condition also induced a mild hypercholesterolemia condition (+52% of total cholesterol; p<0.05). Supplemental diet did not reduce creatinine and phosphate levels as well as vascular calcification, however we observed a significant hypocholesterolemic effect (-18.9% uremic vs supplemental uremic diet; p<0.05). Similarly to simvastatin, incubation of cultured human hepatoma cell line (Huh7) with 7.5 µM and 15 µM of MK7 significantly reduced cholesterol biosynthesis (-35÷40%). At the same concentrations, MK7 induces 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (4 fold), low-density lipoprotein receptor (LDLR) by 3-fold and LDL uptake (+26%). MK7 also induced the mRNA expression of both HMG-CoA reductase and LDLR. However, in contrast to simvastatin, MK7 reduced both intracellular and secreted active form of PCSK9 in Huh7 cell line. The induction on LDLR was reversed by the co-incubation with squalene, suggesting that MK7 may act on an enzymatic step of the mevalonate pathway upstream the squalene synthase. Taken together, these results indicated that MK7 has a significant impact on cholesterol metabolism and its supplementation may help to control the mild hypercholesteremic conditions in CKD patients.

HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: MORE EVOLOCUMAB THAN AUTHORIZED IS NEEDED

F. Bigazzi, F. Sbrana, B. Dal Pino, A. Ripoli, M. Coceani, R. Luciani, M. Pianelli, T. Sampietro
Lipoapheresis Unit, Reference Center for Diagnosis and Treatment of Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa
E-mail: francesco.sbrana@ftgm.it

Background. Evolocumab represents a breakthrough in the treatment of hypercholesterolemia. The TAUSSIG study [NCT01624142] was conducted in subjects affected by Heterozygous Familial hypercholesterolemia (HeFH) already on chronic lipoprotein-apheresis (LA); evolocumab 420 mg was administered every 14 days at the end of each LA procedure, in addition to conventional medical therapy. The aim of the study was to verify if evolocumab efficacy is maintained in HeFH subjects that discontinued chronic LA and began evolocumab 140 mg, according to European Medicines Agency (EMA).
Methods. We retrospectively evaluated 12 HeFH patients enrolled in the TAUSSIG trial (mean age 67±8 years, 9 male) with known cardiovascular disease and on chronic LA. After the end of the trial, all study participants were shifted on Evolocumab 140 mg every 2 weeks, according to EMA recommendations.
Results. During three years of follow-up, a cumulative LDL-C reduction of -56% was recorded; 6/12 patients discontinued LA because they were able to maintain LDL-C level below 70 mg/dl. Three months after the reduction of evolocumab dosage, we registered a significant LDL-C increase in the subgroup of patients who discontinued LA (LDL-C 68±22 mg/dl vs 84±22 mg/dl, $p<0.05$), while subjects on LA showed no significant change in their lipid profile. In the subgroup of patients who discontinued LA, we tried to titrate lipid lowering therapy maximally without success and, in one case, LA was restarted.
Conclusions. Our study reveals the unmet needs of HeFH patients whose phenotypic spectrum is broad and, in many cases, overlaps with that of HoFH, thus needing a higher drug dose.

STATIN-ASSOCIATED MUSCLE SYMPTOMS IN THE PROSISA STUDY: PREVALENCE AND RISK FACTORS

F. Bonaiti[1], M. Casula[1,2], E. Olmastroni[1], M. Gazzotti[1], L. Grigore[3], A. Zambon[4], A.L. Catapano[1,2]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]IRCCS MultiMedica Hospital, esto S. Giovanni, Milan, [3]S.I.S.A. Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Milan; [4]Clinica Medica 1, Department of Medicine - DIMED, University, Hospital of Padua
E-mail: bonaitifederica@gmail.com

Statin associated muscle symptoms (SAMS) are one of the main reasons for poor treatment adherence and/or discontinuation. A definitive diagnosis of SAMS is difficult because symptoms are subjective and there is no 'gold standard' diagnostic test or questionnaire. PROSISA (PROject Statin Intolerance SISA) is an observational, retrospective, and multicentre study aimed at assessing the prevalence of statin intolerance due to SAMS in dyslipidaemic patients. Demographic and anamnestic data, biochemical levels, and potential occurrence of SAMS were collected. Adjusted logistic regression was fitted to estimate odds ratios (OR) and 95% confidence intervals for the association between SAMS onset and several factors.This analysis was carried out on 16,535 patients (mean age 60.5±12.0 years; 52.2% men) on statins. Overall, 57.4% of them had hypertension, 38.1% previous CV events, and 24.6% diabetes. During statin therapy, 9.7% of patients (N=1585) reported SAMS, in particular 8.7% of men, 10.8% of women, 14.5% of patients with liver disease. Symptoms were mainly myalgia (71.7%), cramps (24.8%), and fatigue (16.9%). Risk of SAMS onset was significantly higher for patients who practice physical activity (OR 1.26 [1.09-1.47]), with significant liver disease (OR 1.78 [1.43-2.21]), with previous CV events (OR 1.21 [1.06-1.37]), and for female patients (OR 1.26 [1.13-1.40]). Among patients reported with SAMS, 753 stopped statin treatment (dechallenge), with disappearance of muscular symptoms in 87.5% of cases. Moreover, among 955 patients experienced a rechallenge (different statin/lower dose, with or without washout period), with disappearance of muscular symptoms in 74.2% of cases. This study offers a real-life outlook of SAMS in Italian clinical practice. The reported prevalence of SAMS was 9.65%, but the percentage of patients in whom intolerance has been confirmed by dechallenge/rechallenge was between 23-27%, emphasizing the need for a better management of SAMS to provide a definitive diagnosis of SAMS and treatment re-evaluation.

REPROGRAMMING OF T REGULATORY CELLS AS A THERAPEUTIC TOOL TO DAMPEN THE IMMUNO-INFLAMMATORY RESPONSE ASSOCIATED TO ATHEROSCLEROSIS

F. Bonacina[1], E. Martini[2], M. Cremonesi[2], S. Locatelli[1], J. Nour[1], F. Pellegatta[3], A.L. Catapano[1,4], M. Kallikourdis[2], G.D. Norata[1]
[1]Department of Excellence of Pharmacological and Biomolecular Sciences, University of Milan; [2]IRCCS Humanitas Research Foundation, Rozzano, Milano; [3]Centro SISA, Ospedale Bassini, Cinisello Balsamo, Milano; [4]IRCSS Multimedica, Milano
E-mail: fabrizia.bonacina@unimi.it

Aim. Loss of anti-inflammatory activity of regulatory Tcells (Treg) is a pathogenic feature of immune mediated disease, as atherosclerosis. Our aim was to investigate whether Treg functionality was impaired in patients affected by familial hypercholesterolemia (FH) and test Treg-Adoptive Cell Therapy (ACT) as a treatment of atherosclerosis-related inflammation. To achieve selective targeting, we reprogram Treg to migrate to the plaque as a selective Treg-ACT in models of atherosclerosis.
Methods. Treg from FH patients (HE for LDLR) and LDLR KO mice were phenotypical and functional characterized by flow cytometry. Then, Treg from WT animals were retrovirally (IRES-EGFP vector) transfected with chemokine receptors or an empty vector and i.v. injected (2x105 GFP+ cells/mouse) in 8-week high cholesterol diet (WTD) fed male LDLR KO. Homing of transfected Treg to atherosclerotic plaque, its progression and composition was analysed by flow cytometry and histology.
Results. HeFH patients present increased levels of Treg (CD3/CD4/CD25high/CD127low/FOXP3+) compared to age and sex matched controls (+23%, $p<0.01$). Functional experiments, however, showed that Treg isolated from FH patients present a reduced ability in suppressing conventional Tcells proliferation compared to Treg cells from controls (-60% suppression of T cell proliferation, $p<0.01$), thus pointing to a decreased immunosuppressive function of Treg. Of note, similar impairment of Treg functionality has been confirmed in LDLR KO mice, the experimental model of the disease; these findings allow an easy translation to humans and paves the road for testing Treg treatment as a strategy to dampen the immuno-inflammatory response associated to the atherosclerotic plaque thus limiting disease progression.Therefore, to target Treg function in the plaque, chemokine's signature was evaluated in atherosclerotic LDLR KO mice, the experimental model of FH. mRNA expression revealed that CX3CL1 is selectively expressed in the aorta of KO mice but not in other districts (lymph nodes, spleen and liver), suggesting that the ectopic expression of its matching CX3CR1 would drive Treg to the atherosclerotic plaque. Transduced CX3CR1 or ctrl WT Treg, both overexpressing the fluorescent protein GFP, were injected in atherosclerotic KO mice. 24 h after, CX3CR1-Treg were mainly detected in the aorta (2,5% of GFP+/lived cell compared to 0.6% of ctrl-Treg), while migration was limited and similar to that of ctrl-Treg in other districts, suggesting that the CX3CL1/CX3CR1 axis would represent a strategy to vehiculate Treg to the plaque. To evaluate the effect of engineered Treg on atherosclerosis progression, LDLR KO mice were fed for 4 additional weeks to high cholesterol diet (WTD) after CX3CR1- or ctrl-Treg transfer. Although levels of cholesterol were unchanged, treatment with CX3CR1-Treg significantly reduces plaque progression (-40%, $p<0.05$) and lipid deposition (-11%, $p<0.05$), while ameliorates stability by increasing collagen (+27%, $p<0.05$) and smooth muscle cells content and decreasing pro-inflammatory macrophages.
Conclusion. Our data suggest that dysfunctional Treg could contribute to inflammatory burden of atherosclerotic plaque in FH patients. CX3CL1 is selectively expressed in atherosclerotic plaque and the CX3CL1/CX3CR1 axis may represent a pathway to target selective migration to the atherosclerotic plaque. Overexpressing CX3CR1 appears a promising ACT to promote selective homing of Treg into the plaque thus limiting atherosclerosis progression by reducing inflammatory burden.

THE ACHILLES TENDON ULTRASONOGRAPHY IN THE FAMILIAL HYPERCHOLESTEROLEMIA'S DIAGNOSTIC PATHWAY

M. Biolo[1], P. Scarinzi[1], P. Bigolin[2], C. Panzavolta[1], M.V. Rabuini[1], R. Marin[1], D. Vianello[1], S. Bertocco[1], F. Fabris[1], E. Manzato[1], S. Zambon[1], L. Previato[1], A. Zambon[1]
[1]Dipartimento di Medicina, Università degli Studi di Padova; [2]Ospedale di Castelfranco Veneto
E-mail: marta.biolo@gmail.com

Introduction. For their higher LDL cholesterol levels since birth the patients that suffer from Heterozygous Familial Hypercholesterolemia (HeFH) develop, besides known cardiovascular complications in early age, a progressive tendon thickening until xanthomas formation. Clinical examination, targeted at Achilles tendon (AT) palpation, is really useful for the diagnosis only in advanced stages of disease. AT ultrasonography could be an useful diagnostic tool for clinicians but it doesn't have a clear role yet.
Aim. To analyse the diagnostic value of the AT ultrasonography to identify xanthomas compared with clinical examination in subjects with HeFH.
Materials and Methods. From January 2016 to July 2018 we collected medical history, clinical examination, complete lipid profile, and Dutch Lipid Clinic Network (DLCN) score of 120 subjects, 83 with a confirmed genetic hypercholesterolemia and 37 without genetic mutation. All subjects were submitted to bilateral AT ultrasonography to evaluate AT thickness and presence of hypo-echogenic formations.
Results. No differences were recognized between the two groups about age, sex, BMI, total cholesterol, HDL, triglycerides or cardiovascular risk factors. A ROC curve showed that the best AT thickness diagnostic cutoff was a ≥6 mm value, that provided a 72.3% sensitivity, a 70.2% specificity, a 84.5% positive predictive value, a 53% negative predictive value, a 2.43 positive likelihood ratio and a 0.39 negative likelihood ratio. We confirmed that ultrasonography is more sensitive than clinical examination to identify xanthomas, and it could represent, with medical history, a useful method to reach an early diagnosis of HeFH.

MOLECULAR CHARACTERIZATION OF PATIENTS WITH AND WITHOUT CORONARY ARTERY DISEASE WITH "EXTREME LDL-C PHENOTYPES"

F. Brucato[1], N. Martinelli[2], R. Spina[1], F. Busti[2], V. Ingrassia[1], C. Scrimali[1], G.I. Altieri[1], D. Noto[1], G. Misiano[1], A. Giammanco[1], C.M. Barbagallo[1], F. Fayer[1], A.B. Cefalù[1], O. Olivieri[2], D. Girelli[2], M.R. Averna[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) University of Palermo; [2]Department of Medicine University of Verona;
E-mail: federicabrucato21@gmail.com

Introduction. LDL cholesterol (LDL-C) plasma levels are closely related to the development of coronary artery disease (CAD) and represent one of the main cardiovascular risk factors. The assessment of risk factors must take into account exposure times as well as cholesterol levels. This concept clearly emerges from the study of two monogenic diseases characterized by mutations that are controlled by high levels of cholesterolemia (familial hypercholesterolemia-FH) or low levels of plasma cholesterol (familial Hypobetalipoproteinemia-FHBL) which determine an increase or a reduction to develop CAD.

Materials and Methods. The study sample includes patients of Verona Heart Study (VHS) with angiographically documented CAD (positive CAD) and subjects with a completely normal coronary angiography who underwent coronary angiography for other reasons than suspected CAD (negative CAD). We selected patients with extreme LDL-C phenotype with cut-offs of LDL-C> 98th percentile (222 mg/dl) or with LDL-C cutoff <2nd percentile (66 mg/dl). For the study of the genetic bases of phenotypes characterized by high/low levels of LDL-C, a large-scale sequencing system based on Ion Torrent technology through PGM System has been developed. The analysis was carried out on 13 patients (4 negative CAD and 9 positive CAD) with a high LDL-C phenotype and 12 patients (6 negative CAD and 6 positive CAD) with a low LDL-C phenotype.

Results. Among the patients with LDL-C>98th percentile, the NGS analysis revealed the presence of a pathogenetic mutation in LDLR in one CAD positive subject while the others had an high probability of polygenic-FH (8 CAD positive and 4 CAD negative). The analysis of patients with low levels of LDL-C allowed the identification of two causative mutations in the APOB gene in two CAD negative patients while one missense variant in PCSK9 which correlate with low LDL-C was identified in a CAD positive patient.

CORRELATION BETWEEN DIFFERENT LDL-R MUTATIONS AND RESPONSE TO AB-PCSK9 THERAPY IN A GROUP OF PATIENT WITH GENETIC DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA. PRELIMINARY REPORT

A. Buonaiuto[1], M. Gentile[1], I. Calcaterra[1], C. Giacobbe[2] , M. Tripaldella, F. Forte[1], M.D. Di Minno[1], G. Iannuzzo[1], G. Fortunato[2], P. Rubba[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" di Napoli; [2]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università "Federico II" di Napoli
E-mail: ale.buonaiuto@gmail.com

Familial hypercholesterolemia (FH) is an autosomal dominant disease that leads to cardiovascular disease (CAD). The availability of ab-PCSK9 has changed the approach to therapy. We evaluate the relationship between genetic mutations and response to ab-PCSK9.71 FH patients, 32 women and 39 men (54.04±13.1 yrs), in primary prevention (N=45) and secondary prevention (N=26), were recruited. This sample included patients with mutations in LDLR gene: heterozygotes for missense mutations (N=30), for null mutations (N=30), compound heterozygotes or homozygotes (N=11). At baseline visit, the whole sample had a maximally tolerated lipid lowering therapy (MT-LLT) without ab-PCSK9; 10 patients had statin intolerance. After ab-PCSK9 therapy (160 days from baseline visit) we evaluated the achievement of a goal (LDL<100 mg/dL in patients without CAD or Diabetes Mellitus (DM) or organ damage, LDL<70 mg/dL). After 160 days of therapy with ab-PCSK9 (45 patients on Alirocumab, 26 patients on Evolocumab) and MT-LLT, 46/71 patients (64.8%) of the whole sample achieve the goal of LDL cholesterol. Of them 27/46 (58.6%) were in primary prevention versus 19/46 (41.3%) in secondary prevention, no difference in achievement of the goal (p=0.266). We then evaluated the percent of patients achieving the goal of LDL cholesterol:

- 22/30 (73.3%) patients with missense mutation and 23/30 (76.6%) patients with null mutation, no significant difference among groups;
- 1/11 (9.1%) compound heterozygotes or homozygotes (p<0.001 if compared to heterozygotes);
- 10 patients with statin intolerance, 4/10 (40.0%) achieved the goal of LDL cholesterol (p=0.077 versus those on therapy with statins, 6/10 (60.0%)).

The other main cardiovascular risk factors did not influence of the achievement the goal of LDL cholesterol. These findings indicate lack of correlation between type of mutation in heterozygous FH patients and ab-PCSK9 therapy response. Response was significantly poorest in patients with compound heterozygosis or homozygosis mutation as compared to heterozygotes.

FENRETINIDE TREATMENT ACCELERATES ATHEROSCLEROSIS DEVELOPMENT IN APOLIPOPROTEIN E-DEFICIENT MICE IN SPITE OF BENEFICIAL METABOLIC EFFECTS

M. Busnelli[1], S. Manzini[1], F. Bonacina[1], A. Colombo[1], S. Soldati[2], S.S. Barbieri[3], P. Amadio[3], L. Sandrini[3], F. Arnaboldi[4], E. Donetti[4], R. Laaksonen[5], S. Paltrinieri[2], E. Scanziani[2], G. Chiesa[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Department of Veterinary Medicine, Università degli Studi di Milano; [3]Centro Cardiologico Monzino IRCCS, Milano; [4]Department of Biomedical Sciences for Health, Università degli Studi di Milano; [5]Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
E-mail: marco.busnelli@gmail.com

Aim. Fenretinide, a synthetic retinoid derivative first investigated for cancer prevention and treatment, has been shown to ameliorate glucose tolerance and the plasma lipid profile, and to reduce body fat mass. These effects, together with its ability to inhibit ceramide synthesis, have suggested that fenretinide may display anti-atherosclerotic effects.
Methods. To this aim, 9-weeks-old apoE-knockout (EKO) female mice were fed for 12 weeks a Western diet, without (EKO-Ctrl) or with (0.1% w/w) fenretinide (EKO-Fen). As a reference, wild-type (WT) mice were likewise treated. Growth and metabolic parameters were monitored throughout the study. Atherosclerosis development was evaluated in the aorta and at the aortic sinus. Blood and lymphoid organs were further characterized with thorough cytological/histological and immunocytofluorimetric analyses.
Results. Fenretinide treatment significantly lowered body weight, glucose levels and plasma levels of total cholesterol, triglycerides and phospholipids. In the liver, fenretinide remarkably reduced hepatic glycogenosis and steatosis driven by the Western diet. Treated spleens were abnormally enlarged, with severe follicular atrophy and massive extramedullary hematopoiesis. Severe renal hemosiderin deposition was observed in EKO-Fen. Treatment resulted in a threefold increase of total leukocytes (WT and EKO) and raised the activated/resting monocyte ratio in EKO-Fen. Finally, atherosclerosis development was markedly increased at the aortic arch (34.4±7.3% vs 26.1±5.8%, +32%), thoracic (14.3±4.9% vs 4.9±2.1%, +191%) and abdominal aorta (7.6±3.3% vs 3.3±1.8%, +130%) of fenretinide-treated mice. Plaque extent was further quantified at the aortic sinus and provided similar results (810.000 μm^2 vs. 540.000 μm^2, +50% in EKO-Fen). Plaques of treated mice were characterized by an increased collagen content and a larger necrotic core, whereas the area occupied by macrophages, foam cells and neutral lipids was comparable between EKO-Fen and EKO-Ctrl.
Conclusion. We provide the first evidence that, despite beneficial metabolic effects, fenretinide treatment may prove detrimental for atherosclerosis development.

FAT MASS LEVELS IN OVERWEIGHT/OBESE CHILDREN AND VASCULAR HEALTH

G. Campana[1], M.R. Licenziati[1], G. Iannuzzo[2], G. Valerio[3], D. Morlino[2], M. Renis[4], A. Iannuzzi[5]
[1]Obesity and Endocrine Disease Unit, Department of Neurosciences, Santobono-Pausilipon Children's Hospital, Naples; [2]Department of Internal and Clinical Medicine Federico II University, Naples; [3]Department of Movement Sciences and Welbeing Parthenope University of Naples; [4]Division of Internal Medicine, Cava dei Tirreni Hospital, Salerno; [5]Department of Internal Medicine and Medical Specialties, A. Cardarelli Hospital, Naples
E-mail: giuseppina.campana86@gmail.com

Background. Childhood obesity is one of the most serious public health challenges of the 21st century (1). Adiposity in childhood tracks into adulthood and is associated with later cardiovascular diseases. Body mass index (BMI), the most widely used marker of body fatness, has serious limitations, particularly in children, since it does not accurately discriminate between lean and fat mass, which can be differently distributed in individuals with the same BMI. Recently it was shown that a new prediction model based on height, weight, age, sex, and ethnicity had high accuracy in predicting fat mass in children (2). Aim of our study was to assess in overweight or obese children whether the estimate of fat mass, as derived by the new prediction model, was associated with the carotid cross-sectional area of the intima media complex (CSA-IMC), a parameter that takes into account changes in both wall thickness and lumen diameter and therefore is a more appropriate measure of arterial mass than Intima-Media Thickness (IMT) alone (3).
Subjects and Methods. 485 overweight/obese italian children, 55.2% males, aged 5-15 years, admitted to a tertiary care hospital, were consecutively enrolled in a study on cardiovascular markers of atherosclerosis. All children underwent an ultrasound carotid arterial examination.
Results. Mean weight was 62.5+21.1 kg and fat-mass was 26.3+10.7 kg. CSA-IMC was 8.9+1.6 mm^2. Multiple regression analyses showed a significant association with CSA-IMC (beta 0.216, $p<0.001$); the association remained significant (beta 0.190, $p=0.006$) after controlling for the main cardiovascular risk factors (age, sex, systolic blood pressure, HOMA-index, triglycerides, HDL-cholesterol). Fat mass was also independently associated with other cardiovascular risk factors (blood pressure, HOMA-index, triglycerides, HDL-cholesterol).
Conclusion. Fat mass, as assessed using the new prediction model based on simple anthropometric measures, is associated with subclinical atherosclerosis in overweight/obese children.

References

1. World Health Organization. Childhood overweight and obesity 2014. www.who.int/dietphysicalactivity/childhood/en/
2. Hudda MT, Fewtrell MS, Haroun D, Lum S, Williams JE, Wells JCK, Riley RD, Owen CG, Cook DG, Rudnicka AR, Whincup PH, Nightingale CM. Development and validation of a prediction model for fat mass in children and adolescents: meta-analysis using individual participant data. BMJ. 2019; 366: l4293.
3. Roman MJ, Pickering TG, Schwartz JE, Pini R, Devereux RB. Relation of blood pressure variability to carotid atherosclerosis and carotid artery and left ventricular hypertrophy. Arterioscler Thromb Vasc Biol. 2001; 21: 1507-1511.

PCSK9 REGULATES NOX2-MEDIATED PLATELET ACTIVATION BY CD36 RECEPTOR: IMPLICATIONS IN PATIENTS WITH ATRIAL FIBRILLATION

V. Cammisotto[1], P. Pignatelli[2], C. Nocella[2], S. Bartimoccia[2], V. Castellani[2], D. Pastori[2], F. Violi[2], R. Carnevale[3]
[1]Department of General Surgery and Surgical Speciality Paride Stefanini, Sapienza University of Rome; [2]Department of Internal Medicine and Medical Specialities, Sapienza-University of Rome; [3]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza-University of Rome, Latina
E-mail: vittoria.cammisotto@uniroma1.it

Introduction. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is mainly expressed in the liver, where it LDL cholesterol uptake regulating the expression of LDL receptor (LDL-R). In addition, it was found in other tissues such as the small intestine, kidney, pancreas, and also in the arterial wall, where it could influence local homeostasis and atherosclerosis. The gain of function variants of PCSK9 gene is associated with increased levels of circulating LDL cholesterol, and increased risk for cardiovascular events (CVE) in patients with and without atrial fibrillation (AF). Circulating levels of PCSK9 above 1200 pg/ml were shown to be predictive of cardiovascular events (CVEs) in patients with atrial fibrillation (AF). Because high PCSK9 plasma levels were significantly correlated with 11-dehydro-thromboxane B2 (11-dh-TxB2), a marker of platelet activation, it is is possible to hypothesize a direct effect of PCSK9 on PA, but the mechanism involved is still unclear. The aim of the study was to evaluate the role of PCSK9 on PA in vivo in AF patients, if PCSK9, in the range of concentration achievable in human circulation, may influence PA and the underlying molecular mechanism.
Materials and Methods. Cross-sectional study including 50 patients with baseline PCSK9 below and 50 above the median value of 1200 pg/ml from the ATHERO-AF cohort. The two groups were balanced for age and sex. In vivo PA was assessed by platelet aggregation, platelet recruitment, serum thromboxane B2 (TxB2) formation and sP-selectin levels. As markers of oxidative stress we evaluated sNox2-dp, H2O2 production, urinary isoprostanes and oxLDL. We performed an in vitro study with platelets from healthy subjects (n=5) added with PCSK9 concentrations achievable in human circulation (1000 pg/ml and 2000 pg/ml) measuring aggregation, TxB2, isoprostanes and H2O2 production, Nox2 activation, oxLDL formation. Moreover, to investigate if PCSK9 directly interacts with CD36 and LOX-1, we assessed a co-immunoprecipitation between PCSK9 and these receptors. Finally, p38, p47 and PLA2 phosphorylation was evaluated by western blot analysis.
Results. We observed increased PA, measured by platelet aggregation (49,8±2,5 vs 68,6±1,9%; p<0.0001), platelet recruitment (16,46±1,568 vs 31,20±1,861%; p<0.0001), p-selectin expression and serum levels of TxB2 (7,7±0,6 vs 14,7±0,45 ng/mL; p<0.0001; 121,5 ± 1,9 vs 245,5±4,6 pg/mL; p<0.0001, respectively), and oxidative stress evaluated by sNox2 release (21,4±0,9 vs 34,4±1,1 pg/ml; p<0.0001), H2O2 (29,5±1,3 vs 48,8±1,6 µM; p<0.0001), ox-LDL production (12,0±0,9 vs 33,5±1,1 mU/ml; p<0.0001) and isoprostanes biosynthesis (137,2±22,0 vs 272,0±8,6 pg/mg creatinine; p<0.0001) in patients with PCSK9 levels above median (1200 pg/ml) compared to those below (p<0.05). Circulating levels of PCSK9 significantly correlated with markers of platelet activation (PA, TxB2, sNox2-dp and sP-selectin) and oxidative stress oxidative stress (isoprostanes, H2O2 and oxLDL formation). In vitro study demonstrated that PCSK9 was able to increase PA by binding the CD36 receptor. PCSK9-dependent PA is inhibited by the specific Nox2 blocking peptide and is mediated by p38, p47phox and cPLA2 phosphorylation.
Conclusions. PCSK9, at concentration achievable in blood from patients with AF, directly induce CD36-mediated platelet activation by amplifying Nox2 activity and arachidonic acid metabolism.

THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN

R. Buganza[1], G. Massini[1], M.D. Di Taranto[2], A. Gerace[1], L. de Sanctis[1], O. Guardamagna[1]
[1]Department of Public Health and Pediatrics, University of Turin; [2]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II
E-mail: buganzaraffaele@gmail.com

Introduction. The identification and treatment of familial hypercholesterolemia (FH) since childhood can improve prognosis and reduce the associated cardiovascular mortality. The aim of the study was to evaluate the effectiveness of the international scores Simon Broome Register (SBR), Dutch Lipid Clinic Network (DLCN) and LIPIGEN in predicting the correct FH diagnosis already confirmed by gene mutation detection.
Material and Methods. 213 patients (mean age 7±4.15 years) were retrospectively evaluated. Lipid profiles were analyzed before any treatment, and SBR, DLCN and LIPIGEN scores were applied to all patients; 178/213 patients were screened for mutations in the LDLR, APOB and PCSK9 genes.
Results. Mean levels of total cholesterol (TC) and LDL-cholesterol (LDL-C) were 279±66.5 mg/dl and 211.3±50.16 mg/dl respectively, without significant differences between males and females. Among the 178 patients undergoing molecular investigations, 159 showed causative mutations, while the molecular analysis was negative on 19 subjects. No independent mutation predictors have been identified among the lipoprotein parameters taken into account (CT, LDL-C, HDL-C, non-HDL-C, LDL/HDL ratio and triglycerides). The use of international scores as discriminant for molecular testing would have led to a loss of 25.7%, 40.7% and 27.1% of mutated patients, with SBR, DLCN and LIPIGEN scores respectively.
Conclusions. In the paediatric population with FH, the evaluation of SBR score is the more reliable tool to predict the FH diagnosis in children.

LIPID PROFILE OF MALE AND FEMALE PATIENTS UNDER 18 YEARS OLD WITH A DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA: AN ANALYSIS FROM THE LIPIGEN PAEDIATRIC COHORT

M.E. Capra[1], M. Gazzotti[2], M. Casula[3], A.L. Catapano[3], E. Olmastroni[2], F. Bonaiti[2], C. Pederiva[4]
[1]Centro Dislipidemie in età evolutiva U.O. Pediatria e Neonatologia, Ospedale G. da Saliceto, Piacenza; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [3]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; IRCCS MultiMedica, Sesto S. Giovanni, Milano; [4]U.O. Clinica Pediatrica, Servizio Clinico Dislipidemie per Lo Studio e la Prevenzione dell'Aterosclerosi in età pediatrica, Ospedale San Paolo, Milano

Background. Familial hypercholesterolemia (FH) is one of the most common genetic disorder in childhood, yet is often undetected. High levels of low-density lipoprotein cholesterol (LDL-C) are present since birth, thus leading to the development of early atherosclerosis.
Aim and methods. In 2018, the LIPIGEN paediatric group was born within the LIPIGEN Network, to improve detection, diagnosis and management of paediatric FH patients. In this analysis, we selected LIPIGEN patients <18 years and evaluated biochemical parameters focusing on gender differences.
Results. 567 male and 633 female LIPIGEN patients, age <18 y, were analysed. At diagnosis, the distribution of males/females through the age classes was 48.2%/51.8% for 199 subjects with age 0-5 years, 43.9%/56.1% for 501 subjects with 6-10 years, 52.7%/47.3% for 296 subjects with 11-13 years, and 46.6%/53.4% for 204 subjects with 14-17 years. Mean age at diagnosis was 9.6±3.8 years in both sexes. Excluding 40 males and 44 females under lipid-lowering treatment, in the 0-5y age group, LDL-C was (mean±SD, mg/dl) 198.2±82.4 and 212.1±72.7 mg/dL (for males and females, respectively; p=0.221) and non-HDL-C was (mean±SD, mg/dl) 210.7±85.7 and 225.3±72.0 mg/dL (p=0.205). In the 6-10y age group, LDL-C was 184.6±80.1 and 169.3±64.1 mg/dL (p=0,021) and non-HDL-C was 200.9±81.4 and 185.0±65.1 (p=0.019). In the 11-13y age group, LDL-C was 164.9±60.2 and 168.8±58.9 (p=0.593) and non-HDL-C was 186.14±63.02 and 189.9±62.1 (p=0.630). In the 14-17y age group, LDL-C was 185.2±55.9 and 199.2±61.6 (p=0.118) and non-HDL-C was 202.3±57.2, 218.1±64.0 (p=0.088).
Conclusion. This "snapshot" of LIPIGEN-network pediatric population highlights statistically significant differences in lipid profile in male and female at different ages. These results confirm the variability of lipid profile according to pubertal status, with a transient improvement in female as their reach pubertal status before male. The lipidologist must always be aware of these pubertal-related lipid variations in the management and follow up of pediatric patients.

*Heads of centers of LIPIGEN paediatric group: Francesco Angelico (Roma), Marcello Arca (Roma), Maurizio Averna (Palermo), Andrea Bartuli (Roma), Giacomo Biasucci (Piacenza), Claudio Borghi (Bologna), Paolo Calabrò (Napoli), Francesca Carubbi (Modena), Francesco Cipollone (Chieti), Nadia Citroni (Trento), Giuliana Fortunato (Napoli), Ornella Guardamagna (Torino), Arcangelo Iannuzzi (Napoli), Lorenzo Iughetti (Modena), Graziana Lupattelli (Perugia), Giuseppe Mandraffino (Messina), Lorenzo Maroni (Varese), Giuliana Mombelli (Milano), Sandro Muntoni (Cagliari), Cristina Pederiva (Milano), Fabio Pellegatta (Sesto San Giovanni (MI)), Livia Pisciotta (Genova), Arturo Pujia (Catanzaro), Francesco Purrello (Catania), Paolo Rubba (Napoli), Carlo Sabbà (Bari), Riccardo Sarzani (Ancona), Giovanni Battista Vigna (Ferrara), Josè Pablo Werba (Milano), Sabina Zambon (Padova), Maria Grazia Zenti (Verona) LIPIGEN Steering Committee: Marcello Arca (Roma), Maurizio Averna (Palermo), Stefano Bertolini (Genova), Sebastiano Calandra (Modena), Alberico L. Catapano (Milano), Patrizia Tarugi (Modena) Centralized laboratory: Veronica Zampoleri (Cinisello Balsamo (MI).

