Model Driven Paediatric European Digital Repository

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List of Contributors

Name	Affiliation
A. Taylor	UCL
A. Jones	UCL
M. Manco	OPBG
S. Martin	OPBG
L. Putignani	OPBG

List of reviewers

Name	Affiliation
B. Dallapiccola	OPBG

Abbreviations

Table of Contents

Synopsis of the study

SUMMARY INFORMATION SHEET ABOUT THE STUDY

1. **Title of the project:**
   Model-Driven European Paediatric Repository- MD–PAEDIGREE – WP 4: Risk of cardiovascular disease in obese children and adolescents

2. **Investigator:**
   A. Taylor – M. Manco

3. **Sponsor:**
   funding from the European Commission)

4. **Type of trial:**
Non-therapeutic

5. **Scientific basis and objectives of the research:**
   "Model-Driven European Paediatric Repository", acronym MD – PAEDIGREE, is a Research Project funded by the European Commission in the area of the "Virtual Physiological Human" (VPH). MD-Paedigree aims to improve the state of the art in terms of computational "*patient-specific*" modelling of various paediatric diseases [juvenile idiopathic arthritis (JIA), cardiovascular risk in obese patients, neurological and neuromuscular diseases (NND), cardiomyopathy] and to translate the most recent innovations into clinical evidence, in order to improve understanding of diseases and develop a platform for diagnosis and evaluation of new therapies. MD-Paedigree envisages a) the integration of a specific approach to the various diseases through analysis of heterogeneous biomedical data (clinical, laboratory, genetic and metagenomic, tonometric, ultrasound and magnetic resonance imaging and haemodynamic data and real-time sequencing of musculoskeletal parameters); b) the integration and sharing of various types of biomedical information using an adaptable, modular and reliable VPH system, supporting an evidence-based therapeutic approach.

   MD-Paedigree is organised into various studies (work packages).

   The principal objective of this study is the creation of a system to estimate the cardiovascular risk associated with obesity in adolescent patients and to identify significant predictors for the risk of onset of arterial stiffness, through the collection of clinical, biochemical and imaging information.

   In particular, the objectives associated with modelling of cardiovascular risk in obese children and adolescents are the following:

- the objective and automatic quantification of the principal deposits of adipose tissue (abdominal and thoracic) and their distribution through ultrasound and MRI analysis;
- the collection of information about a series of additional cardiovascular risk factors that could contribute to generating a risk 'score', including metabolic and haemodynamic factors, clinical and family history, and the correlation between those elements;
- the development of personalised models based on the recovery of the data for evaluation of cardiovascular risk through the use of the most modern 'machine learning' techniques, to be used in clinical practice and in both longitudinal and transverse studies;
- the use of these models in order to improve understanding of the pathogenetic mechanisms that cause cardiovascular dysfunction in young and adolescent patients up to adult age; quantitative evaluation of their predictive capacity in cases other than those used for creation of the models, through cross-validation and sensitivity analysis.

On the basis of the results for the data collected and the subsequent analysis, the study will:

- Incorporate, within a single framework, the known variety of biomarkers associated with cardiovascular risk.
- Develop a computational model with significant predictive ability to gain as much insight as possible into the mechanisms associated with the development of cardiovascular risk and to enable simulation of personalised, predictive therapies.
- Improve the use of measurement of distribution of body fat as a biomarker.
- Analyse interdependence among biomarkers.

The project also envisages an ancillary study alongside the principal study funded by the European Community in a subgroup of 60 patients (20 for each centre). The ancillary study is intended to evaluate metabolic and vascular response to a mixed liquid meal (75 g per m2 of body fat surface and 75 g glucose), essentially rich in saturated fats. Metabolic response will be evaluated using blood samples taken using a needle/cannula at 0, 15, 30, 60, 90, 120, 180, 240 minutes) while cardiovascular response will be evaluated using MRI scans in the 90 minutes following consumption of the meal, with evaluation of the principal haemodynamic parameters every five minutes. This information will also be used to create a more specific submodel for simulation of risk.