LIPID PROFILE IN ADOLESCENTS WITH ANOREXIA NERVOSA: A CHALLENGE FOR PEDIATRIC LIPIDOLOGISTS

M.E. Capra, G. Biasucci
Department of Pediatrics and Neonatology, "Guglielmo da Saliceto" City Hospital, Piacenza
E-mail: mel.capra@gmail.com

Introduction. Anorexia nervosa (AN) is epidemically increasing among adolescents. High total-cholesterol (TC) and LDL-cholesterol (LDL-C) values are often reported in AN patients. These alterations might lead some physicians to prescribe a low-fat diet to malnourished AN patients, thus worsening their metabolic and psychological conditions. Pediatric lipidologists play a pivotal role in analyzing and treating lipid alterations in these cohort of patients. Aim of this study is to evaluate the effect of re-feeding on lipid profile in adolescents admitted to our Unit
Matherial and Methods. 235 patients referred to our Pediatric Unit for eating disorders over a 4-year period. 93/235 patients were diagnosed with AN. 37/93 (36 females, age 11-21 years, mean 15 yrs) matched criteria for hospital admission (severe/extreme malnutrition, suicidal thoughts, ineffective/impossible home management). Mean hospital-stay: 78.8 days. Patients were evaluated for anthropometric measures and complete blood lipid profile at admission (T0) and discharge (T1). All patients underwent nutritional and psychological treatment.
Results. Lipid profile (mg/dl) at T0 and T1 was, respectively: total cholesterol 177.7±44.9 and 168.8±38.05, LDL 112.2±36.8 and 99.2±26.5 ($p<0.05$), HDL 55.7±13.1 and 59.2±13.7. Malnutrition-degree at T0 and T1 was, respectively: extreme 20/37 (54%) and 4/37 (11%), severe 8/37 (21.5%) and 3/37 (8%), moderate 8/37 (21.5%) and 10/37 (27%), mild 1/37 (3%) and 13/37 (35%). BMI (mean, range) at T0 and T1 was 14.2 kg/m^2 (range 11.26-18.52) and 16.33 (13.04-19.8). Statistic: Mann-Whitney test.
Conclusions. TC and LDL-C in malnourished AN adolescents were higher than expected before starting re-feeding. This finding may be due to a higher rate of synthesis of cholesterol-rich lipoproteins in such patients. Our preliminary data show that lipid values in AN pediatric patients must be evaluated by a pediatric lipidologist and related to BMI and malnutrition degree, in order to avoid prescription of unnecessary dietetic restriction in an already compromised nutritional and psychological status.

TWO HYPERTRIGLYCERIDEMIC PATIENTS CARRYING A SINGLE VARIANT CAUSATIVE OF HYPERTRIGLYCERIDEMIA AND A RARE APOB VARIANT

G. Cardiero[1], C. Giacobbe[1], D. Palma[1], F. Forte[2], G. Maione[1], M.D. Di Taranto[1], G. Iannuzzo[2], A. Iannuzzi[3], P. Rubba[2], G. Fortunato[1]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, and CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli; [2]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli; [3]UO Medicina Interna, AORN "A. Cardarelli" di Napoli
E-mail: cardiero@ceinge.unina.it

Introduction. Severe hypertriglyceridemia (HTG) is a rare disease consisting in increased plasma triglyceride (TG) levels (>10 mmol/L). Patients can show eruptive xanthomas, lipaemia retinalis, hepatosplenomegaly and pancreatitis. Five genes are causative of the disease with autosomal recessive inheritance: Lipoprotein lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II, Glycosyl-phosphatidyl-inositol-anchored HDL-binding protein and Lipase maturation factor-1. Genetic causes are not identified in a consistent portion of patients.
Patients, Material and Methods. Two HTG patients were recruited and screened for variants in the 5 causative genes by traditional sequencing. Multiplex Ligation-dependent Probe Amplification (MLPA) was used to detect large rearrangements of LPL gene.
First patient: TG 1,456 mg/dL, cholesterol 407 mg/dL, HDL-cholesterol 21 mg/dL, hepatic steatosis, pancreatitis.
Second patient: TG 2,304 mg/dL, cholesterol 280 mg/dL, HDL-cholesterol 8 mg/dL, hepatic steatosis, pancreatitis.
Since the screening revealed only one pathogenic variant in each patient, the next-generation sequencing (NGS) was used to detect variants in a panel of 28 lipid-related genes.
Results. The first patient was heterozygote for the pathogenic variant c.809G>A - p.(Arg270His) in the LPL gene. By NGS, another rare variant was found in the in the exon 29 of APOB gene: c.12382G >A - p.(Val4128Met) that was classified as benign although identified in patients with Familial Hypercholesterolemia (FH).The second patient was heterozygote for the pathogenic variant c.883C >T - p.(Gln295*) in the APOA5 gene. Another variant causative of FH was found by NGS in the exon 26 of APOB gene: c.10672C >T p.(Arg3558Cys). Both patients had the E2/E3 genotype of APOE. No copy number variations were found.
Conclusion. The presence of an APOB variant can be a modifier factor leading to HTG also in patients carrying a single causative variant. Severe HTG is a complex disease that can benefit from NGS to identify variants in a large number lipid-related genes that can act as phenotype modifier.

LOW-GRADE ESCHERICHIA COLI ENDOTOXEMIA ENHANCES ARTERY THROMBUS GROWTH VIA TOLL-LIKE RECEPTOR 4. IMPLICATION FOR MYOCARDIAL INFARCTION

R. Carnevale[1], S. Sciarretta[1], C. Nocella[1], G. Frati, V. Cammisotto[1], M. Forte[2], G. D'Amati[1], R. Cangemi[1], C. Gaudio[1], G. Tanzilli[1], D. Pastori[1], F. Violi[1]
[1]Sapienza University of Rome; [2]IRCCS Neuromed
E-mail: roberto.carnevale@uniroma1.it

Introduction. Escherichia Coli-lipopolysaccharide (EC-LPS), a component of gut microbiota, is detectable in human circulation but its role in thrombosis is still unclear.
Methods and Results. We measured serum EC-LPS concentration and DNA, sP-selectin, a marker of in vivo platelet activation, and zonulin, a marker of gut permeability, in coronary thrombi and intra-coronary blood of patients with ST-elevation myocardial infarction (STEMI, n=50) and stable angina (SA) (n=50) respectively, and in peripheral circulation of matched controls (n=50). Experimental study was carried out in mice to assess if EC-LPS possess thrombotic property. Coronary thrombi from STEMI showed higher concentrations of EC-LPS and sP-selectin versus intra-coronary blood of SA and peripheral blood of controls. Serum zonulin was higher in STEMI compared to the other two groups and correlated with EC-LPS. EC-LPS DNA was detected in 34% of STEMI vs. 12% of SA and 4% of controls. In a subgroup of 12 STEMI, immuno-histochemical analysis of coronary thrombi showed positivity for leucocyte Toll-like receptor 4 (TLR4), cathepsin G and EC-LPS in 100%, 80% and 25% of samples, respectively. EC-LPS injected in mice (n=10) to reach lipopolysaccharides concentrations like those detected in coronary thrombi was associated with enhanced artery thrombosis and platelet activation, an effect blunted by TLR4 inhibitor co-administration. In vitro study demonstrated that EC-LPS, at the same concentrations detected in coronary thrombi, enhanced platelet aggregation via TLR4-mediated leucocyte cathepsin G activation.
Conclusions. STEMI patients disclose an enhanced gut permeability that results in EC-LPS translocation in human circulation and eventually thrombus growth at site of artery lesion via leucocyte-platelet interaction.

ASSOCIATION BETWEEN GLOBAL CARDIAC CALCIFICATION SCORE (GCCS) AND OSTEOPOROSIS

P. Carrai, S. Camarri, P. Fortini, A. AL Refaie, C. Caffarelli, R. Nuti, S.T. Gonnelli
Department of Medicine and Neurosciences, University of Siena, Policlinico Le Scotte, Siena
E-mail: paolo.carrai@libero.it

Background. Lately there is an increasing attention in finding a linkage between bone metabolism and cardiovascular disease. Experimental studies suggest that bone mineral density (BMD) and valvular calcification may be reciprocally related, but with conflicting data.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 84 subjects assessed for bone fracture risk (mean age 71±6,4 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 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.
Results. The results show that there is a significant inverse correlation between BMD-FN, BMD-FT and BMD-LS with GCCS (respectively r=-0,393, p<0,05; r=-0,444, p<0,05 and r=-0,361, p<0,05). Dividing the study population based on BMD, we found that GCCS was higher in osteoporotic and osteopenic subjects respect those who have normal value of BMD. Dividing patients into two groups based on presence of bone fragility fractures (25 patients with fragility fractures and 46 patients without) we observed that the value of GCCS was significant higher (p<0,05) in patients with fractures.
Conclusion. Our data suggest a link between osteoporosis and cardiac calcification. In fact the burden of cardiac calcium is higher in patients with lower BMD and fragility fractures. These findings confirm that osteoporotic patients have an higher risk of cardiac calcification and probably a greater risk of cardiovascular events.

LIPOPROTEIN(A) IS SIGNIFICANTLY HIGHER AND ASSOCIATED WITH INCREASED PREVALENCE OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN MUTATION-NEGATIVE FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

A. Cavicchioli, S. Lugari, P. Vita, A. Mondelli, E. Pellegrini, F. Carubbi, F. Nascimbeni
Unit of Internal and Metabolic Medicine, Civil Hospital, A.O.U. of Modena and University of Modena and Reggio Emilia
E-mail: alessia.cavicchioli.1990@gmail.com

Introduction. High lipoprotein(a) (Lp(a)) concentrations may be a cause of clinical familial hypercholesterolemia (FH) and are associated with an increate risk of atherosclerotic cardiovascular disease (ASCVD) [Langsted, Lancet Diabetes Endocrinol 2016]. We evaluate if Lp(a) concentrations and their association with ASCVD may differ between mutation-negative and mutation-positive FH patients.
Methods. 177 consecutive patients with clinical suspicion of FH from the Lipid Clinic in Modena underwent comprehensive evaluation, including Lp(a) measurement and genetican analysis, within the LIPIGEN Project. We considered high Lp(a) concentrations (hyper-Lp(a)) when more than 50 mg/dl. Patients were defined as mutation-positive if"pathogenic" or "likely pathogenic" mutations were detected in FH-causing genes; mutation-negative patients were defined by the presence of "variants of uncertain significance" or absence of genetic variants.
Results. 135 FH patients (76.3%) were mutation-positive. Mutation-positive patients showed significantly higher LDL-cholesterol(LDL-c) levels (p<0.001) and Dutch Lipid Clinic Network (DLCN) score (p<0.001) than mutation-negative patients, whereas the prevalence of ASCVD was not significantly different between the two groups (15.6% vs. 19.0%; p=0.593). Conversely, Lp(a) concentrations were significantly higher (34.7 [10.7-88.9] vs. 22.8 [8.0-49.6] mg/dl; p=0.043) and hyper-Lp(a) was significantly more frequent (40.5% vs. 23.7%; p=0.034) in mutation-negative than mutation-positive patients. When adjusting LDL-c for Lp(a) cholesterol content (by subtracting 30% of the individuals' Lp(a) total mass from LDL-c), the Lp(a)-adjusted DLCN score tended to be more frequently lowered in mutation-negative than mutation-positive patients (28.6% vs. 15.6%; p=0.059). Hyper-Lp(a) was significantly associated with an increased prevalence of ASCVD in mutation-negative patients (p=0.003), but not in mutation-positive patients (p=0.269). Of note, 7 out of 8 (87.5%) mutation-negative patients with a personal history of ASCVD had hyper-Lp(a).
Conclusions. Our study confirms that high Lp(a) concentrations may represent a significant risk factor for clinical FH. In mutation-negative FH patients hyper-Lp(a) may be a major contributing cause of clinical FH and increased ASCVD risk.

ECHOCARDIOGRAPHIC ALTERATION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AFFECTED BY HEPATIC STEATOSIS: A CROSS-SECTIONAL SINGLE CENTER STUDY

S. Cicco, N. Susca, L. Crudele, M. Guarascio, P. Leone, M. Prete, F. Dammacco, V. Racanelli, A. Vacca
Department of Biomedical Sciences and Human Oncology, Unit of Internal Medicine "Guido Baccelli", University of Bari Medical School, Bari
E-mail: sebacicco@gmail.com

Introduction. Systemic Lupus Erythematosus (SLE) is a chronic inflammatory disease caused by autoimmunity. Systemic inflammation disease related may increase flogosis related damage as steatosis. In this study, we looked for a correlation between disease activity, evaluated by SLEDAI and SLICC-DI score, and heart involvement comparing those who presented a hepatic steatosis to those who do not.
Materials and Methods. We evaluated 19 SLE patients (Steat-SLE: 13 women and 6 men, aged 51.89±10.37 years) between 01/01/2015 and 31/12/2018 affected by hepatic steatosis. As control group, we use 51 patients (No-Steat-SLE: 42 women and 9 men, aged 46.24±14.27 years) that did not evidence of steatosis. Patients came in follow-up for revaluation and therapy adjustment.
Results. Anti-dsDNA Ab, C3, C4, ESR and PCR were not different between the groups. Hepatic dysfunction was not detected in Steat-SLE. No difference were found in SLEDAI, while SLICC-DI results increased if steatosis was detected (Steat-SLE 3.57±3.36 vs No-Steat-SLE 2.27±2.15, $p<0.05$). Patients with Steatosis present a longer disease (Steat-SLE 232.0±129.8 vs No-Steat-SLE 121.4±110.6 months, $p<0.05$). We highlight that patients with steatosis had an increased left ventricular mass (MLV) both as absolute value (MLV: Steat-SLE 226.2±57.53 vs No-Steat-SLE 194.4±58.17 gr, $p<0.05$) and MLV indexed (MLVi) for body surface area (BSA) (MLVi: Steat-SLE 135.0±48.17 vs No-Steat-SLE 112.3±27.47 gr/m^2, $p<0.05$) but no in interventricular septum thickness. At the same time no difference was found in left atrium (LA) as volume absolute value (LAV: Steat-SLE 67.33±25.97 vs No-Steat-SLE 58.45±19.06 ml, $p<0.05$) while it was increased as LA volume indexed (LAVi) for body surface area (BSA) (LAVi Steat-SLE 41.67±19.49 vs No-Steat-SLE 34.12±9.74 ml/m^2, $p<0.05$). Moreover, a correlation was found in both Steat and No-Steat SLE between SLICC-DI, MLV and MLVi.
Conclusions. These data show an increase of LAVi, MLV and MLVi in patients with steatosis. At the same time, these patients present an increased disease damage and duration. This could suggest heart involvement as result of systemic inflammation similarly to that related to hepatic stetosis. At the same time, the detection of hepatic steatosis should suggest a worsening in heart damage in SLE patients.

ARTERIAL STIFFNESS, SUGAR-SWEETENED BEVERAGES AND FRUITS INTAKE IN A RURAL POPULATION SAMPLE: DATA FROM THE BRISIGHELLA HEART STUDY

A.F.G. Cicero[1], F. Fogacci[1], G. Desideri[2], E. Grandi[1], E. Rizzoli[1], S. D'Addato[1], C. Borghi[1]
[1]Hypertension and Atherosclerosis Research Group, Medical and Surgical Sciences Department, University of Bologna; [2]Department of Life, Health and Environmental Sciences, University of L'Aquila
E-mail: arrigo.cicero@unibo.it

Introduction. There are conflicting results linking fruit and fructose intake with cardiometabolic disorders. The main objective of our study was to evaluate the association between fruits and sugar-sweetened beverages intake with pulse wave velocity (PWV), a non-invasive marker of arterial aging, in a large population sample.
Methods. For this study, we selected four age and sex-matched subgroups from the last Brisighella Heart Study population survey, after exclusion of those in secondary prevention for cardiovascular diseases, affected by gout and moderate-to-severe chronic kidney disease (defined as eGFR<60 ml/min), and/or actively treated with direct vasodilating drugs (calcium-antagonists, alpha-blockers, nitrates). The remaining subjects were classified in four groups:
1) low fruit and low sugar-sweetened beverage intake (LFLB);
2) high fruit and low sugar-sweetened beverage intake (HFLB);
3) low fruit and high sugar-sweetened beverage intake (LFHB);
4) high fruit and high sugar-sweetened beverage intake (HFHB).
Results. PWV was significantly more elevated in subjects assuming a higher fructose load, in particular when it was derived from industrially sweetened beverages (8,6±2,3 ms vs. 9,6±2,3 m/s, $p<0,001$). Moreover, the main predictors of PWV values were serum uric acid (B=0,391, 95%CI 0,321-0,486, $p=0,001$), fructose load from both fruits and sugar-sweetened beverages (B= 0,310, 95%CI 0,099-0,522, $p=0,004$), triglycerides (B=0,228, 95%CI 0,117-0,389, $p=0,018$), fasting plasma glucose (B=0,015, 95%CI 0,008-0,022, $p<0,001$) and estimated Glomerular Filtration Rate (B=-0,043, 95%CI -0,052 - -0,035, $p<0,001$).
Conclusion. Our data suggest that increased intake of fructose derived from industrial sweetened beverages, though not from all fruits, is independently associated with higher pulse wave velocity.

AWARENESS OF MAJOR CARDIOVASCULAR RISK FACTORS AND ITS RELATIONSHIP WITH MARKERS OF VASCULAR AGING: DATA FROM THE BRISIGHELLA HEART STUDY

A.F.G. Cicero[1], F. Fogacci[1], G. Tocci[2], F. Ventura[1], V. Presta[2], E. Grandi[1], E. Rizzoli[1], S. D'Addato[1], C. Borghi[1]
[1]Hypertension and Atherosclerosis Research Group, Medical and Surgical Sciences Department, Sant'Orsola-Malpighi University Hospital, Bologna; [2]Hypertension Unit, Division of Cardiology, Department of Clinical and Molecular Medicine, University of Rome Sapienza, Sant'Andrea Hospital, Rome
E-mail: arrigo.cicero@unibo.it

Background. The aim of the present study was to evaluate the vascular aging of subjects aware and not aware to be hypertensive, hypercholesterolemic, hypertriglyceridemic or diabetics in a general population sample.
Methods. We interviewed 1652 subjects without atherosclerotic cardiovascular diseases (M: 46.6%, F: 53.4%) about their awareness of hypertension, hypercholesterolemia, hypertriglyceridemia or type 2 diabetes. Then we compared the augmentation index and pulse wave velocity of subjects aware and not aware of the investigated cardiovascular risk factors.
Results. 1049 participants declared not to be hypertensive, while 32 were not sure. Among them, respectively, 23.5% and 50% were hypertensive. Subjects not aware of their hypertension had significantly higher aortic blood pressure than aware ones ($p<0.001$). 841 participants declared not to be hypercholesterolemic, while 60 were not sure. Among them, respectively, 18.1% and 40% were hypercholesterolemic. Subjects not aware of their hypercholesterolemia had significantly higher augmentation index than the aware ones ($p<0.05$). 1226 participants declared not to be hypertriglyceridemic, while 200 were not sure. Among them, respectively, 19.2% and 44% were hypertriglyceridemic. Subjects not aware of their hypertriglyceridemia had significantly higher TG levels aware ones ($p<0.05$), although this seemed to not related to increased arterial stiffness. 1472 participants declared not to be diabetic, while 20 were not sure. Among them, respectively, 2.0% and 25.0% were diabetics. Subjects not aware of their diabetes had significantly higher augmentation index than the aware ones ($p<0.05$).
Conclusions. In conclusion, the lack of awareness of hypertension and hypercholesterolemia is relatively frequent in general population and associated to significantly higher arterial stiffness.

ENDOCAN LEVELS AS MARKER OF CARDIOVASCULAR HEALTH IN INFLAMMATORY BOWEL DISEASE PATIENTS

M. Cinquegrani[1], M. Scolaro[1], F. Savarino[1], M. Scuruchi[2], G. Costantino[1], C. Zito[1], W. Fries[1], A. Saitta[1], J. Rodriguez Carrio[3], G. Squadrito[1], G. Mandraffino[1]
[1]Department of Clinical and Experimental Medicine, Internal Medicine Unit, University of Messina; [2]University f Messina, Department of Biomedical and Dental Sciences and Morphofunctional Images; [3]Area of Immunology, Department of Functional Biology, Faculty of Medicine, University of Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain
E-mail: mariacinquegrani@gmail.com

Background. Inflammatory Bowel Diseases (IBDs) are systemic disorders characterized by increased CV risk; however, CV risk assessment is not always easy, and it is often underestimated mainly in earlier phases of the ill, when CV involvement is generally subclinical. Endocan, a novel biomarker of endothelial dysfunction, plays a key role in endothelial and systemic inflammatory reaction; recently, Endocan was found significantly increased in IBD patients.
Aim. To evaluate novel potential markers of early cardiovascular involvement in patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) IBD before and after treatment with biological drugs (infliximab, adalimumab, vedolizumab). Consistently, circulating CD34+ cell count and Endocan levels were assessed, along with Carotid-femoral Pulse Wave Velocity (cf-PWV), global longitudinal strain (GLS) assessment.
Methods. Clinical and instrumental examination was performed in 27 IBD patients; PWV, GLS, endocan levels and circulating CD34+ cells were evaluated before (T0) and after (T1) a six-months treatment with biological drugs. GLS was measured by speckle tracking echocardiography. Circulating CD34+ were counted by flow cytometry. In addition, inflammatory indices and EF% were also evaluated. Results: At T1, we found a significant reduction of Endocan levels (-22.16%, $p=0.045$), and CRP (-67%, $p<0.005$) as well CD34+ cell count (+6.5%, $p=0.047$) and GLS (+26%, $p<0.001$) were increased. No statistically significant difference as regards ESR, PWV, EF with respect to T0. The interdependence analysis performed on the mean percent changes showed a significant correlation between ΔEndocan and ΔGLS ($r=0.80$), and a trend with ΔCD34+ ($r=-0.31$).
Conclusions. In our study we have shown that biological drugs may improve inflammatory status, clinical compensation and CV risk as suggested by favorable change of CRP, Endocan and CD34+ plasma levels and GLS values. This study is limited by short patients cohort to confirm this preliminary data. Endocan is involved in a variety of biological processes including cell proliferation, migration, and neovascularization. Its levels are closely related to the development and progression of CVD. Patients with IBD have a greater risk of developing CV disease, especially when IBD is clinically uncontrolled.

SERUM IL-18 INCREASE AND INFLAMMOSOME ACTIVATION IN GLUTEAL ADIPOSE TISSUE AFTER PROLONGED BED-REST

R. Colonna[1], E. Dalla Nora[1], J.M. Sanz[1], M.A. Miselli[1], C.M. Semprini[1], G. Zuliani[2], A. Passaro[1]
[1]Department of Medical Sciences, Section of Internal and Cardio Respiratory Medicine, University of Ferrara; [2]Department of Morphology, Surgery, and Experimental Medicine, University of Ferrara
E-mail: clnrsl@unife.it

Introduction. Sedentary lifestyle is an independent risk factor for cardiometabolic disorders, regardless of concomitant physical activity, body weight and composition. Low-grade systemic inflammation is known to be involved in the pathogenesis of cardiometabolic diseases and IL-18 has been recognized as an actor in the development of atherosclerotic plaque. The aim of this study was to investigate the effect of physical inactivity on gluteal adipose tissue gene expression and systemic inflammation markers.
Methods. 23 healthy men underwent 14 days of total physical inactivity, expressed as absolute bed-rest. At basal time and at the end of bed-rest we performed impedance analysis and obtained blood samples and gluteal adipose tissue biopsy for RNA isolation and subsequent microarray analysis (7 subjects).
Results. After bed-rest we observed a significant modification in body composition (reduction of FFM, BCM, muscle mass; increase of FM) and metabolic profile (reduction of total and LDL-cholesterol). We documented a trend to increase of IL-18 levels, and a direct correlation of IL-18 levels with FM and an inverse correlation with FFM, BCM and MM. Moreover, subjects in the upper tertile of IL-18 variation, showed the greatest increase in FM and the greatest reduction in FFM, BMC e MM. Similar results were observed regarding total and LDL-cholesterol, with the greates reduction in subjects belonging to the upper tertile of IL-18 variation. The microarray analysis showed that, among the 342 coding genes differently expressed after bed-rest, there was a significant up regulation of genes belonging to the inflammosome, such as IL-18, IL1β, and NLRP3.
Conclusion. Our data suggest that subcutaneous adipose tissue may be one of the actors playing a role in the detrimental effect of sedentary behaviour, participating to the activation of the inflammatory cascade and contributing to a low-grade systemic inflammation, a known pathophysiological mechanism in atherosclerosis development.

CARDIOVASCULAR RISK STRATIFICATION THROUGH THE EVALUATION OF COMMON CAROTID STIFFNESS BY SPECKLE-TRACKING ULTRASONOGRAPHY

R. Curcio, N. Mojovic, M.R. Mannarino, E. Cosentini, E. Bortone, M. Pirro, V. Bianconi
Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Medicine, University of Perugia
E-mail: curciorosa90@gmail.com

Introduction. Carotid speckle-tracking ultrasonography is emerging as a non-invasive and feasible method for evaluating the multi-directional mechanics of carotid arteries. The association between carotid stiffness and cardiovascular (CV) risk has not been fully elucidated. We investigated the association between left common carotid (lcc) elastic modulus (lccEM) and pulse wave velocity (lccPWV) and two conventional surrogate markers of CV risk, i.e. lcc intima-media thickness (lccIMT) and aortic PWV (aPWV). Also, we explored the association between lccEM and lccPWV and the 10-year risk of fatal CV events.
Materials and Methods. Seventy-eight dyslipidemic patients were enrolled among those who referred to our tertiary care center for the management of lipid disorders. The 10-year risk of fatal CV events was estimated according to the European Atherosclerosis Society (EAS) recommendations. Carotid speckle-tracking ultrasonography was used to assess lccEM and lccPWV, while lccIMT was automatically calculated through a grey-scale analysis of the ultrasound images. The Sphygmo Cor Vx system was used to assess aPWV.
Results. Seventeen (22%), 24 (31%), 21 (27%) and 16 (20%) patients were in the low, moderate, high ad very high risk categories, respectively. LccEM correlated with aPWV (rho=0.58, p<0.001) and lccIMT (rho=0.25, p=0.025), while lccPWV correlated with aPWV (rho=0.35, p=0.002) but not with lccIMT. In a binary logistic regression model including age, lccEM, lccPWV, lccIMT and aPWV as independent variables, age (OR=1.098, CI=1.017-1.185; p=0.017) and lccPWV (OR=1.717, CI=1.053-2.801; p=0.030) were independent predictors of high/very high CV risk. Carotid speckle-tracking ultrasonography might provide additive information for the CV risk stratification in dyslipidemic patients.

PANCREATIC TISSUE SELECTIVE PCSK9 DEFICIENCY IMPACTS BETA CELLS FUNCTION AND INSULIN RELEASE IN MICE MODELS

L. Da Dalt[1], A. Galli[2], C. Perego[2], L. Arnaboldi[2], P. Cappello[3], A.L. Catapano[1,4], G.D. Norata[2]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [3]Dipartimento di Biotecnologie Molecolari e Scienze della Salute, Università degli Studi di Torino; [4]IRCCS Multimedica Hospital, Sesto San Giovanni, Milano
E-mail: lorenzo.dadalt@unimi.it

Background and Aim. Meta-analysis and mendelian randomization studies have showed that PCSK9 or HMGCoA-R loss of function polymorphisms, although being associated with lower LDL-Cholesterol levels result in increased risk of developing new-onset T2D. Experimental studies in animal models confirmed that PCSK9 deficiency results in endocrine pancreas dysfunction with increased beta cells insulin content and impaired functionality.
Aim of thi study was to investigate the molecular mechanisms responsible for this observation. Methods: Pcsk9 KO, WT, albumin (Alb)Cre+/Pcsk9LoxP/LoxP (liver selective Pcsk9-KO mice) and (Pdx1) Cre+/Pcsk9LoxP/LoxP (pancreas selective Pcsk9-KO mice) were used and fed for 20 weeks with chow diet and standard fat diet. GTT, ITT, insulin and C-peptide plasma levels, pancreas morphology, cholesterol and triglycerides accumulation in pancreatic islets were investigated in the different animal models.
Results. Glucose clearance was impaired in PCSK9 KO mice compared to WT. A similar profile was observed in (Pdx1)Cre+/PCSK9fl/fl compared to their controls (Pdx1) Cre-/PCSK9fl/fl but not in (Alb)Cre+/PCSK9fl/fl mice. Insulin response (ITT test) was preserved in the different animal models. A detailed analysis of pancreas morphology revealed no difference in the islets of (Alb) Cre+/PCSK9fl/fl compared to controls, while (Pdx1) Cre+/PCSK-9fl/fl presented increased cholesterol esters accumulation similar to what previously reported for PCSK9 KO mice.
Conclusion/Discussion. PCSK9 impacts cholesterol and fatty acid metabolism in pancreas thus affecting beta cell functionality and insulin secretion. These findings extend our previous observation, showing that pancreatic PCSK9 but not circulating (liver produced) PCSK9 impact beta cells functionality. This supports the observation that available anti-PCSK9 therapies, which target circulating PCSK9, might have a limited impact on glucose metabolism as opposed to statins.

GENDER RELATED DIFFERENCES IN TWO TYPES OF WEIGHT LOSS INTERVENTION

M. D'Abbondanza[1], E.E. Martorelli[1], G. Mondovecchio[1], M.A. Ricci[1], S. De Vuono[1], S. Ministrini[1], E. Nulli Migliola[1], A.R. Roscini[1], D. Siepi[1], M.T. Paganelli[2], G. Lupattelli[1]
[1]Internal Medicine, Department of Medicine, "Santa Maria della Misericordia" Hospital, University of Perugia; [2]Unit of General Surgery, "Santa Maria della Misericordia" Hospital, Perugia
E-mail: marcodabbo@virgilio.it

Background and Aims. The prevalence of obesity is higher in women than men (1). However, only a few studies (2, 3) described gender effect on surgical and non-surgical interventions, with discordant results.The aim of our study was to investigate whether and how gender influences weight loss in two different populations, after a surgical intervention (sleeve gastrectomy) and dietary intervention (Ketogenic diet).
Methods. We enrolled 125 patients with morbid obesity (BMI 40 kg/m^2 or 35 kg/m^2 + comorbidity) eligible to sleeve gastrectomy (group 1) and a group of 40 patients following a ketogenic diet (group 2). We evaluated anthropometric parameters, peripheral blood pressure, biochemical and serum analysis at the enrollment and at twelve months after interventions.
Results. In the whole population there was a female prevalence (70% in group 1 and 61% in group 2); there were no differences in terms of age and blood pressure between gender in both groups. However, males presented increased adiposity (measured as VFA and fat mass) in both groups and a worse glyco-metabolic profile in group 1. Only after ketogenic diet we observed a significant difference between sexes in total weight loss (7.4% in males vs 5.9% in females, $p<0.05$) and excess of body weight loss (15.7% in males vs 12.1% in females, $p<0.05$) in favour of male population.
Conclusion. Male subjects, beyond a higher weight and BMI, had a worse glyco-metabolic pattern. No significant difference between sexes was found in weight loss in group 1 patients; on the contrary, male patients seemed to have a major benefit from ketogenic diet in comparison to female population. Gender differences in weight loss intervention could be determined by several factors: hormonal profiles, different body composition or higher initial weight in males. Further studies are necessary to investigate the determinants of these differences.

References

1. World Health Organization. Obesity and overweight. Fact sheet. 2018. [WWW document]. URL http://www.who.int/mediacentre/factsheets/fs311/en/
2. Williams RL, Wood LG, Collins CE, Callister R. Effectiveness of weight loss interventions--is there a difference between men and women: a systematic review. Obes Rev. 2015 Feb;16:171-86.
3. Robertson C, Avenell A, Boachie C, Stewart F, Archibald D, Douglas F, Hoddinott P, van Teijlingen E, Boyers D. Should weight loss and maintenance programmes be designed differently for men? A systematic review of long-term randomised controlled trials presenting data for men and women: The ROMEO project. Obes Res Clin Pract. 2016; 10: 70-84.

EFFECT OF DIFFERENT AEROBIC EXERCISES (ENDURANCE TRAINING VS HIGH INTENSITY INTERVAL TRAINING) ON LIPID PROFILE AND LIPOPROTEINS SUBFRACTION IN OBESE PEOPLE: A RANDOMIZED CONTROL TRIAL

A. D'Amuri[1], J.M. Sanz[1], F. Vaccari[2], E. Capatti[3], F. Di Vece[3], E. Dalla Nora[3], G. Zuliani[1], S. Lazzer[2], A. Passaro[1]
[1]Department of Medical Science, University of Ferrara; [2]Department of Medicine, University of Udine; [3]Department of Medicine, Azienda Ospedaliero Universitaria di Ferrara
E-mail: dmrndr@unife.it

Introduction. Exercise and diet are the first line treatment to prevent cardiovascular disease because have proved an evident benefit on cardiovascular morbidity and mortality. Guidelines suggests worldwide to engage at least 150 min/week of moderate intensity continuous physical activity (Endurance Training, ET) in apparently healthy people to preserve physical fitness and prevent non communicable diseases and 250-400 min/sett of the same exercise to induce weight loss in obese people (1). However, compliance to those indications is still low, mainly because lack of time. High Intensity Interval Training (HIIT) is characterized by short bouts of high-intensity exercise alternating with periods of low-intensity exercise. It is a feasible, well tolerated (2) and time sparing training that requires up to 40% less time than ET for an iso-energetic session of exercise (3). Moreover, HIIT appear to be similarly effective respect ET in weight loss (4) and body fat reduction (3) and also more efficient in improving cardio-respiratory fitness (5). Nevertheless, how HIIT affect lipid profile is still unclear. We conduce a randomized control trial to compare the effect of ET and HIIT on lipid profile and lipoprotein sub-fraction in obese people.
Methods. Inclusion criteria were age between 18 and 50 years old and body mass index (BMI) ≥30 kg/m^2. Subjects who had cardiovascular, respiratory, neurologic, muscular-skeletal, metabolic and/or endocrine diseases or those who were taking any drugs known to influence energy metabolism were excluded. Finally, thirty-two obese adults (mean age: 39 years, mean BMI: 36 kg/m^2) were randomized to execute 34 iso-energetic (20 KJ per kg of Fat-Free Mass) different exercise sessions in 16 weeks: ET (60% of the initial VO2peak) or HIIT (3-7 repetition of 3 min bouts 100% of VO2 peak interspersed by 1.5 min 50% of VO2peak). Every session includes 10 min of warm up a 5 min of cool down. Before and after intervention we evaluate Total-Cholesterol (Tot-C), Low Density Lipoprotein-Cholesterol (LDL-C), Triglycerides, High Density Lipoprotein-Cholesterol (HDL-C), noHDL-Cholesterol, Tot-C/HDL-C ratio, Apo-B100, Apo-A1. Lipoprotein sub-fraction were assessed by non-denaturized polyacrylamide gel electrophoresis and the Lipoprint system.
Results. Both exercises improved (all $p<0.05$) Tot-C (-17.8 mg/dl), LDL-C (-15.6 mg/dl), noHDL-Cholesterol (-16 mg/dl), Tot-C/HDL-C ratio (-0.22) and Apo-B100 (-7.4 mg/dl) with no intervention effect. There was no variation in Triglycerides, HDL-C or Apo-A1 but percentage of Large-HDL particles increased in both types of exercise (+2.94%) with no significant differences in percentage of Intermediate- and Small-HDL particles. HIIT sessions required about 22% less time than ET sessions (44.3 vs 33.6 min; $p<0.001$).
Conclusion. We observed that HIIT is non inferior to ET in improving lipid profile and might be an useful strategy to increase patient's compliance in practice exercise. Further studies must assess if HIIT improves cardiovascular morbidity and mortality as ET does despite the modest effect on LDL-C and which biological pathways are involved.

References

1 Appropriate Physical Activity Intervention Strategies for Weight Loss and Prevention of Weight Regain for Adults, 2011. American College of Sport Medicine.
2. Türk, Y., et al. High intensity training in obesity: a Meta-analysis. Obesity science & practice. 2017; 3.3: 258-271.
3. Wewege M, et al. The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. Obesity Reviews, 2017; 18.6: 635-646.
4. Jelleyman, Charlotte, et al. The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. Obesity reviews. 2015; 16.11: 942-961.
5. Milanović, Zoran, Goran, Sporiš, Matthew Weston. Effectiveness of high-intensity interval training (HIT) and continuous endurance training for VO 2max improvements: a systematic review and meta-analysis of controlled trials. Sports medicine. 2015; 45.10: 1469-1481.

DIRECT ORAL ANTICOAGULANTS IN LIPOPROTEIN APHERESIS: HANDLE WITH CARE

B. Dal Pino, F. Sbrana, A. Papa, F. Bigazzi, L. Panchetti, M. Pianelli, R. Luciani, T. Sampietro
Lipoapheresis Unit, Reference Center for Diagnosis and Treatment of Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa
E-mail: francesco.sbrana@ftgm.it

Background. Direct oral anticoagulants (DOAC) are used in clinical practice for the stroke prevention in non-valvular atrial fibrillation and in treatment/prevention of thromboembolism. The aim of the study was to the revised the DOAC use in patients affected by Familial Hypercholesterolemia and ischemic heart disease that undergone chronic Lipoprotein-Apheresis (LA).
Methods. In our Center, 7/34 subjects are on oral anticoagulant therapy, in addition to antiplatelet therapy. Data of these patient were revised.
Results. 6/7 subjects are treated for non-valvular atrial fibrillation and the other case for pulmonary thromboembolism. One patient denied his consent to DOAC therapy and continued vitamin K antagonist. Another subject, after few months of therapy with dabigatran 110 mg BD discontinued DOAC because of vasospastic angina, starting again vitamin K antagonist until the symptom's remission. The symptoms were imputable to lower concentration of verapamil secondary to pharmacokinetic interaction between dabigatran and verapamil extended release formulation. A third patient is currently treated with rivaroxaban 15 mg OD, after pulmonary thromboembolism occurred during LA procedure, imputable to recent transcatheter mitral valve repair. During the follow-up the patient had an hospital admission for severe anaemia (Hb 7.7 g/dl) caused by gastrointestinal bleeding, which required blood transfusions.The other four subjects are regularly treated with dabigatran 110mg BD, but despite a correct plasma peak/trough diluted thrombin clotting time, 3/4 subjects reported a significant decrease in haemoglobin levels and the other subject reported two acute coronary syndromes.
Conclusions. The occurrence of 4 episodes of clinically relevant bleeding (Hb decrease ≥2 g/dl in 3 cases and a major bleeding) is a complication that suggest caution. Our experience, although the small patient group, underlines the need to personalize medical treatment, especially in subjects with comorbidities and multiple drug therapy that undergone extracorporeal procedure.

THE TREATMENT OF THE VERY HIGHLY RISK PATIENT IN ITALY: A PARADOXIAL CASE

D. D'Ardes, I. Rossi, F. Vezzani, F. Cipollone, M. Bucci
Centro Ipertensione e Dislipidemie, Università di Chieti
E-mail: mbucci@unich.it

A 26-yo male came to us in 2018 for suspected familial hypercholesterolemia (FH) treated with atorvastatin 80/ezetimibe 10mg. Treatment was quite effective (LDL-C 94-70 mg/dL) but poorly tolerated for myalgia with a slight CPK rise (2x UNL). He also had hyper-Lp(a), stably between 70-90 mg/dL during treatment. Severe atherosclerotic CAD already complicated his hypercholesterolemia: in 2016, had NSTEMI with PTCA stenting on proximal and mean RCA and Cx/MO). His mother (52-yo) also had high LDL-C (=244 during Lovastatin 40 mg); grandfather had hypercholesterolemia complicated by AMI when was 55-yo and died for re-AMI at 59-yo. Her DLS was ≥10 but couldn't be immediately treated with PCSK9-i for the background therapy, not accepted by the AIFA register to access PCSK9-i. Our patient had DLS=6 and LDL-C 70 mg/dL, so he too couldn't start PCSK9-i. In 2018, ESC/EAS Guidelines' recommended LDL-C target respectively <70 for our very-hi-risk patient and <100 mg/dL for his hi-risk mother, but PCSK9-i could have been started only if LDL-C was ≥30 mg/dL higher than recommended targets. We modified both patients' therapy (rosuvastatin/ezetimibe 20/10 for mother; atorvastatin (poorly tolerated) reduced to 40 mg for son) and immediately performed a genetic test on patients, positive for the variant c.2054C >T, p. (Pro685Leu) of LDLR gene in heterozygosis, pathogenetic regarding FH. No variants associated with elevated Lp(a) have been identified.Recently, patients returned for control: mother had LDL-C =135 and started treatment while son still had 96 mg/dL, which didn't allow to start PCSK9-i despite 2019 Guidelines have reduced LDL-C targets (<55 mg/dL) and now our patient has an LDL-C 80% higher than his recommended target! Due to his characteristics (high Lp(a), very-high CV risk), even more paradoxical is that he could access LDL-Apheresis (refused due to logistical problems) which would cost much more than PCSK9-i treatment. In conclusion: the time has probably come for Italian regulatory Agencies to review access rules for treatments that, even if so expensive, in some cases can no longer be ethically inhibited in patients with similar characteristics.

SANGER VALIDATION OF HIGH-THROUGHPUT SEQUENCING IN GENETIC DIAGNOSIS: STILL THE BEST PRACTICE?

R. De Cario[1], E. Sticchi[1], A. Kura[1], S. Suraci[1], S. Galora[1], A. Magi[1], A. Volta[1], R. Marcucci[1], S. Nistri[2], G. Pepe[3], B. Giusti[1]
[1]Department of Experimental and Clinical Medicine, Section of Critical Medical Care and Medical Specialities, University of Florence; [2]Cardiology Service, Centro Medico Strumentale Riabilitativo (CMSR) Veneto Medica, Altavilla Vicentina; [3]Marfan Syndrome and Related Disorders Regional (Tuscany) Referral Center, Careggi Hospital, Florence
E-mail: decariorosy@hotmail.it

Background. Next-generation sequencing (NGS) is crucial in supporting genetic diagnosis and personalized medicine, this leading to Guidelines for Diagnostic NGS by the ESHG society. Factors of different nature might produce false-positive or -negative NGS data; Sanger validation (costly, time consuming and not completely error-free) is still recommended in genetic diagnosis. We reported the analysis of 4 cases of discrepancy between NGS and Sanger sequencing in a cohort of 229 patients admitted to the Advanced Molecular Genetics Laboratory, Careggi Hospital-University of Florence.
Methods. NGS was performed by Illumina MiSeq® and Haloplex/SureSelect protocols targeting 97 connectivopathies genes and 55 familial hypercholesterolemia genes. Variants passing GATK hard filtering method (QD<2.0, FS>60.0, MQ<40.0, MQ Rank Sum<-12.5, Read Pos Rank Sum <-8.0) were Sanger validated.
Results. Four variants in LTBP2 (n=2), COL5A2 (n=1) and TGFB1(n=1) showed a discrepancy between NGS and Sanger (heterozygous/homozygous or viceversa). Concerning one LTPB2 and COL5A2 variants, they were subjected to a second round of Sanger validation with newly designed primers as the previous primers were designed in a sequence carrying polymorphisms; the second validation confirmed the NGS datum. Concerning the other LTBP2 variant, the second pair of primers in a region not affected by polymorphisms didn't solve the discrepancy; the choice of a further primer combination confirmed the NGS call. Regarding TGFB1, the discrepancy was solved by PCR, using the same primers used for the discrepant Sanger sequencing, followed by restriction fragment length polymorphism approach (AluI enzyme), demonstrating the problem was ascribable to difficulties of Sanger sequencing in that region.
Conclusions. Our data confirm the importance to accurately evaluate the presence of polymorphisms when designing Sanger validation primers and indicate, in case of discrepancy, that Sanger sequencing might not be the gold standard. Our data together with those of literature indicating the growing accuracy of NGS technologies/pipelines of variants calling, raise a discussion on the opportunity to update guidelines on the NGS work-flow in genetic diagnosis.

VASCULAR EFFECTS OF CANAGLIFOZIN ON VISCERAL ADIPOSE TISSUE ARTERIOLES FROM OBESE PATIENTS

A. De Stefano[1], F. Schinzari[2], G.S. Sica[3], P. Gentileschi[3], N. Di Daniele[1], C. Cardillo[2], M. Tesauro[1]
[1]Department of Systems Medicine, Tor Vergata University, Rome; [2]Department of Internal Medicine, Catholic University, Rome; [3]Department of Surgery, Tor Vergata University, Rome
E-mail: alessandrodestefano6@virgilio.it

Sodium-glucose cotransporter (SGLT-2) inhibitors have shown to improve cardiovascular outcomes in diabetes. Whether these drugs have also benefits to induce vasodilation in obese vessels is unknown. Aim of the study is to investigate the possible vasoactive properties of the SGLT-2 inhibitor canaglifozin in obese vessels. Visceral adipose tissue (VAT) is obtained from obese patients undergoing bariatric surgery and from control subjects undergoing laparoscopic surgery. Microarteries (150-450 µm) were dissected from each fat sample and mounted on wire myograph (DMT, Aarhus, Denmark). Vessels were pre-contracted by use of U46619 (1 µM) and the endothelium-dependent (bradykinin, BK 0.1 nM to 10 µM) and -independent (sodium nitroprusside, SNP 0.01 µM to 100 µM) relaxation was assessed. Direct vascular reactivity of canaglifozin (0.5 µM to 100 µM) were assessed by exposing the vessels of obese and lean VAT to cumulative concentrations of this drug. The mechanisms underlying canaglifozin-induced relaxation were investigated by pre-incubating microarteries with either the nitric oxide synthase inhibitor L-NAME (100 µM), or the ciclooxigenase inhibitor indomethacin (10 µM), or the hydrogen peroxide scavenger catalase (500 U/ml). Vascular effect of canaglifozin was tested in the vessels with intact and denuded endothelium. Concentration response curves were analyzed by two-way Anova for repeated measures. Endothelium-dependent relaxation is reduced in microarteries of obese VAT compared to lean VAT (P=0.0002). Endothelium-independent relaxation did not show any difference (P=0.166). Canaglifozin elicits relaxation in arterioles from obese and lean VAT (P=0.0005). Canaglifozin-induced relaxation is not modified after pre-incubating microarteries with inhibitors (P=0.667). Any differences were found when vascular effect of canaglifozin was tested in vessels with intact and denuded endothelium (P=0.551). Canaglifozin showed direct vasodilator properties in microarteries of obese patients through endothelium-independent mechanisms. Our findings support the evolving concept of ancillary cardiovascular properties of SGLT-2 inhibitors, even though further studies are needed to elucidate the signaling pathways involved.

STATINS REDUCE MAJOR ADVERSE LIMB EVENTS AND MORTALITY IN PATIENTS WITH PERIPHERAL ARTERY DISEASE

F. Del Sole[1], D. Pastori[1], A. Farcomeni[2], A. Milanese[2], D. Menichelli[1], W.R. Hiatt[3], F. Violi[1]
[1]I Clinica Medica, Atherothrombosis Centre, Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome; [3]Department of Medicine, Division of Cardiology, University of Colorado School of Medicine, Aurora, Colorado, USA
E-mail: francesco.delsole@uniroma1.it

Background. Statins are guideline recommended in patients with peripheral artery disease (PAD) for prevention of cardiovascular events. However, comprehensive meta-data on the impact of statin therapy on major adverse limb events (MALE) in PAD patients have not been reported.
Objectives. To examine the association of statin use with MALE events, all-cause death, and the composite cardiovascular endpoints cardiovascular death and stroke in patients with PAD.
Methods. We performed a systematic review of literature by searching PubMed (via MEDLINE) and Cochrane (CENTRAL) databases for clinical studies addressing the impact of statin therapy on different outcomes. The main outcomes were MALE (various definitions including amputation, graft occlusion/revascularization), all-cause death, composite cardiovascular endpoints, cardiovascular death and stroke.
Results. We included 51 studies with 138,060 PAD patients, of whom 48,459 (35.1%) were treated with statins. The studies included 2 randomized controlled trials, 20 prospective cohorts and 29 retrospective cohorts. Overall, 11,396 MALE events, 21,624 deaths, 4,852 composite cardiovascular endpoints, 4,609 cardiovascular deaths, and 860 strokes were used for the analysis. Statins reduced the incidence of MALE by 30% (pooled Hazard Ratio [HR] 0.702, 95% Confidence Interval [CI] 0.605-0.815) and amputations by 35% (HR 0.654, 95%CI 0.522-0.819), all-cause mortality by 39% (pooled HR 0.608, 95% CI 0.543-0.680), cardiovascular death by 41% (HR 0.594, 95%CI 0.455-0.777), composite cardiovascular endpoints by 34% (pooled HR 0.662, 95% CI 0.591-0.741) and ischemic stroke by 28% (pooled HR 0.718, 95%CI 0.620-0.831). Sex, age, diabetes and length of follow-up were not significant predictors for MALEs and mortality at meta-regression analysis.
Conclusions. Statins reduce the incidence of MALE, all-cause and CV mortality in patients with PAD. In PAD, a high proportion of MALE events and deaths could be prevented by implementing a statin prescription in this patient population.

TREATMENT WITH VOLANESORSEN (VLN) REDUCED TRIGLYCERIDES AND PANCREATITIS IN PATIENTS WITH FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS) AND SEVERE HYPERTRIGLYCERIDEMIA (SHTG) VS PLACEBO: RESULTS OF THE APPROACH AND COMPASS STUDIES

L. D'Erasmo[1], S.D. Freedman[2], A. Gelrud[3], A. Digenio[4], V.J. Alexander[5], A. Hsieh[4], I. Gouni-Berthold[6], E. Bruckert[7], E. Stroes[8], R.S. Geary[5], S.G. Hughes[5], D. Gaudet[9]
[1]Sapienza University of Rome;[2]Beth Israel Deaconess Medical Center, The Pancreas Center, Boston MA, USA; [3]Miami Cancer Institute, Miami, FL, USA; [4]Akcea Therapeutics Inc, Cambridge, MA, USA; [5]Ionis Pharmaceuticals, Carlsbad, CA, USA; [6]Polyclinic for Endocrinology, Diabetes and Preventive Medicine, University of Cologne, Cologne, Germany; [7]Institut E3M et IHU Cardiométabolique (ICAN), Hôpital Pitié-Salpêtrière, Paris, France; [8]Dept. Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; [9]Dept of Medicine, Université de Montréal, Chicoutimi, QC, Canada
E-mail: laura.derasmo@uniroma1.it

Introduction. FCS is a rare genetic disease characterized by severe chylomicronemia, sHTG and consequent risk of potentially fatal recurrent and acute pancreatitis (AP). HTG-induced AP has a more severe course, causing worse outcomes.
Methods. APPROACH randomized 67 FCS patients (66 dosed), fasting TGs ≥750 mg/dL, 1:1 to 52 weeks of weekly VLN (300 mg) or placebo (PBO). COMPASS randomized 114 sHTG patients (113 dosed), fasting TGs ≥500 mg/dL, 2:1 to VLN or PBO weekly for 26 weeks (dosing adjustments allowed at 3 months). Endpoints included % reduction in serum TGs at 13 weeks and TX-emergent pancreatitis.
Results. Combined COMPASS & APPROACH results showed a significant reduction (p=0.0185) in pancreatitis events (1event/1 patient - VLN; 9 events/6 patients - PBO). Patients in APPROACH with ≥2 pancreatitis events in 5 years (pre-randomization) suffered no attacks during study TX-period (p=0.02). In APPROACH, VLN-group (n=33) month 3 TGs decreased by 77% and increased by 18% in PBO-group (n=33) (p<0.0001). In COMPASS, VLN decreased TGs 71% (p<0.0001) (n=75) after 3 months, compared with 1% decrease in PBO (n=38). The most common AE with VLN was injection site reactions (average % injections w/≥1 LCRIS: 12% FCS/24% sHTG). Declines in platelet counts led to 5 early terminations in APPROACH, 2 had platelets <25,000/µl; platelet counts recovered to normal after VLN was stopped. COMPASS had no serious platelet events, but 1 potentially related SAE reported as serum sickness occurred 2 weeks after the last study dose.
Conclusions. VLN treatment reduced TGs and consequent AP risk in FCS and sHTG patients.

VALUES OF SYSTOLIC BLOOD PRESSURE, DIASTOLIC BLOOD PRESSURE, PULSE WAVE ANALYSIS, PULSE WAVE VELOCITY AND ANKLE BRACHIAL INDEX IN HYPERCOLESTEROLEMIC PATIENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA AND NOT AFFECTED BY HYPERCHOLESTEROLEMIA

C. Ferrari, S. D'Addato, A. F.G. Cicero, S. Palmisano, C. Borghi
Università di Bologna, Ospedale Sant'Orsola
E-mail: caterina.ferrari@studio.unibo.it

The present study investigates the correlation between hypercholesterolemia and hypertension, a correlation that has already been widely examined in previous studies (1). It is an observational and prospective case-control study in which hypercholesterolemic subjects were divided into 2 groups:
- an FH group: a case group made up of 18 patients (7 men, 11 women) with familial hypercholesterolemia with an LDLR mutation. These patients were enrolled starting from the LIPIGEN project database (2);
- a NON-FH group: a control group consisting of 36 patients (14 men, 22 women) not affected by familial hypercholesterolemia.

The average age was 44 years ±11 in the FH group and 45 years ±5 in the NON-FH group.The average total cholesterol values were 189±35 mg/dl in the FH group and 194±18 mg/dl in the NON-FH group.The average LDL cholesterol values were 118±30 mg/dl in the FH group and 119±16 mg/dl in the NON-FH group.Patients below the age of 18 and patients treated with antihypertensive drugs were excluded.The study population was tested with Vicorder (Skidmore Medical Ltd, UK) to collect 5 hemodynamic parameters:
- Systolic Blood Pressure (SBP).
- Diastolic Blood Pressure (DBP).
- Augmentation Index (AIx, a predictor of arterial stiffness recorded within the Pulse Wave Analysis or PWA).
- Pulse Wave Velocity (PWV, another predictore of arterial stiffness).
- Ankle Brachial Index (ABI, a predictor of peripheral artery disease).

Subsequently these values were compared between the two groups, searching for any significant differences: SBP: FH group: 125±11 mmHg; NON-FH group: 122±16 mmHgDBP: FH group: 65±9,5 mmHg; NON-FH group: 69±6 mmHg (p=0,043)AIx: FH group: 28±6%; NON-FH group: 24±8% PWV: FH group: 8,1±1,4 m/s; NON-FH group: 8,3±0,7 m/sABI: FH group: 1,11±0,08; NON-FH group: 1,10±0,09 The results showed that the FH group had significant lower values of diastolic blood pressure compared to the NON-FH group (p<0.05). A possible role of the LDLR mutation in influencing this difference is still to be investigated.The comparison of the other hemodynamic parameters did not reveal significant differences between the two groups.

References

1. Borghi C, Urso R, Cicero AF. Renin-angiotensin system at the crossroad of hypertension and hypercholesterolemia. Nutrition, Metabolism and Cardiovascular Diseases. 2017; 27: 115-120.
2. Averna M, Cefalù AB, Casula M, et al. Familial hypercholesterolemia: The Italian Atherosclerosis Society Network (LIPIGEN). Atherosclerosis Supplements. 2017; 29: 11-16.

IMPAIRED HDL CHOLESTEROL EFFLUX CAPACITY IN SUBJECTS WITH METABOLICALLY- BUT NOT GENETICALLY-DRIVEN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

A. Di Costanzo[1], L. D'Erasmo[1], A. Ronca[2], M. Manfredini[3], F. Baratta[1], D. Pastori[1], L. Polito[1], M. Di Martino[4], F. Angelico[5], M. Del Ben[1], E. Favari[2], M. Arca[1]
[1]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [2]Department of Food and Drug, University of Parma, Parco Area delle Scienze, Parma; [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze, Parma; [4]Department of Radiological Sciences, Sapienza University of Rome; [5]Department of Public Health and Infectious Disease, Sapienza University of Rome
E-mail: alessia.dico@gmail.com

Background and Aims. NAFLD is a common multi-factorial disease characterized by increased hepatic fat. Although NAFLD associates with increased risk of atherosclerotic cardiovascular disease, we speculate that this risk could differ in the different NAFLD subtypes, e.g. metabolically or genetically-driven. Recently, by comparing subjects with NAFLD due to metabolic disturbances to those with genetic NAFLD due to rs738409 PNPLA3 MM genotype, we found that the burden of carotid intima-media thickness was higher in metabolically but not in genetically-driven NAFLD. A mechanistic explanation of this difference is not available. Cholesterol efflux capacity (CEC) and cholesterol loading capacity (CLC), represent a key metric of high-density lipoprotein (HDL) function able to predict atherosclerotic cardiovascular disease (CVD). Therefore, this project was designed to test whether CEC and CLC are altered in metabolically- but not in genetically-determined NAFLD.
Methods. CEC and CLC were measured in three groups:
1) 19 individuals with hepatic steatosis due to metabolic disturbances and wild-type PNPLA3 genotype (metabolic NAFLD - group M);
2) 10 patients with NAFLD associated with the rs738409 PNPLA3 GG genotype (Genetic NAFLD - group G);
3) 10 healthy blood donors, PNPLA3 wild-type and without NAFLD (Controls). Hepatic fat fraction (HFF) was measured using magnetic resonance (MRS/MRI).

Metabolic syndrome (MetS) was defined using the NCEP ATP III definition, with abnormal values for at least 3 of the 5 criteria. HDL CEC was evaluated by radio isotopic techniques, plasma CLC was measured by a fluorimetric assay.
Results. NAFLD cases and controls did not differ with respect to age and sex because they were matched on these criteria. Compared with group G, group M cases had increased adiposity indices, dyslipidemia, insulin resistance and elevated ALT levels (all P<0.05). As expected, group G and controls showed similar anthropometric and metabolic characteristics. Compared with group G, HDL CEC of group M was impaired both as a whole (average reduction of 19%; P<0.0001) and with respect to specific membrane cholesterol transporters (-14.2% for aqueous diffusion; P<0.001; -25% for ABCA1-efflux; P<0.001; -16.3% for ABCG1-efflux; P=0.001; -15.4% for SRBI-efflux P=0.04). No difference in total CEC was found between group G and controls (P=0.82). When the participants in group M were further categorized according to the presence and absence of MetS diagnosis, a steady decrease of total CEC was observed from cases of group G to individuals in group M/MetS- to individuals in group M / MetS+, with the greater reduction of total CEC in the latter group (P for trend =0.001). As total CEC values positively correlated with HDL-C levels in our cohort (P=0.004), we further distributed HDL-C values according to quartiles, showing that plasma isolated from group M individuals promoted significantly lower total efflux from macrophages compared to group G cases with similar HDL-C levels (all P<0.025). To study the overall serum proatherogenic potential, we further evaluated CLC in NAFLD patients and controls. Plasma-induced cholesterol content increase resulted significantly higher in group G compared to group M patients (35.4±2.0 µg/ml vs. 30.0±3.2 µg/ml, P<0.001). After adjustment for BMI, HDL-C, HOMA-IR and ALT levels or HFF%, mean Total CEC and CLC values in group G were significantly higher than those of group M (all Padj <1x10-7).
Conclusions. Our data suggest that metabolically, but not genetically driven NAFLD may be associated with dysfunctional high-density lipoproteins (HDLs). These data led us to concentrate our attention on the functionality of HDL as a potential link between metabolic driven NAFLD and subclinical atherosclerosis.

ASSOCIATION BETWEEN CARDIAC NATRIURETIC PEPTIDES AND LIPID PROFILE: A SYSTEMATIC REVIEW AND META-ANALYSIS

C. Di Pentima[1], F. Spannella[1], F. Giulietti[1], M. Bordicchia[2], R. Sarzani[1]
[1]Internal Medicine and Geriatrics, IRCCS INRCA, Ancona; [2]Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona
E-mail: chiara.dipentima@live.it

Objective. Cardiac natriuretic peptides (NPs) play a fundamental role in maintaining cardiovascular (CV) and renal homeostasis. Moreover, they also affect glucose and lipid metabolism. We performed a systematic review and meta-analysis of studies investigating the association of NPs with serum lipid profile.
Methods. A PubMed and Scopus search (2005-2018) revealed 45 studies reporting the association between NPs and components of lipid profile [total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc) and triglycerides (TG)].
Results. Despite high inconsistency across studies, NPs levels were inversely associated with TC [pooled r=-0.09; I2=90.84%], LDLc [pooled r=-0.09; I2=79.74%] and TG [pooled r=-0.11; I2=94.14%], while they were directly associated with HDLc [pooled r=0.06; I2=87.85%]. The relationship with LDLc, HDLc and TG lost significance if only studies on special populations (works including subjects with relevant acute or chronic conditions that could have significantly affected the circulating levels of NPs or lipid profile) or low-quality studies (NOS<5) were taken into account.
Conclusions. The present study highlights an association between higher NP levels and a favorable lipid profile. This both confirms and extends our understanding of the metabolic properties of cardiac NPs and their potential in CV prevention.

ACETYLSALICYDIC ACID PREVENTS THE PRO-CALCIFIC DIFFERENTIATION OF VALVULAR INTERSTITIAL CELLS THROUGH THE PRODUCTION OF ANTI-INFLAMMATORY LIPOXINS

M. Donato[1], E. Bertacco[2], E. Faggin[1], A. Sala[3], C. Bolego[1], M. Puato[2], G. Arrigoni[4], C. Agostini[2], P. Pauletto[2], M. Rattazzi[2]
[1]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova; [2]Dipartimento di Medicina, Università degli Studi di Padova; [3]Università degli Studi di Milano-Bicocca, Milano; [4]Dipartimento di Scienze Biomediche, Università degli Studi di Padova
E-mail: maristella.donato@studenti.unipd.it

Introduction. Calcific aortic valve disease (CAVD) is the third most common cardiovascular disease in the western world. To date, no pharmacological therapy has proven to prevent or slow down the progression of CAVD.
Aim. To study the effect of acetylsalicylic acid (ASA) and ASA-triggered lipoxins (ATL) on interstitial valve cells calcification (VIC).
Methods. A clone of VIC able to acquire a pro-calcific phenotype was treated with endotoxin (LPS, 500 ng/ml) to induce the calcification and with ASA at two different concentrations (1 mM e 10 mM). Alkaline phosphatase (ALP) activity and calcium deposition were determined through colorimetric assays. Proteins and RNA were extracted from VIC to perform western blotting (WB) and RT-PCR analyses. A RP-HPLC analysis was conducted on the culture medium to assess the production of arachidonic acid metabolites and ATL (such as PGE2, 15HETE, LTB4 e 15-epi-LXA4). Lastly, immunohistochemical analysis was performed on pathological valves to investigate the expression of FPR2 (ATL receptor).
Results. The treatment of VIC with ASA 10 mM reduced ALP activity ($p<0.001$), calcium deposition ($p<0.05$) and the expression of pro-inflammatory cytokines (IL-6, TNF-α and IL-1β, $p<0.001$), induced by LPS. Moreover, the cells treated with ASA showed an increased production of ATL, such as 15-HETE and 15-epi-LXA4 ($p<0.001$). We also observed that FPR2, the main ATL receptor, was expressed within human pathological valves and was increased in VIC treated with LPS. Finally, the in vitro studies showed a significant inhibition of ALP activity in VIC treated with MMK1 (50 µM), a selective agonist for FPR2.
Conclusion. The treatment with ASA inhibits the pro-calcific differentiation of VIC and this effect might be partly due to the action of ATL. The anti-calcific effect of FPR2 activation can offer the opportunity to develop novel therapeutic strategies for CAVD.

EFFICACY AND SAFETY OF BEMPEDOIC ACID: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PHASE 2 AND 3 RANDOMIZED CONTROLLED TRIALS

F. Fogacci[1], M. Banach[2], A.V. Hernandez[3], A.F.G. Cicero[1]
[1]University of Bologna; [2]Medical University of Lodz, Lodz, Poland; [3]University of Connecticut/Hartford Hospital Evidence-based Practice Center, Hartford, CT, USA
E-mail: federicafogacci@gmail.com

Background. Bempedoic acid (ETC-1002) is a first-in-class, small-molecule inhibitor of ATP-citrate lyase (ACLY), a key enzyme that supplies substrate for cholesterol and fatty acid synthesis.
Objectives. To assess the efficacy and safety of bempedoic acid through a systematic review and meta-analysis of phase 2 and phase 3 randomized controlled trials (RCTs).
Methods. A systematic literature search in SCOPUS, PubMed-Medline, Web of Science and Google Scholar databases was conducted up to August 5th, 2019, in order to identify RCTs assessing the efficacy of bempedoic acid on selected biochemical parameters and safety outcomes. Effect sizes for changes in lipids and high-sensitivity C-reactive protein (hsCRP) serum concentration were expressed as mean differences (MD) and 95% confidence intervals (CI). For safety analyses, odds ratios (OR) and 95%CI were calculated using the Mantel-Haenszel method.
Results. We included 10 RCTs (n=3786) comprising 26 arms (active arm [n=2460]; control arm [n=1326]. Bempedoic acid significantly reduced total cholesterol (TC) [MD: -14.94%, 95%CI: -17.31, -12.57, $p<0.001$], non high-density lipoprotein cholesterol (non HDL-C) [MD: -18.17%, 95%CI: -21.14, -15.19, $p<0.001$], LDL-C [MD: -22.94%, 95%CI: -26.63, -19.25, $p<0.001$], LDL particle number [MD: -20.67%, 95%CI: -23.84, -17.48, $p<0.001$], apolipoprotein B (Apo B) [MD: -15.18%, 95%CI: -17.41, -12.95, $p<0.001$], HDL-C [MD: -5.83%, 95%CI: -6.14, -5.52, $p<0.001$], HDL particle number [MD: -3.21%, 95%CI: -6.40, -0.02, $p=0.049$], and hsCRP [MD: -27.03%, 95%CI: -31.42, -22.64, $p<0.001$]. Bempedoic acid did not modify triglycerides (TG) level [MD: -1.51%, 95%CI: -3.75, 0.74, $p=0.189$], very-low-density lipoprotein (VLDL) particle number [MD: 3.79%, 95%CI: -9.81, 17.39, $p=0.585$] and Apo A-1 [MD: -1.83, 95%CI: -5.23, 1.56, $p=0.290$]. Treatment with bempedoic acid was positively associated with an increased risk of discontinuation to treatment (OR: 1.37, 95%CI: 1.06, 1.76, $p<0.05$), serum uric acid (OR= 3.55, 95%CI: 1.03, 12.27, $p<0.05$), liver enzymes (OR=4.28, 95%CI: 1.34,13.71, $p<0.05$), and creatine kinase elevations (OR: 3.79, 95%CI: 1.06, 13.51, $p<0.05$), but was associated with a decreased risk of new onset or worsening diabetes (OR: 0.59, 95%CI: 0.39, 0.90, $p=0.01$).
Conclusions. Bempedoic acid has favourable effects on lipid profile and hsCRP levels. Further well-designed studies are needed to explore its longer-term safety.

EFFICACY AND SAFETY OF VOLANESORSEN (ISIS 304801): A SYSTEMATIC REVIEW AND META-ANALYSIS OF PHASE 2 AND 3 CLINICAL STUDIES

F. Fogacci[1], G.D. Norata[2], P. Toth[3], M. Arca[4], A.F.G. Cicero[1]
[1]Medical and Surgical Sciences Department, University of Bologna; [2]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [3]The Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins School of Medicine, Baltimore, MD, USA; [4]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome
E-mail: federicafogacci@gmail.com

Aim. To assess the efficacy and safety of volanesorsen (ISIS 304801) through a systematic review of the literature and a meta-analysis of the available phase 2 and phase 3 clinical studies.
Methods. A systematic literature search in SCOPUS, PubMed-Medline, ISI Web of Science and Google Scholar databases was conducted up to August 13th, 2019, in order to identify clinical trials assessing the effect of volanesorsen on laboratory parameters and its safety profile. Effect sizes for changes in lipids were expressed as percentage mean differences (MD) and 95% confidence intervals (CI). For safety analysis, odd ratios (OR) and 95%CI were calculated using the Mantel-Haenszel method.
Results. Data were pooled from 3 clinical studies comprising 11 arms, which included overall 156 subjects, with 95 in the active-treated arm and 61 in the placebo one. Meta-analysis of data suggested that volanesorsen significantly affected plasma levels of triglycerides (TG) [MD-67.90%, 95%CI: -85.32,-50.48, P<0.001], high-density lipoprotein cholesterol (HDL-C) [MD=40.06%, 95%CI: 32.79,47.34, P<0.001], very-low-density lipoprotein cholesterol (VLDL-C) [MD-72.90%, 95%CI: -82.73,-63.07, P<0.001], apolipoprotein B (Apo B) [MD=8%, 95%CI: 2.17,13.84, P=0.007], Apo B-48 [MD=-64.63, 95%CI: -105.37,-23.88, P=0.002], Apo C-III [MD=-74.83%, 95%CI: -85.93,-63.73, P<0.001], and VLDL ApoCIII [MD=-83.69%, 95%CI: -94.08,-73.29, P<0.001], without significantly impacting plasma total cholesterol (TC) [MD=-0.65%, 95%CI: -10.70,9.40, P=0.900], non HDL-C [MD=-18.89%, 95%CI: -40.96,3.19, P=0.094], and LDL-C [MD=47.01%, 95%CI: -1.31,95.33, P=0.057] levels.Treatment with volanesorsen was positively associated with a higher risk of injection site-reaction (OR=32.89, 95%CI: 7.97,135,74, P<0.001) and with an increased risk of upper respiratory tract infections (OR=10.58, 95%CI: 1.23,90.93, P<0.05).
Conclusion. Volanesorsen has favourable effects on lipid profile. Further well-designed studies are needed to explore its longer-term safety.