6. **Type of study:**
   - Participating centres:
     - Clinical data collection and analysis centres:
       - OPBG – Manager: Melania Manco
       - University College London - Great Ormond Street Hospital, Manager: Andrew Taylor
       - BMR Genomics, Padua, Manager: Barbara Simionati
       - University of Utrecht, Manager: Hank Shipper
     - Technological centres for anonymised data analysis and model construction:

- Institut National de Recherche en Informatique et en Automatique, France, Manager: Xavier Pennec
- Fraunhofer Research Centre, Czech Republic, Manager: Berent Prakken
- Siemens, Germany, Manager: Michael Suehling

7. **Type of subjects:**
   - Pathology: obesity
   - Sex: 50% male, 50% female
   - Age: 13-18 years

8. **Number of subjects to be included** (in the case of a multicentre trial, also indicate the total number):
   - Total: 180

9. Inclusion criteria:
   - Patients aged between 13 and 18 years, suffering from obesity (BMI percentile ≥ 95th for age and sex or BMI ≥ 30 kg/m2 in patients aged ≥ 16 years)
   - Consent from parent/legal guardian, consent from adolescent

For the ancillary study only, enrolment may also include adolescents with BMI < 95th percentile but with a family history of severe obesity (at least one parent with BMI > 40 kg/m2) or type-2 diabetes mellitus being treated pharmacologically.

10. Exclusion criteria:
   - Endocrine obesity (e.g. administration of glucocorticoids) or genetic obesity (e.g. Prader-Willi syndrome)
   - Pharmacological treatment
   - Alcohol or drug consumption

11. **Trial design:**
   Longitudinal. The study is intended as an observational study in a cohort of obese subjects evaluated on enrolment and 18 months later. Standard nutritional intervention is planned.

12. **Statistics**

   Main goals of this Study are the creation of a data repository for the development of biomechanical models and of workflows.

   For these purposes no inferential statistical analysis is foreseen, nor the application of formal criteria for the definition of the sample size.

However, descriptive data analysis will be performer, both in absolute and in normalized way.

To guarantee quality and usability of the collected data the following procedures will be applied:

Data processing will include the following workflow:

- data *pre-processing*: data validation, discretization, null & outlier removal will be performed using the system "Data Curator & Validator" (DCV) developed in the "*An integrated platform for European paediatrics based on a Grid-enabled network of leading clinical centres*" (Health-e-Child) project;
- mappi*ng: descriptor*s will be cross-mapped to standard data dictionaries (e.g. epSOS value sets, ICD-10 diagnosis, LOINC labs...);
- normalization: source numeric dat*a will be normalized to generate z-values;*
- *data enrichment: normal values will be added from legacy guidel*ines;
- calculation of new variables: aggregated scores will be computed out of various fields (e.g. Body Mass Index out of weight/size);
- data description:
  - standard descriptive statistical tests will be applied for categoric and for continuous variables;
  - distance measures will be applied to generate case-based retrieval application;
  - clustering of instances using statistical & visualization algorithms;
- for imaging data, visual features will be extracted from the images that describe image regions (local image content); data will be aggregated across cases in terms of visual data but also textual or structured data to be able to differentiate normal form abnormal visual data;
- simulations will be performed basad on data-mining tecqniques, using the AITION (*Scalable Platform for Interactive Data Mining)* system, developed in the "*An integrated platform for European paediatrics based on a Grid-enabled network of leading clinical centres*" (Health-e-Child) project.

13. **Duration of the study** (including: enrolment period, any wash-out period, treatment period, follow up, for individual subjects and for the entire study sample).
    - Enrolment period: 18 months;
    - Follow-up period: 18 months.

14. **Treatment plan** (doses, methods of administration):
    The patients will undergo nutritional counselling and, where necessary, pharmacological treatment will be prescribed on the basis of any complications associated with the obesity, in accordance with the guidelines.

15. **Schedule of diagnostic and laboratory tests** (in addition to those normally performed for the condition in question):

Principal study

As part of the research project, the data relating to the tests performed on the patients in relation to their medical condition, i.e. evaluation of blood sugar, blood insulin, lipid profile, liver function, blood count and glucose tolerance, will be supplemented by laboratory and diagnostic data obtained for research purposes as part of the research project, as follows:

- Laboratory tests:
  - On blood samples:
    - measurement of inflammatory adipokines and those related to insulin resistance (i.e. tumour necrosis factor-alpha, high-sensitivity C-reactive protein, etc);
    - genetic analysis: studio of a set of SNPs selected from those previously identified by various Genome Wide Association Studies (GWAS), for creation of a genetic cardiovascular risk score.