IMPACT OF A SHORT-TERM SYMBIOTIC SUPPLEMENTATION ON METABOLIC SYNDROME AND SYSTEMIC INFLAMMATION IN ELDERLY PATIENTS: A RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL

F. Fogacci, A.F.G. Cicero, M. Bove, M. Giovannini, C. Borghi
University of Bologna
E-mail: federicafogacci@gmail.com

Purpose. The connection between gut microbiota imbalance, inflammation and its role in the pathogenesis of metabolic syndrome (MetS) clustering factors has been increasingly recognized. However, data on the efficacy of probiotics supplementation on MetS components are few and almost lacking in the elderly. To address this issue, we conducted a randomized, double-blind, placebo-controlled, parallel-group, clinical study on a large sample of MetS elderly patients.
Methods. After 14 days of diet and physical activity standardization, 60 elderly patients were randomized to treatment with a symbiotic formula of Lactobacillus plantarum PBS067, Lactobacillus acidophilus PBS066 and Lactobacillus reuteri PBS072 with active prebiotics or placebo. Patients were evaluated anamnestically and by the execution of a physical examination and laboratory and haemodynamic analyses at the baseline and after 60 days of treatment. At enrollment and at the end of the trial, all enrolled patients complete the EuroQol- 5 Dimension (EQ-5D) questionnaire.
Results. Through the 2-months period of treatment, patients who received active treatment experienced a statistically significant improvement in waist circumference and in fasting plasma insulin, total cholesterol, high-density lipoprotein cholesterol, non-HDL-C, triglycerides (TG), low-density lipoprotein cholesterol, high sensitivity C-reactive protein and tumor necrosis factor alpha serum levels, compared both to the baseline and the control group. Visceral adiposity index improvement in the symbiotic treatment group was significantly greater than in placebo group. Compared to baseline, treatment with symbiotics also significantly reduced mean arterial pressure and fasting plasma glucose.All treatment groups demonstrated a significant decrease in TG. TG reduction in the symbiotic group was significantly greater than in the control group.The EQ-5D VAS questionnaire significantly improved only in probiotics-treated subjects.
Conclusion. Treatment with a symbiotic formula of Lactobacillus plantarum PBS067, Lactobacillus acidophilus PBS066 and Lactobacillus reuteri PBS072 with active prebiotics decreased MetS syndrome prevalence, several cardiovascular risk factors and markers of insulin resistance in elderly patients.

GENDER DIFFERENCE IN FAMILIAL CHYLOMICRONAEMIA SYNDROME

F. Forte[1], I. Calcaterra[1], G. Iannuzzo[1], A. Buonaiuto[1], M. Gentile[1], M.D. Di Taranto[2], C. Giacobbe[2], M.N.D. Di Minno[3], P. Rubba[1]
[1]Department of Clinical Medicine and Surgery, Federico II University, Naples; [2]Department of Molecular Medicine e Medical Biotechnologies, Federico II University, Naples; [3]Department of Translational Medical Sciences, Federico II University, Naples
E-mail: dr.fortefrancesco@gmail.com

Introduction. Familial Chylomicronaemia Syndrome (FCS) is a subgroup of severe hypertri-glyceridemia disease, characterized by fasting high levels of chylomicrons associated with an in-creased risk of life-threatening pancreatitis. FCS is caused by homozygous or compound heterozy-gous mutations in the gene encoding: lipoprotein lipase (LPL), ApoC-II, ApoA-V, LMF1, GPI-HBP1 and GPD1.
Objective. The aim of our study is to evaluate difference in clinical picture of FCS and in re-sponse to therapy stratifying population according to gender and genotype.
Methods. we evaluated: maximum Triglycerides (maxTG), Triglycerides post-therapy (POST-TG), number of pancreatitis, presence of chronic abdominal pain in consecutive patients with FCS.
Results. Thirteen patients [8 males and 5 females, mean age 36±15 years] with FCS were included in the analysis. All patients followed a low-fat diet, 10 received fibrates+fish oils and 3 fish oils alone. We performed genetic test to assess causative mutation of FCS. We found: homozygous LPL gene muta-tion in 50% of males and 50% of females, compound heterozygous LMF1 gene mutations in 25% of males, heterozygous status in APOA-V gene in 13% of males and heterozygous status in LPL gene in 20% of females. No deleterious variants in the aforementioned genes were identified in 13% of males and 20% of females but their phenotype is similar to FCS. Despite, there were no difference in maxTG between female and males (p=0.114), the TG % reduction was significantly higher in males than in females (49% vs 5%, p=0.044). A history of pancreatitis was reported by 80% of females and 37.5% of males, with chronic abdominal pain being reported by 40% of females and 25% of males.
Conclusions. Despite maxTG and genetic profile were similar between males and females, the TG% reduction was higher in males proving less response to therapy in women.

PREVALENCE OF POTENTIALLY INAPPROPRIATE MEDICATIONS ASSOCIATED WITH RISK OF MAJOR ADVERSE CARDIOVASCULAR EVENTS IN THE ELDERLY

F. Galimberti[1], E. Olmastroni[1], M. Casula[2], E. Tragni[1], A.L. Catapano[2]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; IRCCS MultiMedica, Sesto S. Giovanni, Milano
E-mail: federica.galimberti@unimi.it

Background. Cardiovascular diseases are among the leading causes of death worldwide and are an important cause of morbidity in the elderly. As a result of inappropriate prescribing, this population is more prone to drug related problems, including adverse drug reactions. Therefore, it is crucial to identify potentially inappropriate medications (PIMs) with risk of cardiovascular adverse events in this population.
Aim. We aimed to evaluate the prevalence of PIMs associated with risk of major adverse cardiovascular events (MACE) using Regional administrative databases of the outpatient drug prescriptions (reimbursable by the National Health Service) in 2014, 2015 and 2016.
Methods. The study population was composed by all patients aged ≥65 years followed by a general practitioner of eight local health units (LHUs) in Lombardy (Bergamo, Lecco, Mantova, Monza-Brianza) and Campania (Avellino, Caserta, Napoli-1, Napoli-2). We estimated the prevalence of older patients exposed to selected classes of PIMs to be avoided in the elderly due to their cardiovascular risk, based on drug lists already published in the literature: NSAIDs, antipsychotics, calcium channel blockers with mainly vascular effects, and antiarrhythmics (Class I and III).
Results. For LHUs in Lombardy, the prevalence of PIMs with MACE risk ranged from 34% to 44%. For LHUs in Campania, these values were about two times higher (between 59% and 75%). NSAIDs was the most prescribed class (in 2016, the prevalence ranged between 40.8-44.5% in Lombardy and between 55.9-59.9% in Campania), followed by selective calcium channel blockers (in 2016, 41.2-46.0% in Lombardy and 29.9-33.3% in Campania).
Conclusions. These results show that the prescription of PIMs with MACE risk in older patients is widespread in our national setting, with some remarkable geographical differences. It is therefore necessary to implement local strategies and future interventions to enhance safe prescribing practices in elderly patients.

APOLIPOPROTEIN B AND CARDIOVASCULAR DISEASE RISK: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

F. Galimberti[1], A.L. Catapano[2], J.L. Katzmann[3], A.J. Cupido[4], A.C. Keech[5], A.D. Snidermann[6], B.A. Ference[7]
[1]MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, IRCCS MultiMedica, Milano; [3]MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; Department of Cardiology, University Hospital Leipzig, Leipzig, Germany; [4]MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; Amsterdam UMC, University of Amsterdam, Department of Vascular Medicine, Meibergdreef 9, Amsterdam, Netherlands; [5]National Health and Medical Research Council Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia; [6]McGill University Health Centre, Royal Victoria Hospital, 1001 Decarie Boulevard, Montreal, Quebec, H4A 3J1, Canada; [7]MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
E-mail: federica.galimberti@unimi.it

Background and Aim. Several novel therapies that potently reduce plasma triglyceride levels by targeting the lipoprotein lipase (LPL) pathway are currently in development. Recent evidence from Mendelian randomization studies suggest that lowering plasma triglyceride levels through the LPL pathway and lowering low-density lipoprotein (LDL)-cholesterol through the LDL receptor pathway are associated with very similar proportional reductions in the risk of cardiovascular (CV) events for the same reduction in apolipoprotein B (apoB) containing lipoproteins. This finding implies that the clinical benefit of any lipid-lowering therapy should be proportional to the absolute change in apoB. Therefore, we sought to compare the clinical benefit of different classes of lipid-lowering therapies per unit reduction in apoB, and to estimate the magnitude of the expected clinical benefit of lowering apoB for each class of therapy to inform the design of future randomized trials evaluating novel lipid-lowering therapies.
Methods. We conducted a study-level meta-analysis of randomized trials evaluating 6 different classes of lipid-lowering therapies (statins, ezetimibe, PCSK9-inhibitors, CETP-inhibitors, niacin, and fibrates). We included all randomized trials that reported apoB levels for the entire study population, enrolled at least 1000 participants, had a least 1-year median follow-up and were designed to evaluate CV outcomes. The primary outcome for this meta-analysis was major CV events - defined as the first occurrence of CV death, non-fatal myocardial infarction, stroke or coronary revascularization. We estimated the relative risk (RR) of major CV events standardized for a 30 mg/dl reduction in apoB using fixed effect inverse-variance weighted meta-analysis (adjusted for study duration) separately for each class of therapy and in a combined analysis including all classes of lipid-lowering therapies. Heterogeneity of the observed clinical benefit per unit reduction in apoB between the different classes of lipid-lowering therapies was measured with the I2 statistic.
Results. A total of 25 trials that enrolled 285,241 participants (mean age: 63.3 years; female sex: 24.7%) who experienced 40,244 first major CV events were included in the analysis. Among the included trials, the mean baseline LDL-cholesterol level was 100.7 mg/dL and the mean baseline apoB level was 93.9 mg/dL. The mean absolute difference in apoB between the treatment and comparison groups at one year was 24.1 mg/dL. Among all included trials, the overall RR per 30 mg/dL reduction in apoB levels was 0.79 (95% confidence intervals (CI) 0.77-0.81) for major CV events. Examining separately the components of the composite endpoint, the risk of non- fatal myocardial infarction was reduced by 24% (RR 0.76, 95% CI 0.73-0.79), the risk of coronary revascularization and of stroke by 21% (RR 0.79, 95% CI 0.76-0.81 and RR 0.79, 95% CI 0.74-0.83, respectively), and the risk of CV death by 13% (RR 0.87, 95% CI 0.82-0.92). There was no significant heterogeneity in the clinical benefit per 30 mg/dl reduction in apoB among the six different lipid-lowering therapies, either for the primary composite outcome or for any of the individual components of the primary composite outcome.
Conclusions. Statins, ezetimibe, PCSK9-inhibitors, CETP-inhibitors, niacin, and fibrates are all associated with very similar reductions in the risk of major CV events per unit change in apoB, suggesting that the clinical benefit of all lipid-lowering therapies may be proportional to the achieved absolute reduction in apoB, regardless of the observed changes in other lipids. This study also demonstrates that each 30 mg/dl absolute reduction in plasma apoB concentration is associated with an approximately 20% reduction in the risk of major CV events. These finding can be used to inform the design of randomized trials evaluating novel lipid-lowering therapies.

INTIMA MEDIA THICKNESS AND ACHILLE'S TENDON IN FH CHILDREN

A. Giammanco, C.M. Barbagallo, M. Trevisin, A.M. Cardella, R. Spina, C. Scrimali, F. Brucato, G.I. Altieri, F. Fayer, V. Ingrassia, V. Valenti
Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" - ProMISE - University of Palermo
E-mail: agiamman@gmail.com

Background. Familial hypercholesterolemia (FH) is an autosomal dominant inherited metabolic disorder characterized by high low-density lipoprotein cholesterol (LDL-C) levels from birth and premature coronary heart disease (CHD). Lifetime exposure to LDL-C correlates with increased risk of cardiovascular disease. Although children with heterozygous FH rarely present with identifiable clinical features, carotid ultrasound and the measure of carotid intima media thickness (cIMT) is useful tool for the evaluation of development of atherosclerosis through childhood.
Objective and Methods. We evaluated the effect of the exposure to LDL-C plasma levels on carotid intima media thickness (cIMT) and Achille's tendon thickness (ATT) in a group of FH children (mean age 10.9±4.9) at the Lipid Clinic in Palermo. Non-FH subjects matched for age and gender were enrolled as controls. Anthropometric measures, clinical and biochemical parameters, life style (physical activity), cIMT and ATT were recorded.
Results. A total of 38 subjects (n=20 FH and n=18 controls) were enrolled. ADH subjects were clinically and genetically characterized. Only n=10 FH subjects were on standard lipid lowering therapy (LLT) with statins and/or ezetimibe. Although no differences were found in terms of cIMT and ATT between the two groups, in FH children LDL cholesterol, BMI and age correlated to cIMT.
Conclusions. The finding of our study supports the view that atherosclerosis starts early in life and justifies treatment also beginning early in life.

THE LIPIGEN PAEDIATRIC GROUP: CHARACTERIZATION OF CHILDREN AND ADOLESCENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA IN ITALY

M. Gazzotti[1], M. Casula[2], A.L. Catapano[2], E. Olmastroni[1], F. Bonaiti[1], M.E. Capra[3], C. Pederiva[4]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, IRCCS MultiMedica, Sesto S. Giovanni, Milano; [3]Centro Dislipidemie in Età Evolutiva U.O. Pediatria e Neonatologia, Ospedale G. da Saliceto, Piacenza; [4]U.O. Clinica Pediatrica, Servizio Clinico Dislipidemie per lo Studio e la Prevenzione dell'Aterosclerosi in Età Pediatrica, Ospedale San Paolo, Milano
E-mail: marta.gazzotti@unimi.it

Background. Familial hypercholesterolemia (FH) is a common genetic disorder characterized by elevated LDL cholesterol (LDL-c) concentrations from birth, predisposing to early atherosclerotic lesions and premature coronary heart disease (CHD). Early detection and treatment in childhood or adolescence are crucial to achieve a normal life expectancy.
Aim and Methods. In 2018, the LIPIGEN paediatric group was constituted within the LIPIGEN Network, to improve detection, diagnosis, and management of paediatric FH patients. In this preliminary analysis, we selected LIPIGEN patients <18 years to evaluate clinical characteristics, biochemical parameters, and genetic profile.
Results. The analyses were carried out on 1200 LIPIGEN patients (47.3% males) with valid data. At diagnostic visit, the study population was composed by 16.6% of subjects with age 0-5 years, 41.7% with 6-10 years, 24.7% with 11-13 years, and 17.0% with 14-17 years. The mean age at diagnosis was 9.0±3.7 years. Excluding subjects with missing data, the family history of early CHD was positive in 12.5% of cases, while the prevalence of LDL-c >190 mg/dL among first-degree family members was 68.1%. The mean LDL-c levels among untreated subjects (N=1116), stratified by the four age classes, were 205.5±77.5, 175.9±71.7, 166.8±59.5, and 192.4±59.2 mg/dL, respectively. Among the subjects with genetic testing (N=1023), 53.6% had a positive genetic diagnosis of FH: 95.8% heterozygous for mutations on LDL receptor gene (LDL-R) and one homozygous subject (2 years old with LDL-c 877 mg/dL).
Conclusion. This preliminary analysis offers a general view of all paediatric data collected until now in the LIPIGEN study, providing a characterization of the clinical and genetic features of paediatric FH. This evidence sets the stage to plan an in depth data collection to better understand the specific diagnostic approach required for paediatric patients.

*Heads of centers of LIPIGEN paediatric group: Francesco Angelico (Roma), Marcello Arca (Roma), Maurizio Averna (Palermo), Andrea Bartuli (Roma), Giacomo Biasucci (Piacenza), Claudio Borghi (Bologna), Paolo Calabrò (Napoli), Francesca Carubbi (Modena), Francesco Cipollone (Chieti), Nadia Citroni (Trento), Giuliana Fortunato (Napoli), Ornella Guardamagna (Torino), Arcangelo Iannuzzi (Napoli), Lorenzo Iughetti (Modena), Graziana Lupattelli (Perugia), Giuseppe Mandraffino (Messina), Lorenzo Maroni (Varese), Giuliana Mombelli (Milano), Sandro Muntoni (Cagliari), Cristina Pederiva (Milano), Fabio Pellegatta (Sesto San Giovanni (MI)), Livia Pisciotta (Genova), Arturo Pujia (Catanzaro), Francesco Purrello (Catania), Paolo Rubba (Napoli), Carlo Sabbà (Bari), Riccardo Sarzani (Ancona), Giovanni Battista Vigna (Ferrara), Josè Pablo Werba (Milano), Sabina Zambon (Padova), Maria Grazia Zenti (Verona)LIPIGEN Steering Committee: Marcello Arca (Roma), Maurizio Averna (Palermo), Stefano Bertolini (Genova), Sebastiano Calandra (Modena), Alberico L. Catapano (Milano), Patrizia Tarugi (Modena) Centralized laboratory: Veronica Zampoleri (Cinisello Balsamo (MI))

APPLICATION OF A NEXT-GENERATION-SEQUENCING PANEL FOR THE MOLECULAR DIAGNOSIS OF DYSLIPIDEMIAS

C. Giacobbe, D. Palma, M.D. Di Taranto, G. Maione, G. Cardiero, G. Fortunato
Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II Naples, and CEINGE S.C.a r.l. Advanced Biotechnology, Naples
E-mail: giacobbe@ceinge.unina.it

Introduction. Dyslipidemias are common clinical conditions involved in cardiovascular diseases. Dyslipidemias have often a strong genetic component, even if secondary factors play a key role in a clinical expression. The aim of this study was to evaluated the usefulness of a wide next-generation-sequencing panel of 28 genes for molecular diagnosis of Familial Hypercholesterolemia (FH) and Severe Hypertriglyceridemia (HTG).
Materials and Methods. We preliminary reported data about analysis of 26 patients with different clinical suspect: FH (n=21) and HTG (n=5). These patients were previously analyzed with Sanger sequencing researching pathogenic variants in candidate genes. MLPA was also performed for LDLR and LPL genes in FH and HTG patients respectively. Among FH and HTG, we selected patients carring different type of variants in different genes (single nucleotide variants (SNV) and copy number variations (CNV)). DNA libraries were prepared using Agilent SureSelect target enrichment, and the sequencing was performed using Illumina MiSeq Reagent Micro Kit V2. Sequencing results were analyzed using Agilent SureCall and Agilent Alissa Align&Call and Intrepret.
Results. The average read depth was >200X. All previously identified SNV and CNV variants were confirmed by NGS and 8/21 FH patients and 4/5 HTG patients were positive for pathogenic variants. No additional pathogenic variants in the causative genes were found in patients negative at previous screening. However, in all patients rare variants, in genes not previously analyzed, were found. In some cases, these additional variants were never associated with dyslipidemias and their pathogenicity evaluation is still in progress.
Conclusions. Our results confirm that this NGS-based method is able to detect different type of variants, including CNV. Moreover the simultaneous analysis of many genes involved in lipid metabolism, could be useful to better understand the genetic basis of dyslipidemias and to explain the presence of complex phenotypes.

LIPA GENE MUTATIONS AFFECT THE COMPOSITION OF LIPOPROTEINS: ENRICHMENT IN ACAT-DERIVED CHOLESTERYL ESTERS

M. Gomaraschi[1], L. Arnaboldi[2], A. Ossoli[1], E. Giorgio[1], L. Pisciotta[3], T. Lucchi[4], L. Grigore[5], C. Pavanello[1], A. Granata[2], A. Pasta[3], B. Arosio[6], D. Azzolino[6], A. Baragetti[2], S. Castelnuovo[7], A. Corsini[2]

[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [3]Dipartimento di Medicina Interna, Università di Genova; [4]Unità di Geriatria, IRCCS Fondazione Ospedale Maggiore Policlinico, Milano; [5]Centro SISA per lo Studio dell'Arteriosclerosi, Ospedale Bassini, Cinisello Balsamo, Milano; [6]Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano; [7]Centro Dislipidemie, ASST Grande Ospedale Metropolitano Niguarda, Milano

E-mail: monica.gomaraschi@unimi.it

Background and Aims. Cholesteryl ester storage disease (CESD), due to mutations in the LIPA gene, is characterized by hepatic steatosis, hypercholesterolemia and hypoalphalipoproteinemia, which expose affected patients to an increased cardiovascular risk. Further insights into the impact of LIPA gene mutations on lipid/lipoprotein metabolism are limited. Aim of the study was to investigate the effect of carrying one or two mutant LIPA alleles on lipoprotein composition and function.

Methods. Lipoproteins from 6 CESD patients, 5 relatives carrying one mutant LIPA allele (carriers) and 12 sex/age matched controls were isolated and characterized. Carotid IMT, lipid/lipoprotein profile, LCAT activity and HDL ability to promote nitric oxide release by endothelial cells were also evaluated.

Results. Despite the lipid-lowering therapy, total cholesterol, LDL-cholesterol and triglycerides were increased in CESD patients, while HDL-cholesterol was reduced. Carriers also displayed elevated total and LDL-cholesterol. Mean carotid IMT was higher in CESD patients compared to controls. Very low and intermediate density lipoproteins from CESD patients and carriers were enriched in cholesteryl esters (CEs) compared to the control ones, with a concomitant reduction of triglycerides. Fatty acid composition of CEs in serum and lipoproteins showed a depletion of linoleate content in CESD patients, likely due to the reduced LCAT activity. In CESD HDL, fatty acid distribution of CEs was shifted towards saturated ones, if compared to control HDL. The changes in HDL composition did not affect HDL ability to promote nitric oxide release by endothelial cells.

Conclusions. LIPA gene mutations significantly affected plasma levels and lipid composition of lipoproteins, thus likely contributing to the increased cardiovascular risk of affected patients.

GENETIC DETERMINANTS OF FAMILIAL HYPERCHOLESTEROLEMIA: WHAT ELSE BEYOND LDLR GENE?

A. Kura[1], E. Sticchi[1], A. Volta[1], R. De Cario[1], A. Murri[1], A. Magi[2], S. Suraci[1], A.M. Gori[1], R. Marcucci[3], B. Giusti[1]

[1]Department of Experimental and Clinical Medicine, University of Florence; [2]Department of Information Engineering, University of Florence; [3]Atherothrombotic Diseases Center, Careggi Hospital, Florence

E-mail: ada-kura@hotmail.it

Background. Familial Hypercholesterolemia (FH) represents an autosomal disorder due to pathogenic variants in LDLR or APOB or PCSK9 (dominant form), and in LDLRAP1 (recessive form). Nevertheless, many subjects (about 60%) with FH did not demonstrate functional mutations in these four genes. Aim of the present study was to assess the genetic profile of patients with FH, through targeted 55 genes panel high-throughput sequencing (HTS), thus allowing to delineate the contribution of further major or modifier genes.

Methods. We analyzed 38 subjects with possible/probable or definite FH, according to the most common diagnostic algorithm, the Dutch lipid score. Targeted HTS (55 genes panel, including loci involved in lipid metabolism, genes supposed to be involved in dyslipidemia, pharmacogenetics of statins, genes related to higher susceptibility for the polygenic forms of FH, HDL and triglycerides related diseases) was performed. DNA libraries were prepared using Agilent HaloplexHS enrichment system, and sequencing was assessed using Illumina MiSeq technology.

Results. Thirteen out of 38 patients investigated showed pathogenetic or likely pathogenetic mutations in LDLR gene. Three out 13 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB and LDLRAP1 genes. In patients without LDLR mutations (n=25), at least 2 rare variants were identified in 16 patients (64%), and at least 3 rare variants were identified in 9 patients (36%). Patients with or without pathogenetic mutations in LDLR gene were comparable for age, sex, and LDL cholesterol levels, whereas Dutch score was significantly higher in LDLR mutation positive patients. In these 25 patients, a total of 58 rare variants with uncertain significance/conflicting interpretation of pathogenicity have been identified: 7 (12%) in APOB, 5 (8.6%) in CELSR2, 5 (8.6%) in GHR, 4 (6.9%) in ABCG5, 3 (5.2%) in LMF1, 3 (5.2%) in SLC22A1, 3 (5.2%) in GCKR, 2 (3.4%) in CREB3L3, 2 (3.4%) in ABCB1, 2 (3.4%) in LRP1, 2 (3.4%) in APOA4, 2 (3.4%) in ITIH4, 2 (3.4%) in PON1, 2 (3.4%) in LIPC, 2 (3.4%) in SCARB1, 1 (1.7%) in PCSK9, 1 (1.7%) in DAB2, 1 (1.7%) in PPP1R17, 1 (1.7) in SREBF1, 1 (1.7) in SREBF2, 1 (1.7%) in HFE, 1 (1.7%) in LCAT, 1 (1.7%) in LPL, 1 (1.7%) in ABCG2, 1 (1.7%) in NYNRIN, 1 (1.7%) in SLCO1B1, 1 (1.7%) in LIPI genes. These genes are known to be involved in FH, hypertriglyceridemia, other forms of familial dyslipidemia, polygenic FH or pharmacogenetics.

Conclusions. Results from the present study suggest the possible effect of multiple rare variants on the clinical phenotype, thus supporting the contribution of a polygenic predisposition to lipid profile alteration. Conversely, even if significant, our preliminary data support a less severe weight of cardiovascular manifestations and family history in patients with polygenic forms of FH.

THROMBUS AND PERIPHERAL VENOUS BLOOD GLOBAL GENE EXPRESSION PROFILES IN ACUTE ISCHEMIC STROKE: COMPLEMENTARY OR PARALLEL REALITIES?

A. Kura[1], A. Sodero[2], E. Sticchi[1], A. Sereni[1], S. Galora[1], A. Magi[3], S. Suraci[1], R. De Cario[1], A. Consoli[4], A. Rosi[4], S. Nappini[4], L. Renieri[4], N. Limbucci[4], B. Piccardi[2], C. Sarti[2], D. Inzitari[2], S. Mangiafico[4], A.M. Gori[1], R. Marcucci[1], B. Giusti[1]
[1]Department of Experimental and Clinical Medicine, University of Florence; [2]Stroke Unit, Careggi Hospital, Florece; [3]Department of Information Engineering, University of Florence; [4]Interventional Neuroradiology Unit, Careggi Hospital, Florence
E-mail: ada-kura@hotmail.it

Background. Ischemic stroke remains a major cause of death and disability worldwide. Tissue plasminogen activator and mechanical thrombectomy (MT) represents the standard treatment of acute ischemic stroke (AIS) for recanalization of the occluded intracranial vessel. The use of MT in patients with AIS has allowed for histopathological analysis of clots. Few previous studies suggested a correlation between the composition of thrombus (e.g fibrin percentage and different cellular components such as red blood cells, white blood cells) and pathophysiological mechanisms and outcomes. Further histological and transcriptomic analysis of thrombi obtained during MT may be important in order to acquire useful information about the pathophysiological causes, determinants of clinical outcomes of stroke and to develop therapies in the future. Aim of this study was to evaluate, by using Affymetrix technology, the global gene expression profiles of RNA extracted from thrombus and peripheral venous blood of AIS patients undergoing MT.
Methods. Thirty three consecutive AIS patients (16 males/17 females, mean age±SD 74.5±13.7) undergoing endovascular treatment were collected and followed-up for at least 3 months. The thrombus obtained during MT was collected in RNA later. Blood samples were collected before and 24 hours after MT in tubes with anticoagulants (plasma EDTA and citrated), as well as in tubes without anticoagulant (serum) and with liquid for RNA stabilization. Biological materials were stored for present and future analyses. RNA was extracted by PAX gene blood miRNA kit. The global gene expression was assessed by Affymetrix technology using GeneChip Human Trascriptome 2.0 Array allowing the analysis of 44,699 genes, >285,000 full-length transcripts coverage. Data analysis was performed with TAC 4.0 software (Affymetrix) and in R environment with dedicated pipelines. For statistical analysis we used SPSS v.25 software.
Results. We processed 33 thrombi and 17 peripheral venous blood samples. In order to evaluate the biological processes, the molecular functions and the cellular component that characterize the genes expressed in the biological samples under analysis, we performed a GeneOntology enrichment analysis. In particular, as regards biological processes, 363 terms are significantly enriched ($p<0.01$). In peripheral venous blood samples the biological processes enriched with a $p<0.01$ amount to 1058.The number of probe sets, miRNAs, lncRNAs and genes annotated with an expression greater than 26 is higher in RNA obtained from thrombus than from peripheral venous blood. The GeneOntology enrichment analysis in thrombus highlights among the most significant a series of terms related to activation and mediated immunity of neutrophils, activation of T lymphocytes and platelets, regulation of leukocyte differentiation and apoptosis. These data are consistent with the crucial role reported or suggested in the literature of these processes at various stages of ischemia/reperfusion injury. Although, at peripheral level, differences can be observed in significantly enriched terms, many of the most significant biological processes are common with those observed in thrombi.
Conclusions. Few data are, at present, available on whole gene expression profiles of thrombi and peripheral venous blood of AIS patients after MT. Upon completion of analyses in all enrolled patients, in addition to this important picture that allowed us to obtain important and new data on gene expression profiles in thrombi and peripheral blood of the same AIS patients, we will correlate/associate these profiles with the different pathophysiological mechanisms of AIS, response to treatment and clinical outcomes (eg hemorrhagic transformation, disability, death). It will be interesting to evaluate if the associations observable at the level of injury are the same as those observable at peripheral level. Better understanding of molecular and cellular changes in AIS patients will be useful to acquire information about the pathophysiological bases and outcomes determinants in order to identify and validate new diagnostic/prognostic and therapeutic markers.

THE TREATMENT OF PRIMARY DYSLIPIDEMIA IN PEDIATRICS

G. Massini, R. Buganza, A. Beccaria, L. de Sanctis, O. Guardamagna
Department of Public Health and Pediatrics, University of Turin
E-mail: giulia.massini@unito.it

Introduction. Dyslipidemia in pediatric patients represents a cardiovascular risk factor and the atherosclerotic process progresses if adequate treatment is not started. The aim of the study was to evaluate the impact of different therapeutic approaches on the lipid profile in pediatric patients with primary dyslipidemia.
Material and Methods. Patients with familial hypercholesterolemia (FH) and polygenic hypercholesterolemia, associated or not with hypertriglyceridemia were recruited in the period 2005-2018. The subjects of the study were put on a diet for 6-12 months, then added with food supplements. Consistently with international guidelines, drug therapy was reserved for FH patients. Auxological parameters, food diary and lipid profile were evaluated in basal conditions and after each specific treatment.
Results. Out of the 672 subjects recruited, the efficacy of the diet was considered in 343 compliant patients who achieved a reduction in total cholesterol (CT) and LDL-cholesterol (LDL-C) of 7.3% and 9.8%, respectively. The use of glucomannan (36 cases) led to a significant drop in TC and LDL-C of 6.8% and 9.5% and the drop was higher with the use of phytosterols (130 cases) equal to 11.2% and 14.3%, respectively. The administration of phytosterols significantly reduced apolipoprotein B (13.8%). Administration of pravastatin 20 mg/day, which was well tolerated, reduced LDL-C by 25.4%. LDL-C target of 130 mg/dl was achieved by 18% of patients.
Conclusions. The present study demonstrates the efficacy of diet and supplement intake and confirms the need for early intervention. However, to improve the achievement of the therapeutic target in subjects with high cardiovascular risk is important to evaluate the use of statins.

ASSOCIATION BETWEEN SIZE OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL PARTICLES AND CARDIOVASCULAR EVENTS IN TYPE 2 DIABETES SUBJECTS

G. Lo Coco[1], J. Sanz Molina[1], F. Luciani[1], C.M. Semprini[1], E. Dalla Nora[1], G. Zuliani[2], G.B. Vigna[1], A. Passaro[1]
[1]*Department of Medical Science, University of Ferrara;*
[2]*Department of Morphology, Experimental Medicine and Surgery, University of Ferrara*
E-mail: lccgtn@unife.it

Background. Type 2 diabetes (T2D) is an established independent risk factor for atherosclerotic cardiovascular disease (ASCVD), particularly coronary heart disease (CHD). Moreover, a large number of studies have suggested that diabetic patients are prone to a higher risk of death from ASCVD, such as CHD and stroke, when compared with nondiabetes patients. Until now, however, the incremental cardiovascular risk in diabetic individuals has not been explained fully by hyperglycaemia. Diabetic dyslipidaemia is characterized by elevated fasting and postprandial triglycerides (TG), low-density lipoprotein cholesterol (c-LDL) and decreased high-density lipoprotein cholesterol (c-HDL). However, the characteristics of lipoprotein subfractions distribution in patients with type 2 diabetes (T2D) have not been fully investigated. In addition, guidelines argue about intensive hypolipidemic treatment among T2D patients without CHD as much as those with CHD.
Objective. The aim of present study was to evaluate the distribution of lipoprotein subfractions and the association between size of LDL and HDL particles and cardiovascular events in T2 diabetes patients
Methods. A total of 385 subjects were consecutively enrolled in this study, selected from the database of Emilia Romagna Research Centre of dyslipidemia, whereas controls were selected from general population. Patients were divided into T2D without CHD group (n=116), T2D with CHD group (n=36) and control group (n=233, without T2D and CHD). Both low- and high-density lipoprotein cholesterol (c-LDL and c-HDL) subfractions were analysed using the Quantimetrix Lipoprint System. The distributions of lipoprotein subfractions were evaluated in each group. Subfractions were correlated with selected clinical and biochemical parameters including risk factors for atherosclerotic cardiovascular desease and with cardiovascular events. Variables were analysed with parametric or non-parametric statistical tests as necessary.
Results. Among patients without hypolipidemic treatment serum total-cholesterol, c-LDL, c-HDL and triglicerydes distribution differ significantly between groups ($p<0,05$), whereas patients ongoing hypolipidemic treatment only differ significantly in serum total cholesterol ($p=0,074$), but not in c-LDL, c-HDL and triglycerides serum distribution ($p<0,05$). In addition, among patients without hypolipidemic treatment small-dense c-LDL, large-buoyant c-LDL, c-HDL large subfraction and c-HDL small subfraction distribution differ significantly between groups ($p<0,05$); moreover, lipoprotein subfractions distribution differ significantly between groups also in patients with hypolipidemic treatment ($p<0,05$).
Conclusions. Hypolipidemic treatment in Type 2 diabetes patients, with or without CHD, does not modify lipoprotein subfractions distribution in comparison to control group. Further studies are needed to confirm our results and we ipotize that other therapeutic approaches are necessary to modify lipoprotein subfractions distribution in order to reduce cardiovascular risk. Therefore, our observation provides additional information concerning diabetic dyslipidaemia, which might be useful for future prevention and therapy in patients with Type 2 diabetes.

THE CLINICAL RELEVANCE OF GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE IN FAMILIAL HYPERCOLESTEROLEMIA

S. Lugari, A. Cavicchioli, A. Mondelli, P. Vita, E. Pellegrini, F. Carubbi, F. Nascimbeni
Unit of Internal and Metabolic Medicine, Civil Hospital, A.O.U. of Modena and University of Modena and Reggio Emilia
E-mail: simonetta.lugari@libero.it

Introduction. Next-generation sequencing has resulted in rapid genetic diagnosis of patients with suspected familial hypercholesterolemia (FH); however, genetic variants of uncertain significance (VUS) with an unknown causal link to the disease are being increasingly identified. We aimed at evaluating the clinical significance of VUS in our FH cohort.
Methods. 191 consecutive patients with suspected FH from the Lipid Clinic in Modena underwent comprehensive evaluation, including genetic analysis, within the LIPIGEN Project. Patients were categorized in four groups according to the detection of "pathogenic"/"likely pathogenic" mutations (M) and/or VUS in autosomal dominant FH-causing genes (LDLR, APOB, PCSK9, STAP1, APOE) as follows: M/VUS-, M-/VUS+, M+/VUS-, M+/VUS+.
Results. M and/or VUS were detected in 162 (84.8%) patients (147 M and 50 VUS). The vast majority of M affected LDLR (95.9%), whereas VUS were more heterogeneously distributed across FH-causing genes (LDLR 24%, APOB 60%, PCSK9 14%, APOE 2%). M-/VUS- (n=29) and M-/VUS+ (n=18) patients did not significantly differ for age, LDL cholesterol (LDL-c) and Lp(a) levels, Dutch Lipid Clinic Network (DLCN) score, duration of statin therapy, family and personal history of cardiovascular disease, presence of arcus cornealis or Achilles tendon xanthomas. M-/VUS+ patients showed significantly lower LDL-c levels ($p<0.001$), DLCN score ($p<0.001$) and duration of statin therapy ($p=0.034$) and were less likely to have Achilles tendon xanthomas ($p=0.038$) and a positive family history of cardiovascular disease ($p=0.031$) than M+/VUS- patients (n=116). Of note, polygenic LDL-c score was similar between M-/VUS- and M-/VUS+ ($p=0.134$), but significantly higher in M-/VUS+ than M+/VUS- patients ($p=0.008$). No significant differences were found between M+/VUS- and M+/VUS+ patients (n=28). Restricting the analyses to VUS in LDLR yielded to similar results.
Conclusions. In our FH cohort, VUS carriers had a biochemical and clinical profile similar to that of mutation-negative patients. Moreover, the coexistence of VUS did not increase the phenotype severity in mutation-positive patients.

STUDY OF THE ASSOCIATION BETWEEN CARDIOVASCULAR EVENTS AND SIZE OF LOW DENSITY LIPOPROTEIN AND HIGH DENSITY LIPOPROTEIN PARTICLES IN PATIENTS WITH FAMILIAR HYPERCOLESTEROLEMIA

F. Luciani, J. Sanz Molina, C. Bosi, G. Lo Coco, A. Colangiulo, M. Dall'Agata, E. Dalla Nora, A. Passaro, G.B. Vigna
Department of medical science, University of Ferrara
E-mail: lcnfnc@unife.it

Background. Familial Hypercholesterolemia (FH) is an autosomal dominant genetic disease, characterized by extremely elevated levels of low density lipoprotein cholesterol (LDL-C) and a propensity to early onset Arteriosclerotic Cardiovascular Disease (ASCVD). Low density lipoproteins (LDL) do not show in humans a normal distribution and comprise two different main fractions: large, buoyant (phenotype pattern A) and small, dense (phenotype pattern non-A) particles, that differ not only in size and density but also in physicochemical composition, metabolic behaviour and atherogenicity. On the other hand, HDL particles are also heterogeneous in composition and structure, which may relate to differences in antiatherogenic potential. The aim of present study was to evaluate the distributions of lipoprotein subfractions in FH patients and their role as an important predictor of cardiovascular events and progression of Coronary Artery Disease (CAD).
Methods. A total of 183 patients were enrolled in this study. Based on the evidence of Familiar Hypercholesterolemia, patients were divided into FH group (n=61) and non-FH group (n=122). Controls were taken from the general population and matched with FH patients for age, sex and BMI with a 2: 1 ratio. Both low-and high-density lipoprotein cholesterol (LDL-and HDL-C) subfractions were analysed using the Quantimetrix Lipoprint System®. The distributions of lipoprotein subfractions were evaluated in patients with and without FH. Subfractions were correlated with selected clinical-biochemical parameters including risk factors for atherosclerotic CVD and with cardiovascular events. Variables were analysed with parametric or non-parametric statistical tests as necessary.
Results. Compared with non-FH individuals, the LDL-C levels and TG levels of the FH patients were substantially higher, whereas the levels of HDL-C were almost equal in the two groups. Most FH patients enrolled in the study were already on lipid-lowering treatment. The FH group presented a significant increase in large LDL and small LDL cholesterol subfraction concentration when compared with the non-FH group; the Small-dense LDL-C/Large-buoyant LDL-C ratio is greater in the FH group than in non-FH group, even if the difference is not statistically significant. The pattern of HDL subfractions showed higher levels of large HDL, both in concentration and percentage, in non-FH patients. There were not difference in concentration of small HDL in the two groups. We have also analyzed the correlation between the size of lipoproteins subfractions and the hypolipidemic treatment. There are no significative differences in concentration and percentage of HDL subfractions both in patients not in treatment and in patients in treatment. The situation is different for LDL subfractions. There is a significative difference in concentration, but not in percentage, of LDL subfractions, both small and large, in patients not in treatment (p=0.009 and p=0.001 respectively). Instead the treated patients present a significative difference only for large LDL subfractions (p=0.001); the small LDL subfractions were almost equal in the two group (p=0.292). Eventually we have studied the correlation between the lipoprotein size and the cardiovascular events in patients FH. The small LDL subfractions were higher in patient FH with cardiovascular events (p=0.037). There were not significative differences of HDL subfractions in patient not FH and FH with cardiovascular events.
Conclusions. Small LDL particles are more associated with CVD. The lipid-lowering treatment can reduce the concentration of more atherogenic LDL subfractions.

LIPID ACCUMULATION IMPAIRS LYSOSOMAL ACID LIPASE ACTIVITY IN HEPATOCYTES: EVIDENCE IN NAFLD PATIENTS AND CELL CULTURES

M. Gomaraschi[1], A.L. Fracanzani[2], P. Dongiovanni[3], C. Pavanello[1], E. Giorgio[1], L. Da Dalt[4], G.D. Norata[4], L. Calabresi[1], D. Consonni[3], R. Lombardi[2], A. Branchi[5], S. Fargion[2]
[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano; [3]IRCCS Fondazione Ospedale Maggiore Policlinico, Milano; [4]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [5]Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano
E-mail: monica.gomaraschi@unimi.it

Aims. It has been hypothesized that the activity of lysosomal acid lipase (LAL), a key enzyme involved in lipid metabolism, is involved in the NAFLD phenotype. To clarify the role of LAL in NAFLD, we studied 164 consecutive patients with biopsy-proven NAFLD and fat-loaded HepG2 cells.
Methods. LAL activity was measured:
1) on dried blood spots (DBS) from NAFLD patients and dyslipidemic subjects without fatty liver;
2) on liver biopsies from NAFLD patients.
LAL activity and expression were evaluated in HepG2 cells cultured in the presence of free fatty acids (FAs), with or without a PPAR-alpha agonist.
Results. LAL activity was significantly reduced in patients with NAFLD compared to dyslipidemic subjects. LAL activity measured in liver biopsies from NAFLD patients was highly correlated to that measured on DBS and was independent of LAL expression in the liver. In a fully adjusted model, LAL activity on DBS was associated only with platelets and, when normalized by platelet count, it did not differ according to fibrosis stage. In vitro, FA loading of HepG2 fully replicated the impairment of LAL activity observed in NALFD patients. In these cells, the activation of PPAR-alpha receptors prevented and corrected FAs-induced LAL impairment, by stimulating FA oxidation and LAL expression. In a pilot study, fenofibrate significantly improved LAL activity in NAFLD patients.
Conclusions. LAL activity is reduced in NAFLD patients, independently from disease progression. In vitro, impaired LAL activity induced by FA loading was rescued by PPAR-alpha activation. These data suggest that the pharmacological modulation of LAL should be explored in the management of NAFLD patients.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 ENHANCES VASCULAR CALCIFICATION IN CHRONIC KIDNEY DISEASE

M. G. Lupo[1], E. Faggin[2], M. Rattazzi[2], M. Camera[3], P. Poggio[4], N. Ferri[1]
[1]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova; [2]Dipartimento di Medicina, Università degli Studi di Padova; [3]Dipartimento di Scienze farmacologiche e Biomolecolari, Università degli Studi di Milano; [4]Centro Cardiologico Monzino IRCCS, Milano
E-mail: mariagiovanna.lupo@gmail.com

Background. In patients with chronic kidney disease (CKD), vascular calcification is associated with significant morbidity and mortality. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a pivotal player of cholesterol homeostasis, has been recently associated with a higher rate of calcification in hypercholesterolemic, diabetes and chronic kidney disease patients. In addition, carriers of the PCSK9 R46L loss-of-function variant have a low calcific aortic valve stenosis.
Aim. The aim of this study was thus to investigate the role of PCSK9 in vascular calcification process, under uremic condition, both on in vivo and in vitro experimental settings.
Methods. Sprague-Dawley rats were fed a standard diet (n=11) or uremic diet containing 0.5% adenine (n=11) for 6 weeks. Urine volumes were measured every two weeks by housing rats in metabolic cages for 24 h. At sacrifice, abdominal aortas, plasma, livers and kidneys were collected. Calcium crystals in tunica media of aortas were visualized by von Kossa staining and quantified by a colorimetric assay. Plasma creatinine and phosphate levels were evaluated by clinical standardized methods. PCSK9 expression in kidneys and liver was visualized by western blotting (WB). The overexpression of PCSK9 in vascular smooth muscle cells (VSMCs) was attempted by retroviral infection. PCSK9 overexpression was determined by both ELISA and WB analyses. Wild-type (wt) and PCSK9-overexpressing VSMCs were cultured with low-FCS/high-phosphate media (2.5% FCS in the presence of 2.0 mM or 2.4 mM of Pi) for 7 days, and media were replaced every two days. Evolocumab (Evo) 82 µg/mL was used in combination with 2.4 mM Pi for 7 days. Moreover, wt VSMCs were treated for 7 days with 5 µg/mL recombinant human PCSK9 (recPCSK9). Hydroxyapatite deposition by VSMCs was measured by a calcium colorimetric assay. Matrix GLA protein (MGP) expression was evaluated by WB. Extracellular vesicles (EVs) were determined by flow cytometry.
Results. In vivo experiments: The uremic condition, in Sprague-Dawley rats, was documented by increased urine volume (26 mL/day vs 58 mL/day), plasma creatinine (25.7 µM vs 208 µM) and phosphate levels (2.64 µM vs 6.11 µM). High phosphate concentration was associated to aortic calcification determined by measuring aorta Ca2+ concentrations (0.34 mg/g tissue vs 2.48 mg/g tissue) and by von Kossa staining. This pathological condition was associated to a significant increase of total cholesterol (from 75.3 mg/dL to 107.6 mg/dL) and PCSK9 levels (from 40.1 ng/mL to 109.7 ng/mL). Higher expression of PCSK9 was also observed in kidney (+4.8 fold) and liver (+1.5 fold). In vitro experiments: The overexpression of PCSK9 in VSMCs (from 0.02 ng/mL to 11.3 ng/mL) induced a significant increase of extracellular calcification in response to 7 days exposure to 2.4 mM Pi (+39% compared to control VSMCs). The overexpression of PCSK9 led to a significant dose-response reduction in MGP protein, an important inhibitor of the calcification process, in comparison to wt VSMCs (-30%, -60%, -90% vs control, 2.0 mM Pi and 2.4 mm Pi, respectively), and to an increase in the release of EVs in response to 2.4 mM Pi (+7-fold vs control). The concomitant treatment with 2.4 mM Pi and Evo showed no improvements in the extracellular calcium deposition and the incubation of VSMC with recPCSK9 (5 µg/ml) produced no increase in extracellular calcium deposition.
Conclusions. The present study indicates a direct role of PCSK9 on vascular calcification associated to a CKD condition. We identified MGP as one of the possible mediators of this process. Moreover, we suggest an intracellular-mediated mechanism of action of PCSK9 which promotes the production of EVs.

THE ROLE OF CHEMERIN IN VASCULAR DAMAGE OF MORBIDLY OBESE PATIENTS

S. Ministrini[1], M.A. Ricci[1], E. Nulli Migliola[1], S. De Vuono[2], M. D'Abbondanza[1], M.T. Paganelli[3], A.R. Roscini[4], D. Siepi[1]
[1]Dipartimento di Medicina, Università degli Studi di Perugia; [2]Dipartimento di Medicina, Università degli Studi di Perugia; [3]Chirurgia Generale, Ospedale Santa Maria della Misericordia; [4]Medicina Interna, Ospedale Santa Maria della Misericordia
E-mail: stefano.mns@gmail.com

Background and Aims. Adipokines have been claimed in the progression of atherosclerotic disease progression in obese subjects. Chemerin is an adipokine that affects adipogenesis and glucose homeostasis in adipocytes, whose levels increase with BMI. The aim of this study is to investigate the correlation between circulating levels of chemerin and vascular damage.
Methods. Two-hundred-eighty obese subjects eligible for laparoscopic sleeve gastrectomy (LSG) were enrolled in the study. The following parameters were evaluated: body mass index (BMI), glycemia, insulinemia, HOMA-IR, glycated hemoglobin, lipid pattern, plasma chemerin levels, flow mediated vasodilation (FMV) and intima-media thickness (IMT). One-hundred and twenty-eight underwent LSG and were evaluated 10 months after the intervention.
Results. The study showed a significant association between chemerin and cardiovascular damage. Univariate analysis of chemerin showed a direct and significant correlation with waist circumference (Rho=.276, p=.039), HOMA-IR (Rho=.299, p=.025), glycated hemoglobin (Rho=.428, p=.001), maximum left IMT (Rho=.300, p=.025) and maximum right IMT (Rho=.270, p=.044). At baseline we found that chemerin significantly predicts maximum left IMT according to an exponential model (R2=.082, Beta=.001, p=.032). On analysis with the ROC curve we have identified a threshold of chemerin of 150 ng/ml able to predict cardiovascular risk using IMT as an outcome.
Conclusion. Obese patients show increased chemerin values that correlate with metabolic syndrome and vascular damage expressed by IMT. Patients with plasma chemerin values >150 ng/ml have a higher probability of developing early vascular damage. Bariatric surgery is an effective therapeutic instrument able to decrease cardiovascular risk through reduction of the IMT and of chemerin plasmatic levels, with a concomitant improvement in insulin resistance.

DEPRESSIVE SYMPTOMS AND PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9: EVIDNECE FROM THE SPHERE COHORT

C. Macchi[1], C. Favero[2], M.F. Greco[1], A.C. Pesatori[2], L. Vigna[3], A. Ceresa[4], N. Ferri[5], A. Corsini[6], C.R. Sirtori[7], M. Buoli[4], V. Bollati[2], M. Ruscica[6]
[1]Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]EPIGET Department of Clinical Sciences and Community Health, University of Milan; [3]Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan; [4]Department of Pathophysiology and Transplantation, University of Milan; [5]Department of Pharmaceutical and Pharmacological Sciences, University of Padua; [6]Department of Pharmacological and Biomolecular Sciences, University of Milan; [7]Centro Dislipidemie, A.S.S.T. Grande Ospedale Metropolitano Niguarda, Milan
E-mail: chiara.macchi@unimi.it

Background. Among less frequently evaluated clinical conditions linked to the cardiovascular (CV) risk, anxiety and depression are highly prevalent. Depressed patients are more likely to eventually develop CV disease, with risk of events that raising according to severity of depression. Epidemiological evidence strongly suggests an association between obesity and depressive mood, an evidence which may describe the 50% raised risk of onset of depression in the obese. Proprotein convertase subtilisin/kexin 9 (PCSK9), regulating the number of cell-surface of low-density lipoprotein receptors, may play a major role in CV diseases. Thus, aim of the present study was to verify whether in a population of obese subjects, depression could have an impact on PCSK9 levels and on their association with the Framingham Risk Score (FRS), a well-established predictor of CV events.
Methods. 310 obese subjects were selected among participants of the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miRNAs and Exosomes) study. This was originally aimed at investigating possible molecular mechanisms underlying the effects of particulate matter exposure on health outcomes. All participants were >18 years at enrollment, with a BMI between 25 and 30 kg/m^2 or ≥30 kg/m^2. Depression symptoms were evaluated according to the Beck Depression Inventory II (BDI-II). Univariate and multivariable linear regression models were used to test the relation between circulating PCSK9 levels and BDI-II score. The best models selected to predict these associations were adjusted for age, gender, BMI, smoking habits, non-HDL cholesterol (non-HDL-C), triglycerides, QUICKI, HOMA-IR, % eosinophils, thyroid stimulating hormone (TSH) and use of statins and antihypertensive drugs. A linear regression model was also applied to verify the association between FRS and circulating PCSK9 levels, adjusted for BDI-II and BMI.
Results. Standard laboratory tests (AST, ALT and gamma-glutamyltransferase) were in the normal range as well as glycemic targets, e.g. glycemia, 93.7±15 mg/dL and glycated hemoglobin, 39.6±6.1 mmol/mol. Participants were moderately hyperlipidemic with mean values of total cholesterol, LDL-C and non-HDL-C of 214.5±38.4 mg/dL, 133.1±33.9 mg/dL and 155.8±38.8 mg/dL, respectively. HDL-C and TG levels were in the normal range, being 58.3±14.4 mg/dL and 118.6±78.9 mg/dL, respectively. Thyroid functionality was also normal (TSH=1.8±1.1 U/mL). PCSK9 levels followed a Gaussian distribution and were significantly associated with BDI-II (p=0.049). In individuals with a BDI-II score below 20, the association was positive and statistically significant. For every unit increase of BDI-II, there was an increment of 1.57 ng/mL of PCSK9 levels (β=1.57, SE= 0.80, p=0.049); the opposite being found in individuals with a severe depression, with BDI-II scores above 20 (β= -5.26, SE=1.95, p=0.007). PCSK9 mean concentrations rose in a stepwise manner from the minimal depressive group (252 ng/mL; 95% CI 243;262) to the mildly (264 ng/mL; 95% CI 239; 288) and moderately depressed (276 ng/mL; 95% CI 248; 304). The severe depressive group had a lower PCSK9 levels compared to the others (182 ng/mL; 95% CI 125; 240). Multivariable linear regression analysis showed a 20% rise in FRS for every 100 ng/ml increase in PCSK9 levels (Δ%=20.69, 95%CI: 5.60-35.81, p=0.004), independent of BDI-II score and BMI, both not associated with FRS.
Conclusions. In obese subjects, PCSK9 levels rise in a stepwise manner up to a certain severity of mood symptoms and remain an independent predictor of CV risk, as assessed by the FRS.

RESIDUAL VASCULAR RISK IN PATIENTS WITH LIPOPROTEIN(A) HYPERLIPOPROTEINEMIA ON PCSK9 INHIBITORS THERAPY

E. Paiola, V. Sanga, C. Conforti, C. Maneschi, D. Travia, L. Santi, E. Bonora, M.G. Zenti
Department of Endocrinology, Diabetology and Metabolic Diseases, AOUI Verona
E-mail: elen.paiola@gmail.com

Introduction. Despite significant progress in the identification and treatment of cardiovascular risk (CVR) factors, residual risk represents a challenge to effective prevention. In particular, Lp(a) is involved in processes of vascular damage, its levels cannot be modulated with lifestyle interventions and drugs developed "ad hoc" able to reduce Lp(a) levels are not available yet. PCSK9 inhibitors (PCSK9i) showed only a modest reduction in Lp(a) levels (average -20÷30%), with unclear impact in patients with hyperLp(a). Our study estimated the prevalence of patients with hyperLp(a) in a cohort of subjects with high CVR on PCSK9i therapy and assessed the related effects on Lp(a) plasma concentration.Methods: Monocentric observational cohort study involving 77 patients on PCSK9i therapy.
Results. The cohort included 77 patients (36 women, 41 men), aged 64±10 years; 19 in therapy with alirocumab 75 mg/week, 22 with alirocumab 150 mg/week, 36 with evolocumab 140 mg/week. All subjects had high CVR: 64,9% had a history of CVD, 67,5% had a PVD, 32,5% had a Familial Hypercholesterolemia. 40 patients were statin intolerant (51,9%); 12 subjects (15,6%) were diabetics. At baseline LDL-cholesterol was 174±86,82 mg/dl and Lp(a) median was 58 mg/dl (interquartile range 15-85,3). 38 patients (49,35%) presented hyperLp(a) (>30 mg/dl). After one year, LDL was 68,74±49,27 and Lp(a) 45,2 mg/dl [16-11] in the whole cohort, while in the hyperLp(a) sub-group Lp(a) median was 106 mg/dl [59,75-146].
Discussion. In our experience PCSK9i-therapy has a very heterogeneous effect over Lp(a) serum levels. Intensive management of risk factors (i.e. LDL-C, glucose-metabolism and blood pressure) significantly reduces CVR; however atherogenic dyslipidemia, in particular hyperLp(a), remains an untreated factor. Therefore it would be important to focus on new specific therapies for Lp(a); in the meantime lipoprotein apheresis, which is the golden standard treatment, should be considered.

A NEW VARIANT IN LIPA GENE CAUSATIVE OF LYSOSOMAL ACID LIPASE DEFICIENCY

G. Maione[1], G. Massini[2], C. Giacobbe[3], D. Palma[3], G. Cardiero[1], M.D. Di Taranto[1], O. Guardamagna[2], G. Fortunato[1]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II e CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli; [2]Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università degli Studi di Torino; [3]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, e CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli
E-mail: maione@ceinge.unina.it

Introduction. Lysosomal Acid Lipase Deficiency (LALD) is an autosomal recessive disorder caused by variants in the LIPA gene leading to the complete or partial deficiency of lysosomal acidic lipase (LAL). This enzyme hydrolyzes cholesteryl esters and triglycerides (TG) internalized after endocytosis of plasma lipoproteins. Patients with LALD are characterized by hyperlipidemia, altered liver function, namely increased aminotransferases, hepatic steatosis and hepatomegaly. The prevalence of LALD is not known because the disorder is severely underdiagnosed worldwide.
Patient and Methods. A 55-years-old woman with hypercholesterolemia and high transaminase levels since the age of 22 years (total cholesterol 315 mg/dl, LDL-C 255 mg/dl, HDL-C 39 mg/dL, TG 105 mg/dL, AST 64 UI/L, ALT 125 UI/L) was referred to the lipid clinic of Turin. Liver biopsy revealed the presence of hepatic fibrosis, foamy histiocytes and hepatocytes with foamy and enlarged cytoplasm. The LAL activity resulted decreased <0.02 nmol/punch/hour (n.v. 0.37-2.30 nmol/punch/hour). Treatment with ezetimibe 10 mg/die and Rosuvastatin 10 mg/dL allowed to decrease lipid and transaminase levels. To confirm the clinical suspect of LALD the genetic analysis was performed by a next-generation-sequencing panel of 28 genes involved in lipid metabolism, including LIPA. The rare variants identified were confirmed by Sanger sequencing.
Results. Two rare variants were identified in the LIPA gene, one is the most frequent variant causative of LALD - c.894G>A - an apparent synonymous variant that actually leads to skipping of exon 8 - p.(Ser275_Gln298del). The other variant is the c.260G>A - p.(Gly87Asp) that was never reported as causative of LALD both in HGMD or ClinVar databases. Bioinformatics (Mutation Assessor, SIFT, Polyphen-2, Mutation Taster and PROVEAN) predict that the variant is pathogenic. According to the ACMG guidelines the variant can be classified as "likely pathogenic".
Conclusions. Genetic diagnosis allowed to confirm the LALD disease in the patient and to identify a new causative variant enlarging the spectrum of LIPA pathogenic variants.

ASSOCIATION BETWEEN HIGHER LEVELS OF DISTINCT PLASMA CERAMIDES AND CARDIOVASCULAR MORTALITY IN PATIENTS WITH CHRONIC HEART FAILURE: RESULTS FROM A SUBSET ANALYSIS OF THE GISSI-HF TRIAL

A. Mantovani[1], G. Lunardi[2], J. Meessen[3], S. Bonapace[4], P.L. Temporelli[5], L. Tavazzi[6], A.P. Maggioni[7], R. Latini[3], G. Targher[1]
[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona; [2]Medical Analysis Laboratory, "IRCCS Sacro Cuore - Don Calabria" Hospital, Negrar, Verona; [3]Department of Cardiovascular Medicine, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano; [4]Division of Cardiology, "IRCCS Sacro Cuore - Don Calabria" Hospital, Negrar, Verona; [5]Division of Cardiology, Istituti Clinici Scientifici Maugeri, IRCCS, Veruno; [6]Maria Cecilia Hospital, GVM Care & Research, Ettore Sansavini Health Science Foundation, Cotignola; [7]ANMCO Research Center, Florence
E-mail: alessandro.mantovani24@gmail.com

Background. Ceramides exert several biological activities that may contribute to the pathophysiology of cardiovascular disease and heart failure (HF). However, the association between plasma levels of distinct ceramides (associated with increased cardiovascular risk) and cardiovascular mortality in patients with chronic HF has received little attention.
Methods. In a post-hoc ancillary analysis of the Gruppo Italiano per lo Studio della Sopravvivenza nella Insufficienza Cardiaca-Heart Failure (GISSI-HF) trial, we randomly selected a sample of 200 patients with chronic HF who died for cardiovascular causes during the follow-up of the trial and 200 patients who did not. In these patients, we measured baseline plasma levels of six previously identified high-risk ceramide species [Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0) and Cer(d18:1/24:1) and their individual plasma ratios with Cer(d18:1/24:0)].
Results. Of the six measured plasma ceramides, higher plasma levels of Cer(d18:1/16:0) (adjusted hazard ratio [HR] 1.34, 95% CI 1.13-1.59; p=0.001), Cer(d18:1/20:0) (adjusted-HR 1.23, 95% CI 1.05-1.43; p=0.012) and Cer(d18:1/24:1) (adjusted-HR 1.27, 95% CI 1.08-1.50, p=0.004) were associated with increased risk of cardiovascular mortality, even after adjustment for multiple established cardiovascular risk factors, important comorbidities, medication use, left ventricular ejection fraction and other potential confounding variables. Similarly, increasing plasma ratios of each measured ceramide with Cer(d18:1/24:0) were also independently associated with increased risk of cardiovascular mortality.
Conclusions. In this sub-study of the GISSI-HF trial, we show for the first time that higher levels of specific plasma ceramides [especially when used in ratios with Cer(d18:1/24:0)] are significantly associated with greater cardiovascular mortality in ambulatory patients with chronic HF.

CASE OF A PATIENT WITH ANTIPHOSPHOLIPID SYNDROME AND SEVERE THROMBOCYTOPENIA UNDERGOING LOWER LIMB REVASCULARIZATION

D. Menichelli, A. Maccarone, P. Pignatelli, E. Rossi, F. Violi, D. Pastori
Sapienza University, Roma
E-mail: danilo.menichelli@uniroma1.it

Background and Aim. Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by arterial and/or vein thrombosis or miscarriage in patients with high levels of specific antiphospholipid antibodies (aPL). Anticoagulant therapy with Vitamin K antagonists (VKA) and antiplatelet drugs are commonly used in APS patients with thrombotic events. Here we report the management of antithrombotic therapy in a patient with APS and severe thrombocytopenia undergoing iliac-femoral revascularization.
Case. A 49-year-old male patient with personal medical history of peripheral artery disease (stage IV of Leriche-Fontaine classification), deep venous femoral thrombosis associated with caval thrombosis and pulmonary embolism after appendectomy (1989). After this thrombotic event, a positivity for Lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL) IgM and IgG were found. Thus, the patient was diagnosed with APS and prescribed on VKA with INR range of 2.0-3.0. The patient was not taking any other medications. During VKA therapy, the patient experienced bleeding episodes such as epistaxis, hematoma, massive haemorrhage of the lower limb due to varicose rupture) associated with new-onset severe thrombocytopenia, treated with corticosteroids, cyclosporine, vincristine, cyclophosphamide and rituximab. However, platelet count persistently remained below <50.000 mm^3. Other comorbidities were psoriasis, rheumatoid arthritis and chronic obstructive pulmonary disease. At physical examination, there were pallor, paraesthesia, a weak pulse at the right lower limb associated with claudicatio intermittens that occurred at a walking distance <50 meters. During the pre-operative tests, triple positivity for aPL antibodies was found: aCL IgG and IgM were 466 GPL/ml and 293.50 MPL/ml (normal values <15 GPL/ml and <15 MPL/ml), LAC was 2.44 ratio (normal value <1.35) and anti-beta 2 glycoprotein I IgM and IgG were 256.50 and 867 UA/ml respectively (normal values <15 UA/ml). Other laboratory tests evaluated showed haemoglobin 9,4 g/dL, mean corpuscular volume 68.5 fL, mean corpuscular haemoglobin 21.7 pg, platelet 58.000/mm^3, creatinine 0.88 mg/dL.As part of the pre-surgical work-up, a coronary angiography was performed, showing a stenosis of 80% of the right coronary artery, for which a drug eluting stent was placed. A stent was also placed on the right iliac artery. After stents placement, patient was started on double antiplatelet therapy including aspirin 100 mg/die and clopidogrel 75 mg/die. We decided to not start oral anticoagulation given the high risk of bleeding and the very low platelet count. In the post-surgical period, we observed a worsening of thrombocytopenia, with platelet count reaching a value of 5000 mm^3, which was treated with methylprednisolone 40 mg/die. After 15 days, given the persistence of severe thrombocytopenia (<10.000 mm^3), immunoglobulins were administered (1 g/kg), with platelets increasing to 60.000 mm^3. After 1 month, clopidogrel was discontinued and fondaparinux 5 mg/die was added to Aspirin. No bleeding and thrombotic events occurred during the hospitalization and the following month of follow-up. Patient is currently under clinical observation and VKA therapy has been restarted instead of fondaparinux.
Conclusion. The case describes the difficulty in managing patients with high thrombotic risk such as those with APS and triple antibody positivity, associated with a high risk of bleeding (i.e. severe thrombocytopenia). These patients represent a clinical challenge requiring a tailored therapeutic approach to balance thrombotic and bleeding risk.

LIPOPROTEIN METABOLISM AND FUNCTION ARE ALTERED IN PATIENTS WITH ABDOMINAL AORTIC ANEURYSM

C. Marchi[1], M.P. Adorni[1], F. Zimetti[1], F. Bernini[1], I. Hollan[2], J. Moláček[3], V.Treska[3], N. Ronda[4]
[1]Department of Food and Drug, University of Parma; [2]Lillehammer Hospital for Rheumatic Diseases, M. Grundtvigs veg 6, Lillehammer, Norway and Brigham and Women's Hospital, Cardiology Division, Boston, USA; [3]Department of Vacular Surgery, Faculty of Medicine and University Hospital in Pilsen, Charles University, Plzen, Czech Republic; [4]Department of Food and Drug, University of Parma
E-mail: cinzia.marchi2@studenti.unipr.it

Aims. The pathophysiology of abdominal aortic aneurysm (AAA) development is still unclarified and insufficient tools are available for prevention, diagnosis and early treatment. Serum lipoproteins are known to exert several regulatory functions in tissues in addition to lipid transport. In particular, high density lipoproteins (HDL) are a family of particles undergoing complex metabolism and known for their protective role through the control of cell cholesterol content and intracellular signaling. This study aimed at evaluating lipoprotein functions and metabolism in patients with AAA, independently of the atherosclerotic process.
Material and Methods. We enrolled 30 patients with AAA and 21 control patients with no aortic aneurysm but with the same cardiovascular comorbidities. HDL Cholesterol efflux capacity (CEC) was measured by standardized radioisotopic techniques specific for the membrane transporters ATP-binding cassette G1 (ABCG1) and A1 (ABCA1). Serum cholesterol loading capacity (CLC) was measured by fluorimetric measurement of intracellular cholesterol content in human macrophages. LCAT and CETP activity was evaluated by specific functional assay.
Results. HDL CEC was lower for the ABCG1- (-16%; $p<0.001$) and higher for the ABCA1-pathway in AAA patients (+31.7%; $p<0.0001$) as compared to control patients. Stratification of AAA patients by smoking indicates that smoke contributes to the observed modifications. Altered HDL metabolism was demonstrated by the increased LCAT (+23%; $p<0.0001$) and CETP (+49%; $p<0.0001$) activity in AAA sera. CLC revealed no differences between the two groups. However, the exclusive correlation of this parameter with CEC and CETP only in AAA confirms the relevance of HDL modifications in this pathological condition.
Conclusions. In AAA patients, specific alterations of plasma lipoprotein metabolism and function, possibly involved in tissue inflammation and damage of aortic wall typically occurring during aneurysm formation, were detected. Our data might lead the way to future studies aimed at clarifying the possible causal relationship and validating HDL-related parameters as diagnostic markers or therapeutic targets.