    These evaluations will be performed on residual biological material from laboratory tests performed on the patients for their medical condition and already listed in the introduction. Therefore, no additional blood samples will need to be taken other than the 20 ml on average necessary for performance of routine tests.
  - On faecal samples:
    - analysis of intestinal bacterial flora (metagenomic analysis) with genetic typing, on two faecal samples (approximately 2 g each) provided on enrolment and follow-up.

- Imaging:
  - Ultrasound:
    - abdominal: visceral, subcutaneous, hepatic and pancreatic fat
    - pericardial fat
    - carotid intima-media thickness.
  - MRI: measurement of abdominal adipose tissue (peritoneal, visceral and subcutaneous, pancreatic and hepatic) and intratoracic adipose tissue (epicardial fat). Morphological and functional cardiac resonance imaging.
- Other:
  - Tonometric analysis to evaluate arterial stiffness.

Ancillary study

For the ancillary study only, a quantity of blood not exceeding 100 ml will be sampled in the course of the study for evaluation of cytokine and hormone response to the mixed meal.

The MRI planned in the principal study will be extended in order to evaluate vascular response in the following 90 minutes. The images obtained in the course of the test will also be used to

evaluate body composition. Compared to the period planned for the principal study, this acquisition of images will last approximately 90 min.

16. **Feasibility of the study** (number of subjects/year who can be enrolled at the centre, available facilities):

Principal study

The patients will be enrolled at the educational therapy unit of the OPBG, which treats approximately 500 outpatients annually for obesity. The diagnostic tonometric and abdominal ultrasound tests and the evaluation of the carotid intima-media will be performed at this centre.

The ultrasound evaluations will be performed in the Cardiology Department of the OPBG, while the MRIs will be performed in the Diagnostic Imaging Department.

Ancillary study

The children of severely obese individuals and of patients suffering from type-2 diabetes mellitus are valuable candidates for an evaluation of cardiovascular risk: because of their family history, they exhibit cardiovascular risk factors from a young age. Where our centre encounters difficulties in enrolling patients for the ancillary study, this will conducted only by the two other partners involved in the project (Great Ormond Street Hospital and Johns Hopkins University).

Clinical protocol

TITLE

Model-Driven European Paediatric Digital Repository (MD PAEDIGREE)

WP 4: Risk of cardiovascular disease in obese children and adolescents

Protocol no:

MD PAEDIGREE **WP 4**

Version 3:
May 6 th, 2013

CONFIDENTIAL

Protocol no.:	MD PAEDIGREE **WP 4**
Title:	Data acquisition and processing for the estimation of CVD risk in obese children
Acronym:	MD-Paedigree – WP 4
Multicentric/Monocentric Study	Multicentric
Principal Investigator	Dr. Melania Manco
Sponsor:	Bambino Gesù Children's Hospital (BGCH)
Responsible Work Package 4 :	Prof. Prof. Andrew Taylor (University College London)
Scientific Coordinator of the Project	Prof. Bruno Dalla Piccola(Ospedale Pediatrico Bambino Gesù)
Data Management/Statistical analysis:	Dr. Michael Suehling - Siemens

Protocol approved and signed by:

Scientific Coordinator of the Project

Prof. Bruno Dallapiccola

Responsible Work Package:

Prof. Andrew Taylor

Principal Investigator:

Dr. Melania Manco

Acronym List

AEs	Adverse Events
EC	Ethical Committee
CRF	Case Report Form
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
NSAEs	Non Serious Adverse Events
SAEs	Serious Adverse Events
SOPs	Standard Operating Procedures

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INDEX

1 INTRODUCTION

1.1 GENERAL BACKGROUND OF THE PROJECT

MD – PAEDIGREE *Model-Driven European Paediatric Repository* is a research project funded by the European Commission under the Virtual Physiological and Human Area (VPH) of the ICT Theme of the Seventh Framework Programme (contract no. 600932). The project foresees 7 world-renowned clinical centres of excellence pursuing improved interoperability of paediatric biomedical information, data and knowledge, by developing a set of reusable and adaptable multi-scale models for more predictive, individualised, effective and safer paediatric healthcare. The project is scientifically and technologically supported by one of the leading industrial actors in medical applications in Europe, and operating in conjunction with highly qualified SMEs and some of the most experienced research partners in the Virtual Phisiological Human (VPH) community.