OBESITY-RELATED HYPERPARATHYROIDISM: A NEW PATHOLOGICAL ENTITY WITH POSSIBLE EFFECTS ON CARDIOVASCULAR RISK

S. Ministrini[1], M.A. Ricci[1], G. Daviddi[1], M. Scavizzi[1], S. De Vuono[1], M.T. Paganelli[2], M. Boni[3], A.M. Scarponi[4], G. Lupattelli[1]
[1]Dipartimento di Medicina, Università degli Studi di Perugia; [2]Chirurgia Generale, Ospedale Santa Maria della Misericordia; [3]Chirurgia Generale, Ospedale S. Giovanni Battista; [4]Medicina Interna, Ospedale Santa Maria della Misericordia
E-mail: stefano.mns@gmail.com

Background. Obesity is a risk factor for hypovitaminosis D (HypoD) and hyperparathyroidism (hPTH). Hypo-D is only partially responsible for hPTH and a possible direct effect of leptin on parathyroid glands has been claimed. Hyperparathyroidism could exert a negative effect on glucose metabolism and on vascular function, but evidence about the role of hPTH in morbid obese subjects is lacking. The aim of the study was to evaluate the relationship among parathyroid hormone (iPTH), vitamin D status, leptin, glycometabolic status and markers of early vascular damage in a group of morbid obese subjects before and after laparoscopic sleeve gastrectomy (LSG).
Materials and Methods. We evaluated the levels of iPTH, 25(OH) vitamin D (25(OH)D) and leptin, together with markers of insulin sensitivity and early cardiovascular disease in a cohort of 160 morbid obese patients eligible for a LSG intervention. Sixty-one patients were evaluated again 1 year after the intervention.
Results. Ninety-seven percent of subjects had HypoD and 72% of them had hPTH. After correction for possible confounders, we found a correlation between iPTH levels and carotid intima-media thickness, as well as with HOMA index. Independent predictors of iPTH were age, waist circumference and 25(OH)D. After the LSG, 25(OH)D levels were significantly increased, while iPTH levels were significantly reduced. The reduction of iPTH was significantly correlated with the reduction of BMI, diastolic blood pressure and leptin, which was the independent predictor of iPTH reduction.
Conclusions. Our data confirm the high prevalence of HypoD and hPTH in morbid obese patients. Hyperparathyroidism in obese subjects seems to have a multi-factorial origin, a peculiar calcium-phosphate asset and potential negative effects on glucose metabolism and atherosclerotic disease progression. Therefore, we propose the definition of "obesity-related hyperparathyroidism" as a definite complication of morbid obesity, together with other endocrine and metabolic disorders affecting obese subjects.

ROLE OF MCT1 LACTATE TRANSPORTER IN T LYMPHOCYTES FATE DURING OBESITY

A. Moregola, C. Macchi, M.F. Greco, F. Bonacina, M. Ruscica, G.D. Norata
Department of Pharmacological and Biomolecular Sciences, University of Milan
E-mail: annalisa.moregola@unimi.it

Introducion. Recent studies have shed light on the interconnection between metabolism and immunity in multicellular organisms and their functional coordination for an effective establishment and resolution of immune responses. Imbalance of this delicate signaling network might lead to non-resolving inflammation and consequently to the development of obesity associated chronic inflammation (ObCI). T lymphocytes (T cells) accumulate in the adipose tissue during obesity and their activation lead to a switch in their metabolism from oxidative phosphorylation to aerobic glycolysis which involves the production of elevated amount of lactate. MCT1 is a lactate transporter expressed in different type of cells including T lymphocytes. Aim of this project is to investigate the relevance of T cells lactate transport by MCT1, in the context of adipose tissue inflammation during obesity.
Materials and Methods. MCT1f/f CD4-cre mice, with specific deletion of MCT1 in both CD4+ and CD8+ T lymphocytes, and MCT1f/f littermates were generated and fed with an high-fat diet (HFD; 45% Kcal from fat) for 20 weeks. Body weight was measured weekly; glucose metabolism (glucose-tolerance test (GTT) and insulin-tolerance test (ITT)) was checked at 10 and 20 weeks. Immunophenotyping of different tissues (blood, lymphnodes, adipose tissue, thymus) was performed at 20 weeks by flowcytometry.
Results. T cells activation results in the increase of MCT1 expression in both human and mouse T lymphocytes. Following high fat diet feeding, MCT1f/f CD4-cre mice in spite of a similar weight gain and insulin response compared to MCT1f/f, present a decreased visceral (VAT) and subcutaneous (SCAT) fat accumulation. Moreover, MCT1 deficiency in T cells results in a reduction of CD8+ T lymphocytes number in visceral and subcutaneous adipose tissue (VAT MCT1f/f mice 48098 cells/g±36587, MCT1f/f CD4-cre 18497 cells/g±14508, $p<0.05$; SCAT MCT1f/f 2738 cells/g±1189, MCT1f/f CD4-cre 1669 cells/g±684, $p<0.05$); this profile was associated with a different T cell subsets distribution (T effector memory (Tem) CD8+ VAT: MCT1f/f 84.20%±5.72, MCT1f/f CD4-cre 57.29±8, $p<0.001$; Tem CD8+ SCAT: MCT1f/f 72.2%±14.16, MCT1f/f CD4-cre 44.93%±14.25, $p<0.001$), but a similar number of innate immune cells (monocyte and macrophages) infiltrating adipose depots. The difference in T cells was not the consequence of increased T cell death in MCT1f/f CD4-cre mice.
Conclusions. Our data suggest that MCT1 transporter impacts T lymphocytes activation, in particular CD8+ T lymphocytes, during obesity, independently from systemic metabolism. Whether this difference can affect adipose tissue inflammation during obesity is under investigation.

RESPONSE TO PCSK9-INHIBITORS AMONG PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: A REAL WORD OBSERVATIONAL STUDY - A YOUSISANET PROJECT

M.L. Morieri[1], L. D'Erasmo[2], C. Pavanello[3], A. Buonaiuto[4], E. Paiola[5], R. Scicali[6], I. Rossi[7], N. Cocomello[2], M.R. Mannarino[8], G. Mandraffino[9], M.A. Salvati[10]
[1]Dipartimento di Medicina, Università degli Studi di Padova; [2]Dipartimento di Medicina Interna e Specialità Mediche, Sapienza, Università degli Studi di Roma; [3]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [4]Dipartimento di Medicina e Chirurgia Clinica, Università Federico II, Napoli; [5]AOUI Verona, Dipartimenti di Endocrinologia, Diabetologia e Malattie del Metabolismo, Verona; [6]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania; [7]Università degli Studi Gabriele d'Annunzio" di Chieti; [8]Medicina Interna, Dipartimento di Medicina, Università degli Studi di Perugia; [9]Dipartimento di Medicina Clinica e Sperimentale, Università di Messina; [10]Scienze Mediche e Chirurgiche, Università Magna Graecia, Catanzaro
E-mail: morieri.ml@gmail.com

Background. Despite the well-known efficacy and safety of PCSK9-inhibitors (PCSK9i) few data are available on their effectiveness in a real-world clinical setting. Aims of this study were to describe, in a population of subjects with Familial Hypercholesterolemia (FH), the response to PCSK9i and to assess the prevalence of those experiencing a lower-than-expected-response (LTER) in LDL-cholesterol reduction.
Methods. We conducted an observational, retrospective study, involving 10 clinical Italian centers (part of LIPIGEN network). Patients with clinical or genetic diagnosis of FH and with at least 6 months of follow-up after initiation of PCSK9i were included. Hypo-responders and non-responders subjects were defined by LDL-c reduction from baseline to follow-up ≤25% and ≤5%, respectively.
Results. 320 subjects initiating either Evolocumab (N=131, 41%) or Alircumab (N=189, 59%) were included, 144 (45%) were female and mean age was 56±12 years. Among 280 subjects with genetic analyses, 239 subjects were Heterozygous (HeFH) and 19 Homozygous (HoFH). Baseline LDL-c levels were 178±64 mg/dl, most patients were receiving statins (84%) and ezetimibe (86%). Over a median follow-up of 5.7 months, almost all patients continued the treatment (N=317, 99.1%), with a significant reduction of LDL-c (58%, 44-68%, P<0.0001), with 26 subjects (8.1%) being hypo-responders and 11 (3.4%) non-responders. Similar results were obtained among those 189 subjects with available data after 12 months of treatment. When the analyses were restricted to the 239 subjects with genetic HeFH diagnosis, the median LDL-c reduction was 59% (46-69%), with 14 (5.9%) hypo-responders and 3 (1.3%) non-responders.
Conclusions. In a real-world clinical setting, PCSK9i were confirmed as being highly effective in reducing LDL-c in FH. However, we showed that a small, but consistent, proportion of subjects with FH had a LTER. Further studies are needed to dissect whether the LTER to PCSK9i is linked to modifiable (e.g. adherence) or to unmodifiable (e.g. genetic) factors.

LIPOPOLYSACCHARIDE INDUCES PLATELET ACTIVATION IN HIV PATIENTS: THE ROLE OF DIFFERENT VIRAL LOAD PATTERNS

C. Nocella, I. Mezzaroma, V. Cammisotto, V. Castellani, S. Bartimoccia, P. Pignatelli, D. Pastori, R. Carnevale
Sapienza University of Rome
E-mail: cristina.nocella@uniroma1.it

Introduction. Platelets are key mediators in the pathogenesis of cardiovascular diseases, contributing to the inflammatory process and leading to atherosclerosis and cardiovascular complications. Recent experimental and clinical studies discovered that intestinal microbiota is implicated in the atherosclerotic process. As a product of gut microbiota, lipopolysaccharide (LPS), which is a major component of the outer layer of Gram-negative bacteria, may be involved in the atherothrombotic process. However, the effect of LPS on platelet activation is still controversial. Cardiovascular disease is of clinical importance among individuals with Human Immunodeficiency Virus (HIV) infection and the risk is higher still for those taking antiretroviral therapy (ART). Although ART has suppressed viremia to below the limit of detection of clinical viral load assays, it cannot eliminate viremia completely even after prolonged treatment. Residual viremia could reflect various clinical scenarios such as chronic inflammatory and immunological processes that may contribute to additional risk for cardiovascular events. In HIV patients, gut epithelial integrity is compromised, resulting in enhanced transit of microbial products such as bacterial LPS that can activate immune cells such as monocytes, dendritic cells and other cells including platelets.
Objectives. This study aimed to assess in HIV-1 patients with persistent viremia if gut-derived LPS could affect platelet function and the underlying mechanism.Methods: In a population comprised of HIV treated patients, HIV naïve patients and control subjects, LPS-mediated platelet activation and oxidative stress were assessed. In vitro, platelets from uninfected donors were exposed to plasma from HIV-infected treated and untreated patients. Platelet activation and the underlying mechanism of activation were evaluated.
Results. To evaluate the role of platelet activation during HIV infection, 39 HIV-1 treated patients, 13 naïve patients, and 50 healthy controls were enrolled to study platelet activation and oxidative stress. The results showed that platelet activation and oxidative stress biomarkers were still high in treated HIV-1 patients with persistent viremia, compared to control subjects. Similarly, serum LPS and zonulin were high in HIV-1–infected subjects compared to controls with a significant correlation with platelet activation and oxidative stress. To better understand the link between LPS and persistent viral load in the mechanism of platelet activation, in vitro experiments were performed. The exposure of platelets to plasma from HIV patients with different viral load and plasma from naïve patients increases platelet activation and oxidative stress compared to the exposure to plasma from healthy subjects. This effect was blunted in platelet pre-treated with TLR4 or TLR7 inhibitors.
Conclusions. These data suggest that in HIV patients the combined exposure to HIV-1 itself and LPS derived from the gut might drive platelet toward an increased activation that could lead to atherosclerotic progression. Intervention to modulate endotoxemia could be evaluated to assess if this therapeutic approach, added to conventional HIV therapy, may reduce platelet activation in HIV patients.

ANALYSIS OF A COURT OF DYSLIPIDEMIC SUBJECTS TESTED FOR THE DETECTION OF CAUSATIVE MUTATIONS OF FAMILIAL HYPERCHOLESTEROLEMIA

F. Nota, R. Senkeev, C. Frascaroli, F. Cavalot, K. Bonomo
SSD Malattie Metaboliche e Diabetologia,
AOU San Luigi Gonzaga di Orbassano
E-mail: fabio.nota@unito.it

Introduction. Familial hypercholesterolemia (FH) is one of the most common hereditary metabolic diseases. The Dutch Lipid Clinic Network Score (DLCNS) is the clinical risk score predominantly used to help in the diagnosis of FH. The purpose of this analysis was to evaluate the frequency and type of causative mutations of FH in dyslipidemic subjects with possible (DLCNS 3-5), probable (DLCNS 6-7) or certain (DLCNS ≥8) clinical diagnosis of FH. It is also intended to highlight the main clinical aspects in relation to the genotype.
Materials and Methods. Our Lipid Clinic participates to the LIPIGEN network of the SISA Foundation and the genetic analyses were conducted in the frame of the LIPIGEN project. Genetic analysis was performed to find mutations responsible for FH (mutations of LDL-R, APO-B, PCSK9, STAP1, APOE and LDLRAP1) in 101 subjects with DLCNS ≥3. Since the DLCN score target for the genetic analysis had not been set in the earlier phases of the Lipigen project, we had the chance to examine subjects with relatively low DLCN scores in whom the genetic analysis was equally performed.
Results. Genetic analysis showed the presence of causative mutations for FH in 54% of patients, while in 46% no mutation of the mentioned genes was found. In particular, 24/35 (69%) subjects with DLCNS ≥8 were positive, as well as 13/24 (54%) subjects with DLCNS 6-7 and 17/42 (40%) subjects with DLCNS 3-5. All positive subjects (100%) had a heterozygous mutation in the LDL-R gene. In 3 cases mutations in the PCSK9 gene were associated and in one case a mutation in the LDLRAP1 gene was found (compound heterozygotes). The positive subjects were compared with the negative subjects for the following parameters: age at diagnosis, BMI, fasting blood glucose, total cholesterol, HDL-C, triglycerides and pre-treatment LDL-C. Statistically significant differences in age at diagnosis (positive/negative: 31±15 vs 39±14 years, p=0.05), LDL-C (positive/negative: 270±80 vs 236±58 mg/dL, p=0.02) and triglycerides (positive/negative: 102±45 mg/dL vs 145±74 mg/dL, p=0.001) were found. The BMI was instead superimposable between the two groups (positive/negative: 25.3±4 vs 25.9±4 kg/m^2, p=0,504) as well as the fasting blood glucose (97±16 vs 100±21 mg/dL, p=0,661). HDL-C values were lower in the group of subjects with mutations (54±14 vs 60±16 mg/dL p=0.068); however, for the interpretation of this data, the different distribution of the sexes in the two groups must be considered (positive: 30 M+24 F; negative: 24 M+23 F). The two groups were finally compared for the presence of comorbidity and the response to hypolipidemic therapy. Among subjects with causative mutations of FH there was a significantly higher incidence of CV events compared to subjects without mutations (9/54 vs 2/47, p<0.03). The prevalence of arterial hypertension, DMT2 and peripheral arteriopathy was significantly higher in subjects negative for mutations. Subjects with a documented mutation were more frequently treated with polytherapy (high intensity statin + ezetimibe + PCSK9 inhibitors) than subjects without mutations (p=0.003). In particular, it was necessary to use PCSK9 inhibitors in 25/54 (46%) subjects with causative FH mutations, while the use of PCSK9 inhibitors occurred in 7/47 (14%) subjects with negative genetic analysis.
Conclusions. The typical phenotype of subjects with FH is characterized by a marked form of pure hypercholesterolemia with normal triglyceride and fasting blood sugar values; the diagnosis of FH is usually made earlier and the prevalence of CV events is definetely higher than in non familiar forms. Treatment with PCSK9 inhibitors is frequently needed and represents an excellent therapeutic opportunity for a considerable number of subjects with FH in order to achieve the desired therapeutic targets.

A CASE OF FAMILIAL HYPERCHOLESTEROLEMIA WITH SEVERAL RARE VARIANTS IN DIFFERENT LIPID-RELATED GENES

D. Palma[1], G. Massini[2], M.D. Di Taranto[1], C. Giacobbe[1], G. Maione, G. Cardiero[1], O. Guardamagna[2], G. Fortunato[1]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli e CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli; [2]Amb. Dislipidemie e Prevenzione Cardiovascolare, Ospedale Infantile Regina Margherita, Torino
E-mail: palmad@ceinge.unina.it

Introduction. Approximately 20% to 40% of clinically defined familial hypercholesterolemia (FH) patients do not show a causative variant in candidate genes (LDLR, APOB and PCSK9). Oligogenic FH is a condition in which the accumulation of multiple rare variants in different lipid-related genes could be causative of hypercholesterolemia in FH patients without pathogenic variants.
Patient, Materials and Methods. Here we report a 10 -years-old boy affected by abdominal pain showing severe hypercholesterolemia (total cholesterol 251 mg/dL, LDL-c 184 mg/dL, triglycerides 76 mg/dL and HDL-c 52 mg/dL). The family history was positive for hypercholesterolemia and acute myocardial infarction. Patient was screened by next-generation sequencing (NGS) to detect variants in a panel of 28 lipid related-genes. Rare variants identified by NGS were confirmed by Sanger sequencing. Pathogenicity assessment was performed by ACMG guidelines.
Results. No clearly pathogenic variants in FH-causative genes were identified, although rare variants were identified: c.2312-59C>T in LDLR gene - predicted to not affect splicing and that should be considered as a variant of uncertain significance (VUS); and c.12382G>A - p.(Val4128Met) in APOB, previously described as benign. Other VUS were found in different genes: c.293C>G - p.(Ala98Gly) in ABCG5, a gene causative of sitosterolemia; and c.1232+20G>A in LMF1 gene, a gene causative of hypertriglyceridemia. The ABCG5 variant was previously identified in FH patients in an Argentine study. No copy number variations were found.
Conclusion. Our results supported the idea of an oligogenic basis of FH, i.e. that some patient without clearly pathogenic variants in causative genes can carry several variants in lipid-related genes giving rise to the FH phenotype. In the future, a large use of extended gene panels analyzed by NGS can help to identify oligogenic cases and to determine their frequency among FH patients.

CUMULATIVE EXPOSURE TO BISPHOSPHONATES AND RISK OF CARDIO-CEREBROVASCULAR EVENTS: A POPULATION-BASED RETROSPECTIVE COHORT STUDY

E. Olmastroni[1], M. Casula[2], F. Galimberti[1], E. Tragni[1], G. Corrao[3], L. Scotti[4], A.L. Catapano[2]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan & IRCCS MultiMedica, Sesto S. Giovanni, Milano; [3]National Centre for Healthcare Research & Pharmacoepidemiology, Milan, & Laboratory of Healthcare Research & Pharmacoepidemiology, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan; [4]Unit of Biostatistics, Epidemiology, and Public Health, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan
E-mail: elena.olmastroni@unimi.it

Background and Aim. Since there is conflicting evidence for the protective role of bisphosphonates against atherosclerotic events, we aimed at investigating the effect of bisphosphonates on cardio-cerebrovascular events.
Methods. We carried out a retrospective cohort study selecting from administrative databases of Lombardy (Italy) all patients aged >40 years with a first prescription for bisphosphonates (index date) between 2003/01/01 and 2006/12/31. Subjects were followed from the index date until the date of the first hospitalization for cardio-cerebrovascular event, death, migration, or end of follow-up (2012/12/31), whichever occurred first. Exposure to bisphosphonates was characterized based on cumulative doses (proportion of days covered, PDC). Treatment's adherence was classified as low (PDC≤40%), intermediate (PDC 41%-80%), or high (PDC>80%). Cox models were fitted to estimate the association between time-dependent exposure to bisphosphonates and cardio-cerebrovascular events (adjusted hazard ratios [HRs] and 95% confidence intervals). The model was adjusted for age, sex, comorbidities, and concomitant treatments.
Results. Among 82,704 incident bisphosphonates users (females 87.0%; mean age±SD 70.7±10.6 years), we identified 13,337 individuals (16.1%) with a first hospitalization for cardio-cerebrovascular event, occurred on average (SD) 3.5 (2.3) years after the index date. Compared with individuals with an exposure ≤40% of follow-up, those exposed for 41-80% or more than 80% showed HRs of 0.95 [0.91-0.99] and 0.75 [0.71-0.81], respectively. In the sub-analysis based on type of event, a PDC>80% was associated with a reduction of both cardiovascular and cerebrovascular events (HRs 0.75 [0.68-0.83] and 0.76 [0.70-0.83], respectively). The protective effect was confirmed both in the analyses stratified by sex and age classes, and in those performed at 1 and 3 years of follow-up.
Conclusions. Although further studies to investigate possible mechanisms are warranted, it is important to consider bisphosphonates, and a strict adherence to this treatment, as having a potential effect also in the prevention of cardiovascular events.

IMPACT OF CARDIAC SURGERY TIME-OF-THE-DAY AND FASTING METABOLISM ON PERIOPERATIVE SYSTEMIC INFLAMMATION

C. Panzavolta[1], E. Woitrain[2], S. Aghessaf[2], H. Dubrulle[2], S. Ninni[2], T. Modine[2], F. Pontana[2], A. Coisne[2], A. Zambon[3], B. Staels[2], D. Montaigne[2]
[1]Clinica Medica 1, Department of Medicine - DIMED, University Hospital of Padua; [2]Department of Clinical Physiology and Echocardiography, Heart Valve Clinic, University Hospital CHU Lille & Univ. Lille & Institute Pasteur de Lille & EGID, France; [3]Clinica Medica 1, Department of Medicine - DIMED, University Hospital of Padua
E-mail: panzavolta.chiara@gmail.com

Background. Patients undergoing cardiac surgery still face a significant incidence of postoperative complications.Aim: To investigate whether peri-operative systemic inflammation induced by cardiac surgery is impacted by of time-of-the-day (morning vs afternoon) of surgery and fasting metabolism.
Matherials and Methods. Patients were included in the prospective POMI-AF Cohort Study (NCT03376165) referred to the University Hospital of Lille for coronary artery bypass grafting and/or valve surgery, from September 2018 to June 2019. Clinical data and blood samples were collected on surgery day (J0), after 24 h (J1) and after 7 days (J7), for standard metabolic profile and for PBMC (peripheral blood mononuclear cells) isolation. Moreover, Multiplex Assay for 29 serum cytokines and qPCR for NLRP3, IL1ß, IL18 on PBMC at J0-J1-J7 were performed in a subgroup of 24 patients.
Results. 87 patients were split according to surgery time-of-the-day (morning 8-11am n=51 patients; afternoon 14-16pm n=36 patients). Despite no difference was observed regarding the two groups baseline characteristics, surgery was associated with higher CRP increase in the morning vs afternoon patients ($p<0.001$ by two-way ANOVA). For 24 patients inflammatory cytokines were dosed at J1 after surgery, showing increased blood IL6, G-CSF ($p<0.001$), IL-8 ($p=0.0078$), IL-10 ($p=0.0037$) and IL-18 ($p=0.0025$). Metabolic profile of 47 patients revealed lower glucose and higher beta-hydroxybutirate (BHB) serum concentration on afternoon vs morning surgery ($p=0.0043$ by post-hoc t test), as a result of the prolonged fasting. An inverse correlation was observed between fasting BHB concentration and post-operative peak plasma CRP concentration after surgery ($r=-0.30$; $p=0.041$).
Conclusions. Cardiac surgery induces a systemic inflammation with increase of CRP, IL6, G-CSF and IL-18. CRP levels observed after afternoon surgery might result from related prolonged fasting and subsequent increase in blood ketone bodies.

ROLE OF SPHINGOSINE 1-PHOSPHATE AND ITS RECEPTORS S1P1 AND S1P3 IN THE REVERSE CHOLESTEROL TRANSPORT

B. Papotti[1], I. Zanotti[1], E. Scalera[1], D. Greco[1], S. Battista[1], F. Bernini[1], J.-R. Nofer[2], M. Simoni[3], F. Potì[4]
[1]Department of Food and Drug, University of Parma; [2]Center for Laboratory Medicine, University Hospital of Münster, Münster, Germany; [3]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena; [4]Department of Medicine and Surgery, Unit of Neurosciences, University of Parma
E-mail: bianca.papotti@gmail.com

Background and Aim. Sphingosine 1-phosphate (S1P) is an integral constituent of High-Density Lipoprotein (HDL) particles and has been proposed to contribute to many of the cardiovascular and atheroprotective effects of HDL. Indeed, S1P is a bioactive lysosphingolipid, which binds to its 5 specific G protein-coupled receptors, particularly expressed in cardiovascular system. To date, we have no direct evidence connecting endogenous S1P with cellular and systemic cholesterol handling. This study aims to investigate the role of endogenous S1P in the modulation of reverse cholesterol transport (RCT), a relevant physiological anti-atherogenic process.
Materials and Methods. We evaluated the role of S1P receptors employing a transgenic mouse model overexpressing S1P1 or S1P3 receptor in myeloid lineage (S1P1-Lyz or S1P3-Lyz, respectively). In vivo RCT was measured through a radioisotope technique by injecting 3[H]-Cholesterol-enriched MPM isolated from both CTRL and S1P1-Lyz or S1P3-Lyz mice in C57BL/6 recipient. Cholesterol efflux from cultured MPM was evaluated in CTRL and S1P1 and S1P3-Lyz MPM through a radioisotope technique, adding HDL (12,5µg/ml) or murine plasma (0,5% and 2% v/v) as cholesterol acceptors.
Results and Conclusions. Mice injected with S1P1-Lyz MPM displayed an increased 3[H]-Cholesterol elimination in faeces compared to CTRL MPM-injected mice (0,38%±0,009 vs 0,30%±0,01; p<0,001). Upon incubation with acetylated LDL, S1P1-Lyz MPM are characterized by an increased cholesterol efflux to plasma compared to CTRL MPM (5,42%±0,16 vs 4,33%±0,38; p<0,001). S1P1-Lyz MPM stimulated with LXR/RXR agonists showed an increased cholesterol efflux to HDL (5,24%±0,77 vs 4,34%±0,38; p<0,05). In vivo total RCT resulted higher in S1P3-Lyz MPM injected mice compared to CTRL MPM-injected group, as 3[H]-Cholesterol found in plasma (0.99%±0.32 vs. 0.60%±0.12; p<0.05), liver (2.66%±0.41 vs 1.99%±0.35; p<0.01) and faeces (0.99%±0.19 vs 0.66%±0.10; p<0.01) was higher in the former. Consistently, acLDL-loaded S1P3-Lyz MPM displayed an increased cholesterol efflux to HDL (8.47%±0.63 vs 5.93%±0.46; p<0.001) and mouse plasma compared to CTRL (8.04%±0.43 vs 10.49%±1.2; p<0,001, and 22.99%±1.2 vs 37.09%±5.43; p<0,001, to 0.1% and 2% mouse plasma, respectively). Similarly, S1P3-Lyz MPM stimulated with LXR/RXR agonists showed an increased cholesterol efflux to HDL (7,45%±1,36 vs 5,31%±0,37; p<0,01) and plasma (6,17%±1,17 vs 3,76%±0,46; p<0,01) compared to CTRL MPM. Endogenous S1P, through the interaction with its receptors S1P1 and S1P3 on macrophages, positively modulates cholesterol metabolism by improving RCT, thus exerts a potentially anti-atherogenic function in vivo.

ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN LIVERS OF KNOCK-IN MOUSE MODELS BY MICROARRAY TECHNOLOGY

C. Parolini[1], G.S. Ganzetti[1], E. Rigamonti[2]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]IRCCS Ospedale San Raffaele Scientific Institute, Milan
E-mail: cinzia.parolini@unimi.it

The natural variant of human apoA-I, i.e. the apoA-IMilano (A-IM), is the result of a point mutation, with an arginine to cysteine substitution at position 173. Carriers of this mutation exhibit hypertriglyceridemia with markedly reduced HDL and apoA-I plasma levels, a condition generally associated with a high risk of premature coronary disease. Evaluation of the cardiovascular status in A-IM carriers, compared with control subjects from the same kindred, did not reveal any evidence of increased CVD. In addition, whether A-IM may impart a "gain of function" compared to wild-type apoA-I still an open question. This study was aimed at investigating intrinsic differences in the livers of mice expressing human apoA-I or A-IM, by using the Affimetrix GeneChip Mouse Gene ST system. To this aim, previously generated A-I (A-I k-in) or A-IM knock-in mice (A-IM k-in) were crossed with transgenic mice expressing human apoA-II but lacking of murine apoA-I (hA-II) to generate hA-II/A-I k-in, and hA-II/A-IM k-in, respectively. hA-II/A-IM k-in mice were characterized by lower HDL cholesterol and A-I/A-IM plasma levels and by higher triglyceride concentrations compared to both hA-II/A-I k-in and A-IM k-in mice. The expression of 871 genes was significantly altered between the hA-II/A-I k-in and hA-II/A-IM k-in mouse lines, of which 373 up- and 498 down-regulated in hA-II/A-I k-in compared to hA-II/A-IM k-in mice. 1018 differentially expressed genes, 434 up- and 584 down-regulated, were instead found in A-IM versus hA-II/A-IM k-in animals. Comparison of the up-regulated genes by Venn diagrams revealed 46 genes in common to hA-II/A-I k-in, hA-II/A-IM and A-IM k-in mice. Among these, the Elovl6 gene, a key lipogenic enzyme, has been discovered. Protein association networks (STRING database) of Elovl6 highlighted that A-IM could be associated with a modulation of fatty acid (FA) metabolism (i.e. FAs synthesis and catalysis) and biosynthesis of unsaturated FAs.

EFFECT OF THE RS1051338 POLYMORPHISM OF LIPA GENE ON LIPID PHENOTYPE, LIVER ASSESSMENT AND LYSOSOMAL ACID LIPASE ACTIVITY: POSSIBLE ASSOCIATION WITH NAFLD

A. Pasta[1], A.L. Cremonini[1], P. Borro[2], A. Picciotto[3], R. Fresa[3], G. Tozzi[4], L. Pisciotta[5]
[1]Department of Internal Medicine, University of Genoa; [2]IRCCS S. Martino Polyclinic Hospital, Genoa; [3]Department of Internal Medicine, University of Genoa;IRCCS S. Martino Polyclinic Hospital, Genoa; [4]Bambino Gesù Children's Hospital, Rome; [5]Department of Internal Medicine, University of Genoa; IRCCS S. Martino Polyclinic Hospital, Genoa
E-mail: andreapasta93@gmail.com

Background. Lysosomal Acid Lipase deficiency (LAL-D) is an autosomal recessive disease characterized by hypoalfalipoproteinemia, mixed hyperlipemia and hepatic steatosis. The rs1051338 polymorphism in LIPA gene has been previously associated with increased risk of atherogenic dyslipidemia, metabolic syndrome, obesity and cardiovascular disease and, in vitro, could adversely affect the LAL activity (LAL-A). Non-Alcoholic Fatty Liver Disease (NAFLD) is the presence of fat in the liver (FL) after the exclusion of other secondary causes. The aim of the study was to value the impact of rs1051338 rare allele (c.46C) on lipid phenotype, hepatic steatosis and LAL-A. Hepatic fibrosis and liver transaminase were also evaluated.
Materials and Methods. We included 45 subjects with hypoalphalipoproteinemia and, at least, one of the following conditions: hypertriglyceridemia, transaminase elevation or hepatic steatosis. Demographic and anthropometric parameters, lipid and glycemic profiles and liver transaminases were evaluated for all patients. Genetic analysis was performed with the length of the restriction fragments method and LAL-A was measured on dry blood spot with standard technique. All included patients underwent ultrasonographic evaluation with the "Controlled Attenuation Parameter" (CAP) technique and hepatic stiffness evaluation with "shear wave" emitter. Statistical analysis was performed with IBM SPSS.
Results. The presence of rare c.46C (genotypes c.46AC or c.46CC) allele resulted in statistically higher levels of triglycerides and hepatic transaminase and lower levels of HDL cholesterol compared to wild type genotype (c.46AA). The rare c.46C allele is associated to higher values of CAP compared to controls (332 308 - 346 vs 295 275 - 325 between c.46AC+c.46CC vs c.46AA with p=0.033) and the multivariate analysis highlighted the independent association between the rare C allele and the severity of hepatic steatosis in subjects with NAFLD. Among genotypes none differences in the other parameter evaluated were identified. In conclusion, the rs1051338 is associated with NAFLD and atherogenic dyslipidemia.

DIFFERENT CLINICAL MANIFESTATIONS DESPITE A COMMON LIPID PHENOTYPE IN THREE NOVEL CASES OF TANGIER DISEASE

C. Pavanello[1], L. Pisciotta[2], R. Spina[3], C. Scrimali[3], S. Castelnuovo[4], G. Mombelli[4], A. Branchi[5], E. Cassinerio[6], A. Baldassare Cefalù[3], S. Bertolini[2], M. Gomaraschi[1]
[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Dipartimento di Medicina Interna, Università di Genova; [3]Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), Università di Palermo; [4]Centro Dislipidemie, Dipartimento Cardiotoracovascolare, ASST Grande Ospedale Metropolitano Niguarda, Milano; [5]Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano; [6]Dipartimento di Medicina Interna, Fondazione IRCCS Policlinico, Milano
E-mail: chiara.pavanello@unimi.it

Tangier disease (TD) is a very rare autosomal recessive disorder caused by mutations in the ABCA1 gene. It is characterized by near absence of plasma high-density lipoprotein cholesterol (HDL-C), accumulation of cholesterol in multiple tissues, peripheral neuropathy, and accelerated atherosclerosis. However, clinical manifestations have a high variability. Here we report three new cases of Tangier disease harboring both known and novel mutations in ABCA1 gene. Case 1, a 25-year old male of Iranian origin, is homozygote for a novel intronic variant, c.721-2A>G, predicted to produce a short truncated protein; case 2, a 45-year old male, is homozygote for the novel nonsense mutation W1526X. Interestingly, he is also heterozygous carrier of the common variant E8SJM in LIPA gene. Case 3, a 56-year old female, is compound heterozygote for two previously reported missense variants (Asn1800His/Arg1068His). All the patients show severe hypoalphalipoproteinemia with nearly absence of both apoA-I and apoA-II. Plasma LDL-C levels are also reduced; in addition, case 3 displays hypertriglyceridemia. None of them is receiving lipid-lowering treatment. Besides the common lipid abnormalities, subjects display different clinical manifestations. Indeed, thrombocytopenia and hepatosplenomegaly are present in case 1 and in case 2, consistent with the diagnosis of TD. However, neither of them displays signs of preclinical atherosclerosis. Conversely, case 3 has no signs of blood cells abnormalities and normal liver and spleen, but she has increased carotid IMT, with several stenotic plaques. Our findings provide insights into the diverse clinical phenotype of this rare disorder and suggest that ABCA1 deficiency must be considered in patients with low HDL-C, regardless the presence of other clinical manifestations.

SYSTEMATIC LAB KNOWLEDGE INTEGRATION FOR MANAGEMENT OF LIPID EXCESS IN HIGH-RISK PATIENTS: RESULTS FROM THE SKIM LEAN PROJECT

C. Pavanello[1], M. Parolini[2], A. Alberti[3], M. Carenini[4], P. Maino[4], G. Mombelli[3], F. Pazzucconi[1], G. Origgi[5], F. Orsi[5], M.G. Trivella[2], L. Calabresi[1], R. De Maria[2]
[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Istituto di Fisiologia Clinica del CNR, Dipartimento Cardiotoracovascolare, ASST Grande Ospedale Metropolitano Niguarda, Milano; [3]SSD Diagnosi e Cure Territoriali per Malattie Cardiache, Dipartimento Cardiotoracovascolare, ASST Grande Ospedale Metropolitano Niguarda, Milano; [4]Dedalus S.p.A., Bologna; [5]Sistemi Informativi Aziendali, ASST Grande Ospedale Metropolitano Niguarda, Milano;
E-mail: chiara.pavanello@unimi.it

Despite the big improvements of the recent years in the clinical management of cardiovascular disease, the rate of underdiagnosed and undertreated high-risk subjects contributes to the still raised CVD burden worldwide. Implementation of a systematic approach in the management of CV risk may represent a strategy to reduce the social and economic impact of this disease. Methods: The SKIM LEAN project exploits the big data deriving from electronic health records of the Niguarda Hospital catchment area, integrated with clinical database and routine laboratory tests for the identification and early referral to specialist care of patients with hypercholesterolemia, who may be inadequately controlled according to their risk level. The project included a retrospective epidemiological analysis and a prospective assessment phase in subjects with suspected familial hypercholesterolemia and in patients with a previous atherothrombotic event (ASCVD). In addition, the population-level changes in lipid profile between the first (2016 and 2017) and second (2018) data extraction were assessed. A total of 617,793 laboratory records were processed between January and December 2016 and 25,248 patients (females 43.7%) with at least one valid LDL-C value available were identified. Seven hundred and fifty-eight subjects with suspected familial hypercholesterolemia and 2253 with previous ASCVD were eligible for the prospective phase.
Major findings:
1) At follow-up, 12% of FH patients achieved the recommend target of LDL-C <100 mg/dL or at least a 50% reduction from baseline.
2) At follow-up, the proportion of patients in secondary prevention with LDL-C levels <100 mg/dL increased from 39% to 69%.
3) In the overall population, a significant decrease in LDL-C concentrations was observed between 2017 and 2018.

Conclusions. The SKIM LEAN project provided evidence for the feasibility and usefulness of the use of a systematic approach in the identification and treatment of high-risk patients, finally improving prevention of costly CV events.

VASCULAR ACCESS IN LIPOPROTEIN APHERESIS: SAVE THE VEIN, SAVE THE LIFE

M. Pianelli, R. Luciani, F. Sbrana, B. Dal Pino, F. Bigazzi, T. Sampietro
Lipoapheresis Unit, Reference Center For Diagnosis And Treatment Of Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa
E-mail: francesco.sbrana@ftgm.it

Background. Vascular access is life-saving for patients who depend on Lipoprotein Apheresis (LA), such as those suffering from inherited dyslipidaemias.
Methods. Since 1994, we performed more than 500 treatment/year and a total of 67 patients (male 73%) were treated in 25 years (34 current patient and 33 former patients). We revised data regarding vascular access in these patients.
Results. LA techniques were: dextran-sulphate absorption from plasma (Liposorber®-LA MA-03 systems; Kaneka, Osaka, Japan; 19/34 current patients), heparin-induced LDL precipitation apheresis (HELP®; B. Braun, Melsungen, Germany; 10/34 current patients), immunoadsorption (TheraSorb™ - LDL pro Adsorber, Miltenyi biotec, Bergisch Gladbach, Germany; 5/34 current patients). Thirty-three patients discontinued LA because: therapeutic target was reach by PCSK9i therapy (17/33); treatment refusal (8/33), death (5/33), terminal renal failure requiring dialysis (2/33), lack of reliable vascular access (1/33).Only in 5 cases arteriovenous fistula were performed before LA treatment. It occurred in 4 female and in an 8-year-old child affect by Homozygous Familial Hypercholesterolemia who started LA 26-years ago. In this patient the venous arterial fistula was subsequently surgically closed due to a good development of brachial venous system. Out of 4 females, a patient had failure of arteriovenous fistula secondary to thrombosis; this patient discontinued LA for inability to obtain reliable vascular access and started PCSK9i drug. All the other patients chronically continued LA without significant long-term problems on vascular access.
Conclusions. In own Center we paid particular attention to nurse skills in vascular access management and in the chosen needle: we mostly used the Diacan® Set (B. Braun, Melsungen, Germany) because is extremely thin-walled (diameter 18G; length 20 mm), allow a laminar flow, ensures a high blood flow rate (90-95 ml/minute) with a minimal trauma to the vascular access. An incorrect management of vascular accesses can lead to discontinuation of a life-saving treatment as LA.

USE OF COMPLEMENTARY MEDICINE IN A SAMPLE OF PATIENTS IN CARDIOVASCULAR PREVENTION CLINIC

M. Pulicanò, A. Maretta, V. Spagnuolo
Lipid Center Cosenza
E-mail: vitalianos@yahoo.com

Complementary medicine (CM) can play a role improving health and well-being and can be integrated with traditional medicine. It is often self-prescribed and it can expose to potential risks from drug interactions with conventional medicine drugs.We evaluated, in a group of outpatients in a dedicated clinic to prevention of cardiovascular diseases, the use of CM.We enrolled the first hundred outpatients, aged over 50, of a Cardiovascular Prevention Clinic (Lipid Center Cosenza). All hundred patients were given a questionnaire (validated questionnaire proposed in an AARP/NCAAM study) concerning use of CM in the last 12 months. Fifty-five were females. Average age was 66.6±8.45 years; 68.2±6.4 (females), 64.7±10 (males). Twenty-five patients reported use of CM approved by our legal system. Osteopathy and chiropractic were the most used (15 patients); posturology (5 patients), herbs (5 patients). Females were more prone to use CM (20 vs 5). The most frequently given motivation to use CM was to solve a certain pathological condition (77%) rather than prevent a disease. Regarding to full awareness in carrying out CM, 15 patients out of 25 fully knew the indication for use before starting it, and the same fifteen had received precise indications from their doctor. Seventy-five were already being treated with at least one drug before enrollment (average 2.35±1.9). Of the 25 patients who used CM, all took at least two drugs (average 3.6±1.2).The fact that they have taken substances without having informed a healthcare professional has potential for risk, given the possible interaction between medicines also in the field of CM. CM is widely used in our reality, but often without the patient informing the doctor. Better management of counseling is therefore necessary to improve the indication and safety of the use of CM.

BARIATRIC SURGERY IMPROVES, BUT DOES NOT COMPLETELY RESTORE BETA CELL FUNCTION IN OBESE DIABETIC PATIENTS

E. Rinaldi[1], M. Trombetta[1], G. Ceradini[1], A. Altomari[1], M.L. Boselli[1], L. Santi[1], M. Battistoni[2], R. Bonadonna[3], E. Bonora[1], M.G. Zenti[1]
[1]Endocrinologia, Diabetologia e Malattie del Metabolismo, Università e AOUI Verona; [2]Chirurgia Generale e dell'Obesità, AOUI Verona; [3]Endocrinologia e Malattie del Metabolismo, Dipartimento di Medicina e Chirurgia, Università e Azienda Ospedaliera di Parma
E-mail: elisabettarinaldi@hotmail.com

Background. Impact of bariatric surgery(BS) on the different components of glucose homeostasis is a matter of active investigation. Aim of the study was to investigate the changes induced by BS on beta-cell function (BCF) insulin clearance (IC) and I-Sen during time in a group of type 2 diabetic (TD2) patients.
Methods. 10 T2D-obese patients (M/F=5/5, mean age: 54.3y) underwent BS (3 sleeve gastrectomy, 7 RYgastric bypass). We used a standardized 5h-mixed-meal-test (MMT: 186 Kcal; polenta plus parmesan cheese) at baseline(MMT0), at 1 month (MMT1) and at 12 months (MMT12) after surgery to assess:
1) glucose, lipid levels, insulin, C-peptide, GIP and GLP-1 concentrations;
2) BCF expressed as derivative (DC) and proportional control (PC);
3) I-Sen expressed as Oral Glucose Insulin Sensitivity index (OGIS);
4) IC. Then we analyzed the net effect of BCF, IC and I-Sen in determining variations of glucose tolerance over time. Glucose metabolism was also compared with 9 healthy subjects with normal glucose tolerance.

Results. BMI and waist circumference decreased at 1-month (Δweight: -13.5 kg; Δwaist: -10 cm; $p<0.01$ for both) and at 12-months (Δweight: -36.2 kg; Δwaist: -31 cm; $p<0.001$). Diabetes remission occurred in 80% of patients. The area under curve(AUC) of glucose was reduced by 23% (MMT1) and 32% (MMT12) ($p=0.007$ for both) and the AUC insulin by 40% and by 56% during MMT1 ($p=0.017$) and MMT12 ($p=0.005$). The overall work of the beta-cell decreased by 14% ($p=0.047$) and by 47% ($p=0.007$), respectively. I-Sen significantly improved after surgery ($p=0.005$). An improvement of the adaptation of the beta-cell secretion to the degree of I-Sen was observed, mainly one year after surgery. A complete restoration of the relation between I-Sen and I-Sec was not observed.
Conclusions. BS in T2D-obese subjects determines an improvement in I-Sen and a restoration of the dynamic of I-Sec after meal. The effect was more evident 1 year after surgery but already detectable after 1 month. However, the relation of insulin availability and I-Sen improved a little only in the long term and remained subnormal. This data highlights the necessity of a regular follow-up of patients in whom a remission is achieved.

STATIN THERAPY IS ASSOCIATED WITH BETTER AMBULATORY BLOOD PRESSURE CONTROL: A PROPENSITY SCORE ANALYSIS

L. Ristori[1], F. Spannella[2], A. Filipponi[2], F. Giulietti[2], C. Di Pentima[2], V. Bordoni[2], R. Sarzani[2]
[1]Internal Medicine and Geriatrics, "Hypertension Excellence Centre" of the European Society of Hypertension, IRCCS INRCA, Ancona, Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona; [2]Internal Medicine and Geriatrics, "Hypertension Excellence Centre" of the European Society of Hypertension, IRCCS INRCA, Ancona, Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona
E-mail: letizia.ristori@gmail.com

Objective. Statin therapy was associated with lower blood pressure (BP) in some but not all studies. We evaluated the association between statin therapy and 24 h BP in a large hypertensive population using "propensity score matching".
Methods. Retrospective observational study on 1827 consecutive essential hypertensives evaluated with 24 h ambulatory BP monitoring. Anti-hypertensive treatment intensity (ATI) was calculated to compare different drug associations. We used a propensity score matching to compare two equally-sized cohorts of patients with similar characteristics according to statin therapy. Matching was performed on log-transformed propensity score in a 1:1 fashion with a caliper of 0.1, in order to account for the different baseline characteristics between statin and no-statin group.
Results. Mean age: 58.1±13.8 years; male sex: 55%. Patients on statin therapy: 402 (22%). These patients showed lower 24 h BP (-2.8/-7.1 mmHg), daytime (-3.3/-7.6 mmHg) and night-time BP (-2.5/-6.0 mmHg, all p<0.001). They also showed better ambulatory BP control, even after adjustment for confounding factors. The analyses on the groups derived from the "propensity score matching" (369 patients in each group) confirmed these results (OR 1.8 for 24 h BP control; OR=1.6 for daytime BP control; OR=1.7 for night-time BP control, all p<0.001).
Conclusions. Statin therapy is associated with better ambulatory BP control in essential hypertensives. This result is not affected by the intensity of anti-hypertensive treatment or by the several cofactors analyzed.

23-MONTHS FOLLOW-UP TO ASSESS THE EFFICACY AND TOLERABILITY OF EVOLOCUMAB IN A YOUNG PATIENT WITH CHRONIC AND PROGRESSIVE ISCHEMIC ARTERIOPATHY

I. Rossi, D. D'Ardes, F. Vezzani, F. Cipollone, M. Bucci
Centro Ipertensione e Dislipidemie, Università di Chieti
E-mail: mbucci@unich.it

Background. PCSK9 inhibitors represent a revolution for patients who do not reach their LDL-C target and need invasive and expensive treatments such as LDL-apheresis.
Aim. We evaluated efficacy and tolerability of Evolocumab 140mg (E140) Q2W during a follow-up period of 23 months in a 57-yo patient with a history of early coronary heart disease (recurrent episodes of unstable angina and myocardial infarction treated with PTCA+ stent) and non-target LDL-C values despite standard lipid-lowering therapy (LLT) at the maximum tolerated dose (LDL-C consistently >100-120 mg/dL, Lp(a) >100 mg/dL).
Case report. E140 Q2W was added on top of the patient's LLT (rosuvastatin 20 and ezetimibe 10mg OD) in October 2017, when his LDL-C was 144 mg/dL and his Lp(a) 104 mg/dL. Lipid profile and liver function were constantly monitored. Target LDL-C was rapidly achieved (lowest LDL-C =1mg/dL in March 2019), with an average reduction of 92,9%. The average Lp(a) reduction was 65,55% (lowest value: 54 mg/dL). Symptoms related to coronary atherosclerosis were stabilized for the first time in the last 7 years, without need for additional revascularization procedures. The exercise stress test showed a good effort tolerance in June 2018. Adherence and tolerability were excellent. LDL-C remained between 1-23 mg/dl during the observation period without adverse events; this allowed to suspend ezetimibe and reduce the statin dosage. A genetic analysis was performed to confirm the existence of a familial hypercholesterolemia and polymorphisms of Lp(a) (DLCN=6): no mutations were detected; however the LDL-C score according to FerrerInCode (Lipigen Project) was 1.23, suggesting a high probability of a polygenic cause1.
Conclusions. In this difficult case E140 Q2W has led to an LDL and Lp(a) reduction much greater than expected according to literature2,3, symptoms' stabilization with a considerable improvement in QoL and also a relevant money saving for the National Health Service.

References

1. Pirillo A, et al. Spectrum of mutations in Italian patients with familial hypercholesterolemia: New results from the LIPIGEN study. Atheroscler. 2017; (Suppl. 29), 17-24.
2. Ference BA, Cannon CP, Landmesser U, Lu TF. Reduction of low density lipoprotein-cholesterol and cardiovascular events with proprotein inhibitors and statins : an analysis of FOURIER, SPIRE, and the Cholesterol Treatment Trialists Collaboration. Eur. Hear. J. 2018; 39: 2540-2545.
3. Qamar A, et al. Interindividual Variation in Low-Density Lipoprotein Cholesterol Level Reduction With Evolocumab An Analysis of FOURIER Trial Data. JAMA Cardiol. 2018; 2115: E1-E5.

CHOLESTEROL LDL LEVELS AND ESTIMATION OF THE PREVALENCE OF FAMILY HYPERCHOLESTEROLEMIA IN A POPULATION OF THE AREA OF BOLOGNA

A. Rossolini[1], A.F.G. Cicero[1], F. Ventura[1], E. Ramazzotti[1], R. Mancini[1], F. Tombariv[3], D. Patron[2], S. Palmisano[1], C. Borghi[1], S. D'Addato[1]
[1]Università di Bologna; [2]Laboratorio Unico Metropolitano di Bologna; [3]Farmacia Clinica AOSP di S. Orsola, Bologna
E-mail: arianna.rossolini@studio.unibo.it

Objectives. Although Familial hypercholesterolemia is the most common genetic cause of early coronary artery disease, its recognition only occurs in a few cases (<1% in Italy) and only 20% of the affected patients reach the recommended LDL cholesterol levels. The purpose of this work is to estimate the prevalence of probable and possible Familial Hypercholesterolemia according to the DLCN among people who have checked their lipid profile at Laboratorio Unico Metropolitano di Bologna between 01/06/2017 and 05/31/2018. Using DLCNs, we only have considered the levels of c-LDL as the analysis of these parameter alone is enough to define the diagnosis of possible or probable FH.
Materials and Methods. This is a retrospective observational study in which anonymous data from the biochemical exams included in the normal care procedure were used. Age, sex and total cholesterol, c-HDL, triglyceride and d-LDL c-levels were extrapolated for each patient. Patients with triglycerides >=300 mg/dL and / or aged <20 years were excluded. The calculated LDL cholesterol was derived through the Friedwald formula. Since DLCNs are based on pretreatment c-LDL values and the pharmacological history is missing in this study, each c-LDL value is increased by 3.45%. This correction consists in the dilution of the effect of cholesterol-lowering drugs on the entire population: the value was obtained by multiplying the defined daily dose of cholesterol-lowering drugs in the province of Bologna by the average capacity of this therapy to reduce the c-LDL; on the basis of lipid-lowering drugs mostly dispensed by the Sant'Orsola hospital pharmacy, the average lipid-lowering power is -40%. Calculated c-LDL values were analyzed by the DLCNs and the population was divided according to the probability of diagnosis of familial hypercholesterolemia (possible with 3 and 5 points: c-LDL =191-250 mg/dL and 251- 325 mg/dL, probable with 8 points: c-LDL >325 mg/dL).
Results. The sample of patients considered is made by 109188 subjects with a maximum age of 109 years and a minimum of 20 years (mean: 62.6 years ± ds 16.4) and a maximum total cholesterol level of 621 mg/dL and minimum of 24 mg/dL (average: 199.8 mg/dL ± ds 44.2). Correcting the effect of the possible therapy shows that 77.14% of subjects have c-LDL <155 mg/dL, 17.63% have c-LDL =155-190 mg/dL, 4.94% has c-LDL =191-250 mg/dL, 0.27% has c-LDL =51-325 mg/dL and 0.03% has c-LDL >325 mg/dL. Applying the DLCN scores to these values we found out that, with only the analysis of calculated c-LDL levels, 5.23% of subjects meets the possible diagnostic requirements (of which 4.94% with 3 points and 0, 27% with 5 points) and 0.03% those of probable diagnosis (with 8 points). Analyzing these results it is evident that 0.29% (1 on 340) of our sample has a high risk of being affected by Familial Hypercholesterolemia.
Conclusions. Our results confirm the prevalence of FH estimation emerged from other studies and can be reasonably extended to the entire Bologna's population aged >=20 years and with triglycerides <300 mg/dL.

COMPARATIVE EFFECTS OF ADD-ON THERAPY WITH PCSK9 INHIBITORS OR EZETIMIBE ON LIPID PROFILE AND ON ARTERIAL STIFFNESS IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS WITH INADEQUATELY CONTROLLED LDL CHOLESTEROL DESPITE HIGH INTENSITY STATINS: TWO-CENTER EXPERIENC

F. Savarino[1], G. Mandraffino[1], R. Scicali[2], J. Rodriguez-Carrio[3], F. Mamone[1], M. Scuruchi[1], M. Cinquegrani[1], E. Imbalzano[1], A. Di Pino[2], S. Piro[2], A.M. Rabuazzo[2],
[1]Department of Clinical and Experimental Medicine-University of Messina; [2]Department of Clinical and Experimental Medicine-University of Catania; [3]Area of Immunlogy, Department of Functional Biology, Faculty of Medicine, University of Oviedo, Oviedo, Spain; Instituto de Investigacion Sanitaria del Principado de Austrias (Ispa), Ovieo, Spain
E-mail: francescasavarino@hotmail.it

Background. FH is characterized by elevated LDL-C levels and high CV risk. Prognosis correlates with lifelong LDL-C levels, and therapy aims to achieve and maintain LDL-C target over years. Despite high intensity statins, only few FH patients achieve the recommended low-density lipoprotein cholesterol (LDL-C) targets.Vascular dysfunction occurs years before atherosclerosis is detectable by instrumental tests. PWV is considered an early, reliable marker and independent prognostic predictor for CV mortality.
Aim. We aimed to evaluate the efficacy of six-month add-on therapy with proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-i) or ezetimibe on lipid profile and on vascular arterial function, in a setting of LDL-C off-target HeFH patients (primary/secondary prevention).
Methods. We enrolled 98 genetically confirmed FH patients with an LDL-C off-target despite high-intensity statins; of these, 45 patients added ezetimibe (EZE group) and 53 added PCSK9-i (PCSK9-i group). At baseline and 6 months after add-on treatment we tested for blood chemistry, arterial stiffness (SphygmoCor) and anthropometric parameters.
Results. After 6 months of add-on therapy, the majority of patients achieving LDL-C targets were in the PCSK9-i group (77.3% PCSK9-i group vs 37.8% EZE group, p<0.001). The PCSK9-i group also achieved a significant PWV reduction compared with the EZE group [-51% vs -22.8%, p<0.001 and -15% vs -8.5%, p<0.01, respectively]. In a linear regression analysis we showed a beta index of 0.334 for the relationship between ΔPWV and ΔLDL (p<0.05); particularly, this relationship was stronger in FH patients without cardiovascular events (β=0.422, p<0.01).
Conclusions. The addition of PCSK9-i or ezetimibe to high intensity statin therapy significantly improves lipid and PWV profiles in FH patients; moreover, ΔPWV is associated with ΔLDL. Our study confirms the additional beneficial role of these novel therapies in FH subjects.

A LARGE ITALIAN COHORT ON PCSK9-INHIBITORS: A SINGLE CENTER EXPERIENCE

F. Sbrana, B. Dal Pino, F. Bigazzi, A. Ripoli, E. Volpi, M.P. Fogliaro, G. Lo Surdo, M. Pianelli, R. Luciani, S. Biagini, T. Sampietro
Lipoapheresis Unit, Reference Center for Diagnosis and Treatment of Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa
E-mail: francesco.sbrana@ftgm.it

Background. PCSK9-inhibitors (PCSK9i) represents a breakthrough in the treatment of hypercholesterolemia as efficiently and safely reduce LDL-C and cardiovascular events.Aim of this study was to perform a single-center retrospective analysis on the effects of PCSK9i, introduced in Italy on 2017.
Methods. During an observation period 122 patients affected by primary hypercholesterolemia (mean age 60±11 years, male 75%) add PCSK9i (evolocumab 83/122; alirocumab 39/122) to the maximally tolerated lipid-lowering therapy. Patient's clinical characteristics, lipid value modification, acute cardiovascular events (ACVE) and adverse events (AEs) reported during the follow-up were analysed.
Results. 49/122 patients had previously participated in the PROFICIO program of evolocumab and the other 73/122 patients started PCSK9i therapy in accordance with Italian prescription rules. Lp(a)-hyperlipoproteinemia was present in 40/122; 86/122 had ischemic heart disease (IHD) and 34/122 subject were chronically on Lipoprotein Apheresis (LA). At the end of follow-up (13±6 months), a significant decrease in total cholesterol (-38%, p<0.001), triglycerides (-15%, p<0.001), LDL cholesterol (-52%, p <0.001) and Lp(a) levels (-8%, p0.006) was observed. PCSK9i therapy allow the discontinuation of LA in 16/36 patients but only 27/86 (31%) of patients with IHD reached an LDL cholesterol below 55 mg/dl. A sub analysis on HeHF patients with IHD, do not reveal significant difference on lipid modification in subjects treated, every 14-days, with alirocumab 150 mg or evolocumab 140 mg. During the follow-up 10/122 patients had an ACVE and 33/122 had an AEs which led, in 4 cases, to discontinuation of PCSK9i therapy. Furthermore, other 4 subjects discontinued PCSK9i therapy: 2 patients due renal failure worsening and 2 patients were lost in the follow-up.
Conclusions. Our data are in agreement with the large evidence on tolerability, effectiveness and cardiovascular event reduction.

ASSOCIATION BETWEEN ACHILLES TENDON XANTHOMAS AND CARDIOVASCULAR DISEASE IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: A PILOT STUDY

P. Scarinzi[1], M. Biolo[1], P. Bigolin[2], C. Panzavolta[1], M.V. Rabuini[1], R. Marin[1], D. Vianello[1], S. Bertocco[1], F. Fabris[1], E. Manzato[1], S. Zambon[1], L. Previato[1], A. Zambon[1]
[1]Dipartimento di Medicina, Università degli Studi di Padova;
[2]Ospedale di Castelfranco Veneto
E-mail: scarinzi.paolo@gmail.com

Background. Patients with familial hypercholesterolemia (FH) have an increased lifetime risk of cardiovascular disease (CVD) due to high levels of plasma low-density lipoprotein cholesterol (LDL-C). Achilles tendon xanthomas (ATX) are acknowledged clinical diagnostic criteria of FH. Despite recent studies analyze the association between ATX and atherosclerotic cardiovascular disease, their clinical predictive value remains unknown.
Aim. To investigate ATX clinical significance and their possible association with CVD.
Methods. From January 2016 to July 2018, 95 subjects with FH were included: 83 with FH genetically confirmed, 12 with clinical diagnosis based on Dutch Lipid Clinic Network (DLCN) score >8. For each patient we collected: clinical examination, blood lipid profile, familial and personal medical history considering CVD risk factors and adverse cardiovascular events including acute coronary syndrome, angina, coronary intervention, stroke and peripheral arterial disease. The presence of ATX were detected also with bilateral ultrasonography (US), considering as US positive sign hypoechogenic formations or a tendon's maximum diameter thickness >6.0 mm (Sensibility 72%, Specificity 70%).
Results. ATX was observed in 71 patients (M=26, F=45). These patients showed higher mean age (48.1±14.8 vs 40.3±16.7 years, p<0.05) and LDL-C concentration (320.9±107.5 vs 235.9±50.7 mg/dl, p<0.05) compared to the group without ATX These characteristics could explain the greater DLCN score (9.1±4.6 vs 5.0±2.7, p<0.05) and the longer treatment time (88.8±92.5 vs 35.8±57.5 months, p<0.05) observed in these patients. By the way traditional CV risk factors in the two groups did not show any significant difference. Moreover, in multivariate logistic regression analysis the AT thickness was positively associated with CVD (OR 1.39, 95% C.I. 1.10-1.75, p<0.05).
Conclusion. In FH subjects we observed the association between Achilles tendon thickness and CVD, that seems to be independent of other known risk factors. These results emphasize the importance to explore the presence of tendon xanthomas and their implication in the setting of atherosclerotic cardiovascular disease.

ANALYSIS OF HDL-MICRORNA PROFILE IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITH LDL RECEPTOR NULL OR DEFECTIVE MUTATION

R. Scicali[1], A. Di Pino[1], C. Pavanello[2], A. Ossoli[2], A. Strazzella[2], A. Alberti[2], S. Di Mauro[1], A. Scamporrino[1], F. Urbano[1], A. Filippello[1], S. Piro[1], A. M. Rabuazzo[1], L. Calabresi[2], F. Purrello[1]
[1]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania; [2]Centro E. Grossi Paoletti, Dipartimento i Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano
E-mail: robertoscicali@gmail.com

Background and Aims. In the last few years increasing attentionhas been given to the connection between genotype/phenotype andcardiovascular events in subjects with familial hypercholesterolemia(FH). MicroRNAs (miRs) bound to high-density lipoprotein (HDL) maycontribute to better discriminate the cardiovascular risk of FH subjects. Our aim was to evaluate the HDL-miR profile in heterozygous FH (HeFH)patients with an LDLR null or defective mutation and its association withpulse wave velocity (PWV).
Methods. We evaluated lipid panel, HDL-miR profile and PWV in 32 LDLRnull mutation (LDLR-null group) and 35 LDLR defective variant (LDLRdefectivegroup) HeFH patients. All subjects were on statin treatment atthe time of enrollment.
Results. The LDLR-null group exhibited a significant increase of HDL-miR486 and 92a than the LDLR-defective group ($p<0.001$ for both).When wefurther stratified the study population into three groups according toboth the LDLR genotype and history of ASCVD (LDLR-null, LDLRdefective andLDLR-ASCVD group), both the LDLR-ASCVD and the LDLR-null groups had ahigher expression of HDL-miR 486 and 92a than the defective group (forLDLR-ASCVD versus LDLR-defective group $p<0.05$ for both; for LDLR-nullversus LDLR-defective group $p <0.05$ for both). Finally, HDL-miR 486 and92a were independently associated with PWV ($p<0.01$ for both).
Conclusion. The LDLR-null group exhibited an increase of HDL-miR 486 and92a with respect to the LDLR-defective group. Further studies are neededto evaluate these HDL-miRs as predictive biomarkers of cardiovascularevents in FH patients.

MUTATION IN CANDIDATE GENES ACCOUNT FOR A SMALL MINORITY OF HYPOBETALIPOPROTEINEMIAS AND NGS ANALYSIS SUPPORT POLYGENICITY IN MUTATION-NEGATIVE PATIENTS

C. Scrimali, R. Spina, V. Ingrassia, F. Brucato, V. Valenti, A.B. Cefalù, G. Misiano, G.I. Altieri, F. Fayer, D. Noto, A. Giammanco, C.M. Barbagallo, A. Ganci, M.R. Averna
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) University of Palermo
E-mail: chiara.scrimali@libero.it

Introduction. Hypobetalipoproteinemias (HBL) represent an heterogeneous group of disorders characterized by plasma levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. Two major monogenic forms of HBL are well characterized:
1) FHBL1 (familial hypobetalipoproteinemia 1, OMIM#615558), a co-dominant disorder due to mutations in APOB gene or, less frequently, in PCSK9 gene.
2) FHBL2 (familial hypobetalipoproteinemia 2, OMIM#605019) due to mutations in ANGPTL3 gene.

The approach to molecular diagnosis of HBL has changed in the last years with Next-generation sequencing approach (NGS) replacing the traditional Sanger Sequencing method.
Materials and Methods. In this study, we described a novel diagnostic workflow for molecular characterization of HBL disorder by NGS approach. We designed a custom panel in order to analyze known genes involved in HBL by Ion Torrent PGM. The analysis was conducted on 23 patients.
Results. The HBL-custom panel allowed to identify 4 novel pathogenic APOB gene mutations in four probands. In 19 patients, targeted next generation DNA sequencing revealed several rare variants ($MAF \leq 0.005$) predicted by informatics tools to be possible/probably damaging allowed to filter different significant variants in APOB, PCSK9 and ANGPTL3 genes and in other genes known to regulate LDL-C levels through different pathways (ie, ANGPTL8, LDLR, MYLIP, PEMT, SORT1). In several patients the HBL phenotype could be due to the oligogenic interaction involving multiple rare variants in different genes, each of which may contribute to determine the clinical and biochemical phenotype.

SERUM ENDOCAN LEVELS IN CARDIOVASCULAR RISK CLINICAL CONDITIONS

M. Scuruchi[1], A. D'Ascola[1], G.M. Campo[1], S. Campo[2], A. Avenoso[2], J. Rodríguez Carrìo[3], A. Saitta[4], G. Mandraffino[4]
[1]Department of Clinical and Experimental Medicine, Internal Medicine Unit, University of Messina; [2]University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Images, University of Messina; [3]Area of Immunology, Department of Functional Biology, Faculty of Medicine, University of Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain; [4]Department of Clinical and Experimental Medicine, Internal Medicine Unit, University of Messina
E-mail: mscuruchi@unime.it

Background. Endocan is a small soluble proteoglycan produced and secreted by human endothelial cells, detectable in peripheral circulation, considered an indicator of angiogenesis and endothelial cell activation. Studies have shown that Endocan levels increase in cancer and sepsis; its increase during chronic inflammatory conditions is under evaluation so far, because its expression may be influenced by VEGF-α and TNF-α, cytokines involved in pathogenic pathways in chronic inflammatory disease.
Aim. To evaluate plasma Endocan levels in patients with different degree of vascular and/or systemic inflammation; here we present the results of an extensive evaluation of serum Endocan in different clinical conditions characterized by chronic inflammatory status and increased endothelial reaction.
Methods. We measured Endocan in healthy controls (10), statin-treated hypercholesterolemic (HC) patients (28), untreated scleroderma (SSc) patients (30), patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) IBD (20), by a commercially available ELISA kit. CRP levels were also measured by routine methods.
Results. Endocan levels in controls were 337.73±64.01 pg/ml; in statin treated HC we found a mean value of 183.1±59.41; in SSc patients 497.15±281.43; in refractory IBD patients 348.9±196.63. HC patients presented with lower Endocan levels with respect to controls ($p<0.0001$). Endocan levels in SSc and IBD patients did not differ from controls. Endocan correlates to CRP levels in HC patients ($r=0.515$), while no significant relationship we found in controls, in IBD and in SSc patients.
Conclusions. Endocan is involved in a variety of biological processes including cell proliferation, migration, and neovascularization. Its levels are reported to be correlated with inflammatory cytokines, and to be closely related to the development and progression of CVD. However, while in statin-treated HC patients we found a significant correlation between CRP and Endocan plasma levels, we cannot confirm this relationship in controls, IBD and SSc patients.