MD-Paedigree validates and brings to maturity patient-specific computer-based predictive models of various paediatric diseases (cardiomyopathy, risk of cardiovascular disease in obese children and adolescents, Juvenile Idiopathic Arthritis (JIA) and neurological and neuro-muscular diseases (NND), thus increasing their potential acceptance in the clinical and biomedical research environment by making them readily available not only in the form of sustainable models and simulations, but also as newly-defined workflows for personalised predictive medicine at the point of care.

MD-Paedigree aims to advance the state-of-the-art of patient-specific computational modelling of different paediatric diseases and translate the latest advances into clinics to improve disease understanding, therapy outcome, and provide an infostructure platform for assessing new therapies at the point of care.

MD-Paedigree's goals, therefore, are to integrate and share highly heterogeneous biomedical information, data and knowledge, using best, jointly develop reusable, adaptable and composable

multi-scale VPH workflow models and to support evidence-based translational medicine at the point of care practices from the biomedical semantic Web.

Ilness in infants, children, and adolescents are a large and under-appreciated public health problem. Because paediatric patients now have a much longer life expectancy, the burden and costs are substantial for families and society. For instance, recent studies have shown that the number of adult patients with congenital heart disease is already similar to that of the paediatric population and will continue to grow. It can therefore be stated that in the longer term the VPH scientific approach requires a fundamental research investment in paediatrics, where the conceptual revolution which is underway, transforming the nature of healthcare from reactive to preventive, can best be applied, moving towards an approach based on personalised, predictive, preventive, and participatory (P4) medicine, increasingly focused on wellness.

MD-Paedigree represents a major step towards personalised paediatric e-Health, based on data-driven models, patient-specific simulations and a sustainable data and model repository. The project can in fact impact the way healthcare is practiced in the future. MD-Paedigree's impact on dealing with very concrete and exemplary clinical cases is demonstrated through the following applications in the diseases scenarios. MD-Paedigree encompasses complete services for storage, similarity search, outcome analysis, risk stratification, and personalised decision support in paediatrics within its innovative model-driven data and workflow-based models repository, leveraging on service and knowledge utilities. It fosters the state-of-the-art of patient-specific computational modelling of the selected diseases and translates the latest advances into clinics to improve disease understanding and provide a platform for testing new therapies at the point of care. In fact, MD-Paedigree demonstrates how a dedicated VPH repository can provide full accessibility to existing and further developing knowledge in paediatrics to all interested bio-medical researchers precisely through linking data with models, thus proving the large scale benefits of having both the data and models readily available at the point of care.

The data collection performed within Health-e-Child and Sim-e-Child projects (already funded by the European Commission in previous calls for proposals) is still available to the MD-Paedigree consortium thanks to the continuity in the eHealth platform which is shared by all three projects. In addition, the new patients' recruitment to be performed within MD-Paedigree consists of:

Pathology	No of patients/Time	
Cardiomyopathies	180 children, by month 33: 60 patients (among which 30 girls) for each clinical centre,90 for BGCH	**Genetic and meta-genomic:** 180 patients with cardiomiopathies, 180 with CVD risk in obesity, 200 with JIA, and 100 unaffected
	180 patients , by month 36: 60 (among which 30 girls) for each	

CVD risk in obese children	clinical centre, 90 for BGCH.	subjects (control group).
Juvenile Idiopathic Arthitis (JIA)	Altogether 200 patients by month 28.	
NND	**Cerebral Palsy**: 50 patients for each clinical centre for probabilistic modelling, as well as 600 retrospective patients from KU Leuven and OPBG.	
	Spinal Muscular Atrophy (SMA) 20 ambulant patients (severity grade type 3); 10 patients for each centre for biophysical modeling; 10 patients among the 3a subgroup (symptoms of weakness appearing before age 3 years); 10 patients among the 3b subgroup (weakness appearing after the age of 3 years.	
	Duchenne Muscular Dystrophy (DMD) Clinical data will be collected by OPBG, KU Leuven and VUA from 20 ambulant genetically confirmed DMD Patients. 10 patients with an age ranging between 5 and 6 years, additional 10 patients with an age ranging between 7 and 8 years.	