EFFECT OF ENDURANCE TRAINING AND HIGH INTENSITY INTERVAL TRAINING ON BODY COMPOSITION AND CARDIO-METABOLIC RISK FACTORS IN A POPULATION OF OBESE ADULTS: A RANDOMIZED CONTROLLED TRIAL

C.M. Semprini[1], A. D'Amuri[1], J. M. Sanz[1], F. Vaccari[2], S. Lazzer[2], E. Capatti[1], F. Di Vece[1], E. Dalla Nora[1], G. Zuliani[3], A. Passaro[4]
[1]Department of Medical Sciences, Section of Internal and Cardio Respiratory Medicine, University of Ferrara; [2]Department of Medicine, University of Udine; [3]Department of Morphology, Surgery and Experimental Medicine, Section of Internal and Cardio Respiratory Medicine, University of Ferrara; [4]Department of Medical Sciences, Section of Internal and Cardio Respiratory Medicine, University of Ferrara
E-mail: smpcrm@unife.it

Aim. The prevalence of obesity is constantly increasing and the mainstay of therapy remains the association of diet and exercise. The guidelines recommend 250-400 min/week of continuous aerobic exercise of moderate intensity (Endurance Training, ET) but the adherence remains generally low. High Intensity Interval Training (HIIT) allows shorter training sessions than ET, with the same energy expenditure, but its role in the treatment of obesity remains unclear. The aim of this study is to analyze the effects of two different exercise protocols (ET vs HIIT) on anthropometric parameters and cardiovascular risk factors, in a population of obese subjects.
Patients and Methods. 32 obese subjects (aged 18-45 years), with no history of cardiovascular and respiratory disease nor diabetes, were enrolled. Subjects were randomized into two iso-energetic exercise arms (3 times/week for 16 weeks): ET (60% training VO2MAX) and HIIT (3-7 series of 3 min repeats at 100% VO2MAX interspersed with 1,5 min at 50% of VO2MAX). Before and after intervention we evaluated: arterial pressure, anthropometry (weight, BMI, waist and hip circumference), body composition, lipids and glucose metabolism.
Results. In both arms we observed a statistically significant reduction of diastolic blood pressure, weight, waist and hip circumference. Impedance analysis showed a reduction in FM and an increase in FFM. There was a reduction in total cholesterol, C-LDL and ApoB100. There were no changes in HDL-C, triglycerides, glycemia, insulinemia or HOMA-IR index. In men, waist decreased more with HIIT than ET. The average duration HIIT was significantly shorter than ET.
Conclusions. HIIT is at least as effective as ET in the treatment of obesity and associated cardiovascular risk factors, although it requires smaller exercise volumes. This could result in higher compliance. Moreover, HIIT could be more effective in reducing visceral adipose tissue, at least in men.

ENDOCAN LEVELS ARE ASSOCIATED TO CD34+ CIRCULATING CELL NUMBER IN SYSTEMIC SCLEROSIS

D. Sinicropi[1], A. Lo Gullo[1], M. Cinquegrani[1], F. Savarino[1], M. Scuruchi[1], S. Loddo[1], J. Rodriguez-Carrio[2], A. Saitta[3], G. Squadrito[3], G. Mandraffino[3]
[1]Department of Clinical and Experimental Medicine, Internal Medicine Unit, University of Messina; [2]Area of Immunology, Department of Functional Biology, Faculty of Medicine, University of Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias (Ispa), Oviedo, Spain; [3]Department of Clinical and Experimental Medicine, Internal Medicine Unit, University of Messina
E-mail: sinicropidavide@gmail.com

Background. Systemic sclerosis (SSc) is an autoimmune chronic disease characterized by vascular alterations of small arteries and microvessels. Circulating CD34+ cell number is acknowledged to be associated to cardiovascular health status in several chronic conditions, including chronic immune-inflammatory disease. CD34+ cell number was found inconstantly reduced in SSc. Endocan is a proteoglycan expressed by endothelial cells likely interacting with white blood cells, recently suggested as a marker of vascular stress.
Methods. We selected 27 (26 female, 61±13.5 years) patients out of 36 SSc patients (35 female) we have previously enrolled; CD34+ cell number (2.6±0.73), CRP (0.59±0.97), ESR (22.09±17.57), serum uric acid (SUA, 3.90±1.05), creatinine (0.76±0.37), Vitamin D3 (27.26±10.98), Rodnan skin score (28.89±10.24) and two frozen plasma samples should be available for all of these patients. Then, we randomly selected 15 patients to determine plasma Endocan levels.
Results. We found no correlation between Endocan and Rodnan skin score, ESR, fibrinogen or SUA; we found a trend of correlation between Endocan and Vitamin D levels (r= -0.315), red blood cells distribution width (r=0.310), CRP (r=0.310), but statistical significance was not reached due to the small sample size. We found an association between endocan levels and PAPs (rho=0.442, p=0.045). The only significant (inverse) correlation we found was between Endocan and CD34+ cell number (rho=-0.605, p=0.017).
Conclusion. In our study population, we found a significant correlation between CD34+ cell number and Endocan plasma levels; Endocan and CD34+ progenitor cells can be suggested as potential marker of disease status.

PCSK9-D374Y MEDIATED LDL-R DEGRADATION CAN BE FUNCTIONALLY INHIBITED BY EGF-A AND TRUNCATED EGF-A PEPTIDES. AN IN VITRO STUDY

R. Spina, C. Scrimali, V. Ingrassia, F. Brucato, G.I. Altieri, D. Noto, V. Valenti, G. Misiano, A. Giammanco, F. Fayer, C.M. Barbagallo, A. Ganci, A.B. Cefalù, M.R. Averna
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) University of Palermo
E-mail: rossella.spina@libero.it

Introduction. PCSK9 (proprotein convertase subtilisin/kexin type 9) is a modulator of the cellular LDL receptor (LDL-R) and plasma cholesterol levels. Gain-of-function mutations of PCSK9 cause hypercholesterolemia and early-onset coronary heart disease therefor inhibition of PCSK9 is being used as an approach to reduce plasma LDL cholesterol levels. Although the molecular mechanism of action of PCSK9 is not yet completely clear, various approaches for inhibiting the PCSK9/LDL-R binding interaction have therefore investigated and developed. Different therapeutic approach as RNA interference, antisense DNA and monoclonal antibodies are not the most desirable for chronic asymptomatic conditions such as hyperlipidemia. Peptides are now emerging as therapeutic candidates to bridge the gap between small-molecule drugs (<500 Da in mass) and large biologics, including antibodies (>5,000 Da) and they may offer potentially more convenient routes of administration and lower costs.
Materials and Methods. In this study, we evaluated whether a synthetic EGF-A domain of LDL-R could inhibit LDL-R degradation mediated by the D374Y-PCSK9 mutant and restore LDL uptake using HuH7 liver cells as a model system. A similar set of experiments were performed by adding a shorter 25-amino acid truncated EGF-A analog (P1). HuH7 cells were treated with serum free media or media containing EGF-A peptide/P1 at three different concentrations. Western blot analysis and internalization assay were performed in order to evaluate the expression and the functionality of LDL-R. As control, we also tested the effect of treatment with alirocumab in cells transfected with PCSK9-D374Y.
Results. Transient transfection of cells with PCSK9-D374Y expression vector very effectively enhanced degradation of mature LDLR in Huh7. The treatment with both EGF-A and EGF-A truncated peptide (P1) inhibited this effect and showed increased LDLR protein in Huh7 cells transfected with PCSK9-D374Y in a clear concentration dependent manner. Huh7 transfected cells treated with increasing concentration of EGF-A analogs also showed an increase internalization of labeled Dil-LDL. In our in vitro system the treatment with EGF-A analogs appeared to be more effective in the inhibition of the degradation of LDL-R compared to the treatment with monoclonal antibody against PCSK9. The result of our study shows that EGF-A analogs are able to effectively hamper the enhanced degradation of LDLR in liver cells expressing PCSK9-D374Y. The binding between PCSK9 and LDL-R can be modulated by peptides administration and that our therapeutic approach is suitable for patients affected by familial hypercholesterolemia due to GOF mutations in PCSK9 gene.

ROLE OF TALMUD POLYGENIC SCORE IN A TUSCANY COHORT OF FAMILIAL HYPERCHOLESTEROLEMIA WITH AND WITHOUT LDLR GENE MUTATIONS

E. Sticchi[1], A. Kura[2], A. Volta[2], R. De Cario[2], A. Murri[2], A. Magi[3], S. Suraci[2], A.M. Gori[1], R. Marcucci[1], B. Giusti[1]
[1]Department of Experimental and Clinical Medicine, University of Florence, Atherothrombotic Diseases Center, Careggi Hospital, Florence; [2]Department of Experimental and Clinical Medicine, University of Florence; [3]Department of Information Engineering, University of Florence
E-mail: elenasticchi@hotmail.com

Background. Familial Hypercholesterolemia (FH) represents an autosomal disorder due to pathogenic variants in LDLR or APOB or PCSK9 (dominant form), and in LDLRAP1 (recessive form). Previous data showed that in about 60% of patients who are mutation-negative the clinical phenotype can be associated with an accumulation of common small-effect LDL-C-raising alleles using a 12- Single nucleotide polymorphisms (SNPs) score. Aim of this study was to evaluate Talmud (2013) genetic score in patients with FH with and without variants in LDLR gene.
Methods. We analysed 45 patients with clinically possible/probable or definite FH using the most common diagnostic algorithm, Dutch Lipid Clinic Network Score. SNPs included in Talmud score were detected through high-throughput (HTS) or Sanger sequencing. HTS was performed using Illumina MiSeq Reagent Kit. Sequencing results were analyzed using a pipeline optimized by the Florentine bioinformatics group. The possible pathogenicity of variants was evaluated using five different in silico tools. For statistical analysis we used SPSS v.25 software.
Results. Among 45 patients analyzed, 20 had a pathogenetic variant in LDLR and 25 did not carry mutations in this gene. Significantly higher Talmud score mean value in patients without LDLR mutation with respect to those with LDLR variant was observed [mean±SD: 1.015±0.140 vs 0.894±0.196, p=0.02]. Mean Talmud score value found in LDLR mutation negative group relates to a risk ratio (95% CI) of LDL-C>4.9 mmol/L 2.87 (2.04-4.05) (7th LDL-C gene score decile). Conversely, in patients carrying LDLR mutations mean score value relates to a risk ratio (95% CI) 2.21 (1.55-3.17) (5th LDL-C gene score decile).
Conclusion. The results suggest a possible contribution of common variants in modulating the lipid profile in FH patients. Further expansion of present data will allow to confirm the hypothesis that FH can also be caused by an accumulation of common small-effect LDL-C-raising alleles.

ACCUMULATION OF PRE-β HDL IN GENETIC LECITHIN: CHOLESTEROL ACYLTRANSFERASE DEFICIENCY: ROLE IN RENAL DISEASE

A. Strazzella, A. Ossoli, M. Turri, L. Calabresi
Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli studi di Milano
E-mail: arianna.strazzella@unimi.it

Genetic LCAT deficiency is a rare disorder due to "loss of function" mutations in the LCAT gene, which codifies for the only human enzyme able to esterify cholesterol in plasma.Carriers' lipid and lipoprotein profile shows deep alterations, in particular a dramatic reduction in HDL-C, the accumulation of small, discoidal and immature preβ-HDL and the appearance of the abnormal lipoprotein, called LpX. The major cause of morbidity and mortality in LCAT deficient carriers is the kidney disease, and the renal damage has been described both at glomerular and tubular level. While the role of LpX in the onset of glomerular damage has been already demonstrated, the impact of the accumulation of preβ-HDL in the pathogenesis of the renal disease is still unknown; thus, the aim of the work is to evaluate the potential role of pre-β HDL in the onset of renal damage and to investigate the involved molecular mechanisms.
Methods. For in vivo study, Lcat-/- and Wild Type mice were injected with 0.05 mg of synthetic preβ-HDL every 48 hours for four weeks. The lipid profile and Blood Urea Nitrogen (BUN) were measured and the HDL subclasses distribution was evaluated by bidimensional electrophoresis. For the in vitro study in tubular kidney cells, HDL were isolated from LCAT deficient carriers and controls' plasma by ultracentrifugation or synthesized (rHDL) starting from apoA-I and phospholipids. Oxidative stress and apoptosis were evaluated.
Results. After four weeks of treatment, Lcat-/- mice lipid profile showed deep alterations compared to WT mice, with an increase of total cholesterol (+14,64%, p=0,008 vs basal), triglycerides (+176%, p=0,008 vs basal) and phospholipids (+111,94%, p=0,002 vs basal). Moreover, the plasma renal damage marker BUN was enhanced (+19,07%, p=0,006 vs basal), and the urine albumin to creatinine ratio slightly increases. To further investigate the molecular mechanisms of the renal damage, tubular cells were incubated with homozygous carriers' HDL and ROS production was evaluated; the result showed a significant increase of the oxidative stress (+28,57%, p=0,0489 vs HDL control) after the incubation with homozygous' lipoproteins. Similar results were obtained with rHDL (+30,10%, p=0.028 vs control), resembling the endogenous preβ-HDL for shape and size, confirming the role of these particles in the increased ROS production.The evaluation of apoptosis, which is reported to be related to oxidative stress, highlighted an enhanced expression of the pro-apoptotic genes BAX and BAD (+169,44%, p=0.034 vs control; +83,6%, p=0.033 vs control, respectively) after the incubation with rHDL, suggesting an increase of this process; the result was confirmed also by the enhanced caspase-3 and -7 activity (+23,2%, p=0.034 vs control).
Conclusion. In conclusion, the accumulation of preβ-HDL is involved in the onset of tubular damage in LCAT deficient carriers and some possible nephrotoxic mechanisms could be represented by the increase in oxidative stress and apoptotic process.

EFFECT OF WESTERN TYPE DIET ON PROTEOGLYCANS SIGNATURE IN THE AORTA OF LDL-R KO MICE

M. Svecla[1], G. Garrone[2], F. Fare[2], P. Uboldi[1], F. Bonacina[1], A.L. Catapano[1,4], G. Beretta[3], G.D. Norata[1]
[1]*Department of Pharmacological and Biomolecular Sciences, University of Milan;* [2]*Unitech Omics platform, University of Milan;* [3]*Department of Environmental Science and Policy ESP, University of Milan;* [4]*IRCCS MultiMedica, Sesto S. Giovanni, Milan*
E-mail: Monika.Svecla@unimi.it

Introduction. Proteoglycans (PG) are essential components of the extracellular matrix. They are composed from a protein core and glycosaminoglycans (GAGs, polysaccharides with repeatable disaccharide units). GAGs play an important role in pathogenesis of atherosclerosis as contribute to lipoprotein retention in the subendothelial matrix of the vascular wall. Aim of this work is to assess the protein expression of proteoglycans and the signature of GAGs N-glycosylation in the aorta of LDL-R KO mice fed with a western type diet (WTD).
Materials and Methods. 8 weeks old LDL-R KO mice were fed chow or western type diet (WTD, 0,15% of cholesterol) to induce atherosclerosis, then plasma and aorta were collected. Aorta was processed for shotgun proteomics analysis in Orbitrap Fusion™ Tribrid™ Mass Spectrometer to profile the proteome and to identify N-glycoproteins differentially expressed. Glycopeptidase F allowed to compare N-glycosylated profile in the different samples. OpenMS, Proteome discoverer and MaxQuant software were used to perform protein interference and quantification while Network Analyst, KEGG pathway database and David 6.7 were used for gene ontology and pathway enrichment analysis.
Results. Plasma cholesterol and triglycerides levels chow were (Chol. 211,64±24,85 mg/dl, TG 126,76±0,92 mg/dl) and in WTD (Chol. 518,9±53,41 mg/dl, TG 266,16±56,86 mg/dl. Proteomics from aorta identified 1.166 proteins, n=592 proteins reached the minimum parameters established for quantification. Among them, 94 proteins were significantly modulated in the aorta of LDL-R fed a WTD (p-value <0,05, Log2FC >1). Among different GAGs identified there was an altered expression of dermatan sulfates (Decorin, downregulated, Biglycan, upregulated), chondroitin sulfate (Perlecan, downregulated) and keratan sulfate (Lumican, downregulated). Deamination in aspargine has shown more than 10 N-glycosylation sites for each of proteoglycans.
Conclusions. Our data suggest that hypercholesterolemia affects not only proteoglycans expression within the arterial but also impact their N-glycosylation signature. How a different glycans signature in GAGs correlates with atherosclerotic plaque development is under investigation.

LOSS-OF-FUNCTION ABCG5 MUTATION IN A HYPERCHOLESTEROLEMIC PATIENT TREATED WITH COMBINATION THERAPY

C. Trenti, E.A. Negri, S. Canovi, L. Vecchia, T. Fasano
AUSL, IRCCS Reggio Emilia
E-mail: Tommaso.Fasano@asmn.re.it

A relevant proportion of patients clinically diagnosed as having familial hypercholesterolemia (FH) do not harbor causative mutations in FH-causing genes such as LDLR, APOB, and PCSK9. Recent findings suggest that rare and deleterious mutations in ABCG5/ABCG8 genes contribute substantially to mimicking the FH phenotype. We investigated a patient with clinical diagnosis of probable FH with no mutation in FH-causing genes. A 42 yo male patient presented to the lipid clinic with hypercholesterolemia (TC 310 mg/dl, LDL-C 243 mg/dl, HDL-C 41 mg/dl, TG 129 mg/dl) and a family history of premature CHD, with two sons (respectively 15 and 9 yo) having LDL-C above the 95th percentile for sex and age. Dutch score of the patient was 6. Next generation sequencing for a panel of FH-related genes was performed on patient's DNA. Lipid-lowering therapy was established and lipid profile monitored.Statin therapy was started (atorvastatin 20 and 40 mg/daily) with a modest LDL-C reduction (LDL-C 204 mg/dl, -16% compared to no therapy). Genetic analysis was negative for mutations in LDLR, APOB and PCSK9 genes. A heterozygous mutation in ABCG5 gene was discovered in patient's DNA (Exon 7, c.805G>A, Gly269Arg). The two sons were found to share the same ABCG5 mutation with the father. Ezetimibe (10 mg) was added to statin therapy and a robust LDL-C reduction was observed (LDL-C 118 mg/dl, -42% compared to atorvastatin alone). Surrogate markers of cholesterol absorption were not quantified.A dominant pattern of segregation for a deleterious ABCG5 mutation with hypercholesterolemic phenotype was found in the family described. In our patient, net LDL-C reduction was only modest with statin therapy. Based on the results genetic analysis, ezetimibe was added to therapy and a significant reduction of LDL-C was observed. Our data support the idea that genetic testing is important to establish a correct lipid-lowering therapy.

LIPOPROTEIN (A), METABOLIC SYNDROME COMPONENTS AND CIGARETTE SMOKING ARE INDEPENDENT PREDICTORS OF CARDIOVASCULAR EVENTS IN MEDITERRANEAN WOMEN (PROGETTO ATENA)

M. Tripaldella[1], M. Gentile[1], V. Simeon[2], G. Iannuzzo[3], A. Mattiello[3], A. Buonaiuto[3], F. Forte[3], A. Iannuzzi[4], M.D. Di Taranto[5], S. Panico[1], P. Rubba[1]
[1]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" di Napoli; [2]Unità Statistica Medica, Dipartimento di Salute Mentale e Fisica e Medicina Preventiva, Università degli Studi della Campania 'Luigi Vanvitelli', Napoli; [3]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" di Napoli; [4]UO Medicina Interna, AORN "A. Cardarelli" di Napoli; [5]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli and CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli
E-mail: mariatripaldella@gmail.com

Background. Cardiovascular disease (CVD) is the primary cause of mortality globally and ischaemic heart disease is responsible for one third of all female deaths.
Methods. Twenty-five years after a baseline visit, blood glucose and Lipoprotein a), (Lp [a]) were measured, in a group of 296 women participating in Progetto Atena (N=5062). Women were divided into a control group (group 1, N=147) and a group (group 2, N=148) who experienced cardiovascular events after the initial visit. Low density lipoprotein (LDL) diameter and LDL score, a marker of small dense LDL, were also determined.
Results. The two groups were of comparable age, group 1 (55.9±7.6 yrs), group 2 (55.8±7.6 yrs). LDL Cholesterol (p=0.014), Triglycerides (p=0.006), Systolic blood pressure (p=0.004), Diastolic blood pressure (p=0.003), LDL score (p=0.012) and Lp(a) (p=0.026) were higher in the group with cardiovascular events, while HDL-cholesterol (p<0.001) and LDL diameter were lower (p=0.021). There were more smokers (p=0.001) in the group reporting cardiovascular events. In conditional logistic regression analysis, elevated Lp(a) (Rate ratio, RR 1.26, p=0.03), smoking (RR 4.45, p<0.001) and Metabolic Syndrome components were independently associated with increased risk of having CVD. Among individual Metabolic Syndrome components, low HDL cholesterol (RR 0.97, p=0.05), high Systolic Blood pressure (RR 1.02, p=0.002) and presence of diabetes mellitus (RR 4.8, p<0.05) were independently related to CVD.
Conclusions. In this group of women, elevated Lp(a), smoking habits and Metabolic Syndrome components were independent predictors of CVD.

CAN FAMILIAL HYPERCHOLESTEROLAEMIA BE DIAGNOSED AT BIRTH? A CASE REPORT

C. Viggiano, R. Stracquadaino, S. Paci, E. Verduci, G. Banderali, C. Pederiva
Clinica Pediatrica, Ospedale San Paolo, ASST-Santi Paolo e Carlo, Università degli Studi di Milano
E-mail: claudiaviggiano79@gmail.com

Introduction. Heterozygous Familial Hypercholesterolaemia (FH) is a common codominant monogenic dyslipidaemia associated with premature cardiovascular disease (CVD) due to lifelong elevation of plasma levels of LDL-C. Around 95% of FH cases are caused by mutations in LDL-R. Timely identification and treatment of children with severely elevated cholesterol levels, can be an important step in reducing cardiovascular events
Aim. It is increasingly recognised that childhood end early adolescence offer the most favourable timeframe for diagnosing FH as well as introducing and maintaining lifelong treatment and management strategies. We report a case of heterozygous familial hypercholesterolaemia diagnosed at birth.
Results. It is an Italian child, born in our Hospital, with important family history for hypercholesterolemia and premature CVD (father and first cousin with FH in drug therapy, grandfather with miocardial infarction at 34y) that was blood-sampled for lipid profile and genetic screening when it was four days old. The lipid profile was normal (mg/dl): TC: 98, LDL-C:34, HDL-C:29, triglycerides:109, Lp(a):2. The genetic screening showed a pathogenetic mutation in heterozygous in the LDL-R gene, eson 10, c.1466A >G (also present in the father and first cousin). At subsequent checks, the lipid profile remained normal up to 18 months of life when it reached atherogenic values (mg/dl): TC:212, LDL-C:150, HDL-C:40, triglycerides: 110. Diet intervention with less consumption of fat, more fruit, vegetables and whole grain, was started very early, with excellent compliance of the whole family. The stature-ponderal growth has always been within limits. At the last check (June 2019), LDL-C was very high (mg/dl): TC:250, LDL-C:195, HDL-C:43, triglycerides: 61 and we planned to start drug therapy.
Conclusions. Family history is one of the keys to early diagnosis, but the analysis of the candidate genes for FH helps in the clinical management and in the screening of affected family members, gives a definitive diagnosis and avoids delays in the diagnostic process and in the implementation of appropriate treatment.

THREE NEW ITALIAN CASES OF LCAT DEFICIENCY

M. Turri[1], B. Dal Pino[2], F. Sbrana[2], F. Bigazzi[2], L. D'Erasmo[3], . Minicocci[3], B. Arosio[4], D. Azzolino[4], C. Pavanello[1], T. Lucchi[5], M. Arca[6], T. Sampietro[2], L. Calabresi[1]
[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Lipoapheresis Unit and Reference Centre for Inherited Dyslipidaemias, Fondazione Toscana Gabriele Monasterio, Pisa; [3]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [4]Metabolic Disease Clinic-Geriatric Unit, Fondazione IRCCS Ca' Granda , Ospedale Maggiore Policlinico, Milan, Department of Clinical Sciences and Community Health, University of Milan; [5]Metabolic Disease Clinic-Geriatric Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; [6]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome
E-mail: marta.turri@icloud.com

Background. Lecithin:cholesterol acyltransferase (LCAT) is the unique human enzyme capable of catalysing the formation of cholesteryl esters in plasma. The reaction takes place both in HDL, preferential substrate for LCAT, and in LDL. LCAT deficiency is a rare recessive disorder caused by loss-of-function mutations in the human LCAT gene. More than 90 LCAT mutations have been described, which lead to two different syndromes, familial LCAT deficiency (FLD, OMIM #245900) and fish-eye disease (FED, OMIM #136120). Diagnosis of FLD and FED is based on biochemical parameters: in FLD subjects, the lack of LCAT activity is complete; in FED subjects, LCAT loses its ability to esterify cholesterol on HDL, but it retains its activity on LDL. Biochemical alterations in LCAT deficiency are characterized by very low HDL cholesterol levels with deep modifications of HDL subpopulation distribution, high triglyceride levels, and the presence of lipoprotein X, an abnormal lipoprotein absent in physiological conditions. Clinical manifestations are different in FLD and FED cases: they both show corneal opacity, while anemia and renal disease, the major cause of morbidity and mortality, affect mainly FLD carriers. The role of LCAT in atherogenesis is still debated; FLD carriers normally do not show subclinical atherosclerosis (measured as carotid Intima-Media Thickness, IMT), while FED cases show an increased carotid IMT.
Methods. Blood samples have been collected after an overnight fast and plasma separated by low-speed centrifugation at 4 °C. A complete lipid–lipoprotein profile, has been determined using a Roche Integra c311 analyzer. It includes plasma total and unesterified cholesterol, VLDL, LDL and HDL cholesterol, triglycerides, phospholipids, apoA-I, apoA-II and apoB. Plasma cholesterol esterification rate (CER) and LCAT activity, that reflect the ability of endogenous LCAT to esterify cholesterol within endogenous lipoproteins and exogenous HDL, respectively, have been assessed by using standardized methods. Genetic analysis have been performed on carriers' blood samples.
Results. Three new carriers of LCAT deficiency (1M/2F) have been recently identified and characterized. Two not related subjects (1M, 63 y.o.; 1F, 62 y.o.) are homozygotes for the Val309Met mutation, and one (1F, 20 y.o.) is a compound heterozygote for the Val139Ala stop 6 and Ile202Thr. Based on absence of LCAT activity and cholesterol esterification rate, the three cases have been diagnosed FLD. They all show very low HDL-C levels (7- 9 mg/dL) and reduced apoA-I (28-30 mg/dL). Plasma LDL-C is also reduced in all of them (33-56 mg/dL). The unesterified to total cholesterol ratio is very high (76%-94%); one carrier shows high plasma triglycerides (337 mg/dL). HDL subclasses analysis shows a high content of small native HDL particles, and absence of large HDL. The 3 carriers show corneal opacity and anemia. Two of them (1M/1F, carriers of the Val309Met mutation) present severe renal disease (serum creatinine 3.5 and 2.9 mg/dL) and they are waiting for kidney transplantation. The compound heterozygote display no sign of atherosclerosis, but unexpectedly the homozygous carriers for the Val309Met mutation, show dramatic multivessels atherosclerosis and have history of severe cardiovascular disease, contrary to what it has been described for FLD carriers, and despite the low LDL-C levels. This peculiar phenotype, which might be related to the specific LCAT mutation, is presently under investigation.

A DOUBTFUL CASE OF RABDOMIOLYSIS

F. Vezzani, I. Rossi, D. D'Ardes, F. Cipollone, M. Bucci
Centro Ipertensione e Dislipidemie, Università di Chieti
E-mail: mbucci@unich.it

Case report. A 52-yo female patient, suffering from systemic atherosclerotic disease (aortic ulcer and common iliac artery stenosis), came to our Center 5 years ago for a diagnostic reassessment of a severe hypercholesterolemia. In her history there were a cerebellar cavernous angioma and a previous episode of rhabdomyolysis due to fluvastatin hesitated in a myotonic myopathy with a consequent absolute contraindication to statin use. Her family history was positive for premature cardio-cerebrovascular events. The genetic test we performed was positive for the LDLR variant c.661A>G (p.Asp221Asn) in heterozygosis. She was referred to the Transfusion Center of Pescara to perform LDL-apheresis Q2W from 2014 (without other lipid-lowering therapy) with only partial efficacy and persistence of moderately elevated LDL-C values. Ezetimibe, introduced in her therapy, was suspended due to an increase in CPK (3-4xUNL). In 2015 she had a stroke and a MI treated with PTCA+stent. In 2016 she started taking Alirocumab 150 mg Q2W in association with LDL-apheresis without reaching the LDL-C recommended target. To increase patient compliance, in 2018 she was directed to her home nearest Aferesis Center, but since her pre-apheresis LDL-C remained >150 mg/dL, with the patient's informed consent, we tried to reintroduce a low dose of pravastatin obtaining an improvement in LDL-C (80 mg/dL) with an asymptomatic slight increase in CPK.
Discussion. Patient's LDL-C target <551 (<40) mg/dL, according to the current guidelines, is difficult to reach considering baseline values. However, with the current strategy, we have obtained a stable reduction from the basal values >50%. Meanwhile, we are evaluating and reconsidering the possibility of a genetic neurological disorder (mild neuromotor symptoms and a daughter with a neuromotor problematic not yet defined) perhaps responsible for the symptomatology and the rise in CPK. Despite the moderate increase in CPK during pravastatin, our patient is currently asymptomatic. The perspective is to titrate a well-tolerated and sufficiently effective statin and perhaps associate ezetimibe in order to limit the need for LDL-apheresis.

Reference

1. Society, E. et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur. Heart. J. 2019; 0, 1.78.

INTRAMUSCULAR INJECTION OF HD-AD VECTOR EXPRESSING A SECRETED LDLR/TF CHIMERIC PROTEIN AMELIORATES LIPID PROFILE IN LDLR-DEFICIENT MICE

M. Vitale[1], E. Leggiero[2], A. D' Agostino[3], G. Labruna[4], B. Lombardo[1], L. Tripodi[3], L. Pastore[5]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli; [2]CEINGE-Biotecnologie Avanzate, Napoli; [3]SEMM-European School for Molecular Medicine, Naples; [4]CEINGE-Biotecnologie Avanzate, Napoli; [5]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli
E-mail: leggiero@ceinge.unina.it

Familial hypercholesterolemia (FH) is an inherited disorder of lipid metabolism mainly due to mutations in the LDL receptor (LDLR) gene and is characterized by premature onset of cardiovascular disease due to high plasma LDL cholesterol concentrations. FH patients homozygous for LDLR mutations do not always respond to conventional therapies and have often a poor prognosis. Therefore, more effective therapeutic strategies, such as gene therapy, are still of main interest. We have recently developed a safe and effective gene therapy strategy based on liver expression of a secreted chimeric protein composed of the extracellular portion of the LDLR linked to a transferrin dimer using a helper-dependent adenoviral (HD-Ad) vector. This chimeric protein binds LDL and removes them from the bloodstream by receptor-mediated endocytosis through the transferrin receptor (TfR). As previously demonstrated, intravenous administration of this HD-Ad vector in LDLR-deficient mice resulted in an amelioration of the lipid profile and a reduction of aortic atherosclerosis. In order to increase safety of this strategy for a possible clinical application, we have recently generated a HD-Ad vector for a muscle-restricted expression of the mLDLR/mTF chimeric protein using a muscle-specific promoter and intramuscular administration of the vector. We observed expression of the chimeric protein after infection of myoblast C2C12 cell lines with our HD-Ad vector, whereas expression was absent after infection of other cell lines derived from different tissues. In preliminary experiments, expression of the chimeric protein after intramuscular administration in LDLR-deficient mice ameliorated the lipid profile compared to controls. In summary, we developed an innovative strategy for FH therapy based on the expression of a secreted chimeric protein after intramuscular administration of an HD-Ad vector. This approach reduces risks associated to systemic administration of viral vectors and, in principle, is applicable to other genetic diseases; collection of additional efficacy and safety data will further define its applicability in clinical settings.

PREVALENCE OF FAMILIAL HYPERCHOLESTEROLAEMIA (FH) IN ITALIAN PATIENTS WITH CORONARY ARTERY DISEASE: THE POSTER STUDY

V. Zampoleri[1,2], M. Casula[3,10], D. Lucci[4], G. Fabbri[4], M.G. Abrignani[5], M. Averna[6], S. Calandra[7], F. Colivicchi[8], M.M. Gulizia[4,9], A.P. Maggioni[4], A.L. Catapano[1,10] on behalf of POSTER Investigators
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Milano; [3]Epidemiology and Preventive Pharmacology Centre (SEFAP), Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan; [4]ANMCO Research Center of the Heart Care Foundation, Florence; [5]Operative Unit of Cardiology, S. Antonio Abate Hospital, ASP Trapani; [6]Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy; [7]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena; [8]Division of Cardiology, San Filippo Neri Hospital, Rome; [9]Cardiology Division, Ospedale Garibaldi-Nesima, Azienda di Rilievo Nazionale e Alta Specializzazione "Garibaldi" Catania; [10]IRCCS MultiMedica, Sesto S. Giovanni, Milano
E-mail: veronica.zampoleri@unimi.it

Background and Aims. Familial hypercholesterolaemia (FH) is a risk factor for cardiovascular (CV) events. High levels of low density lipoprotein cholesterol (LDL-C) since birth are linked to the early onset of atherosclerotic disease. A genetic mutation determining FH is about in one subject out of 250; FH should be more represented among subjects with a documented diagnosis of coronary artery disease (CAD). The POSTER Study evaluated the prevalence of FH in Italian patients with recent cardiovascular event.
Methods. Eighty-two cardiology centers enrolled patients with a documented CAD event; CV risk profile, drug therapy and biochemical parameters were collected. Patients with a Dutch Lipid Clinic Network (DLCN) score ≥6 underwent to a whole blood sample withdrawal to perform a genetic test based on full gene sequencing of genes involved in FH.
Results. Overall, 5415 patients were enrolled and the main index event was an acute coronary syndrome, myocardial infarction with or without ST-elevation, or a recent coronary revascularization (34.8%, 37.2% and 28% respectively). Mean age was 66±11 years, men were 78%; about 40% were already treated with statins, proportion that increased after the acute event (96.5%). Based on the DLCN score, the prevalence of potential FH (score ≥ 6) was 5.1%, 0.9% had a diagnosis of definite FH (score >8). These patients were younger than patients with a score <6 (56±10 vs 66±11, p<0.001), and LDL-C levels were in most of them (~87%) >190 mg/dL. The genetic test has been performed in 259 patients: 37 patients (14,29%) were positive for the diagnosis of FH, in 63 (24.3%) patients there was a mutation, but the genetic diagnosis was defined as not-conclusive for FH.
Conclusions. Results underscore a prevalence of FH in patients with a recent CAD. Therefore, an early identification of these subjects may help to improve their high cardiovascular risk.

INDICE DEGLI AUTORI