1.2 BACKGROUND OF WP 4 - "DATA ACQUISITION AND PROCESSING FOR THE ESTIMATION OF CVD RISK IN OBESE CHILDREN"

The World Health Report 2002 revealed that, in developed countries, approximately one third of all coronary heart diseases and ischaemic strokes and almost 60% of hypertensive diseases can be directly attributed to obesity [WHR2002]. These figures confirm obesity as one of the primary risk factors for cardiovascular disease (CVD), a risk factor that originates early in life. As autopsy studies have shown, the levels of lipids, blood pressure, and obesity in the young are directly associated with the extent of early atherosclerosis of the aorta and coronary arteries [Berenson et al.,1998]20. For this reason, it is of particular concern that there has been a significant increase in childhood and adolescent obesity over the last decade. In the United States, 32% of children and adolescents are now at or above the eightyfifth percentile of the 2000 BMI-for-age growth charts [Ogden et al., 2008; Kuczmarski et al., 2000], but also in the United Kingdom, the prevalence of obesity in children is approaching one third [BHF2008]. One of the challenges concerning the study of childhood obesity and its influence on CVD risk is the required time span for longitudinal studies: cardiovascular events occur mostly later in adulthood, which means that longitudinal studies have to comprise several decades. Nonetheless, cross-sectional studies are able to show correlation between childhood obesity and established surrogate markers for CVD, such as atherosclerosis and cardiac hypertrophy. The Strong Heart Study [Chinali et al., 2006; Chinali et al., 2008], which analysed data from over 450 adolescents, demonstrated that in patients with obesity and/or metabolic syndrome a significantly higher prevalence of left ventricular hypertrophy and left atrial dilation paired with impairment in both systolic and diastolic function is observed. Insulin resistance (IR) is an

established determinant in the pathogenesis of CVD; it is constantly observed in patients with hypertension, dyslipidemia and atherosclerosis. Evidence supports firmly that body fat distribution (subcutaneous, visceral, muscle and hepatic fat) modulates IR and cardiovascular risk more than total body adiposity, thus explaining why some individuals who are seemingly equally obese and share common lifestyle and dietary habits tend to have higher IR and CVD risk than others.

MD-Paedigree will integrate the variety of known biomarkers for CVD risk assessment into one common framework, enhance body fat distribution biomarker measurement, and analyse interdependencies between the biomarkers. In addition, MD-Paedigree will develop computational models with high predictive power to better understand the mechanism of CVD development. These models will also allow the simulation of interventions to make personalised predictions for the optimal therapy.

Obesity is commonly acknowledged as a major risk factor for cardiovascular disease (CVD). However, the precise mechanism leading to the development of cardiovascular risk in obesity from childhood to adolescence to adulthood remains largely unsolved [Lloyd et al., 2010; Cornier et al., 2011]. In particular, it is still unclear whether childhood obesity increases CVD risk simply because of the tracking of obesity from childhood to adulthood or via the development of CVD risk factors already present in childhood and adolescence. Many structural and functional changes in the adolescent heart, such as left ventricular (LV) hypertrophy, left atrial (LA) enlargement, and subclinical impairment of LV systolic and diastolic function are believed to be precursors to more overt forms of cardiac dysfunction and heart failure [Abel et al., 2008].

In order to rate the degree of obesity for clinical diagnostics and studies, the body mass index (BMI) is still the primary measure, also in children [Lloyd et al., 2012]. However, BMI only estimates the general adiposity of a subject, while it does not take into account the distribution of adipose tissue within the body. Specifically, visceral adipose tissue (VAT), the fat between the abdominal organs, has shown to correlate highly with CVD [vanGaal et al., 2006]. In addition, subjects with normal BMI may still have high body fat content, which has proved to be a significant CVD risk factor for adults [Romero-Corral et al., 2010].

Complementary to BMI, imaging modalities such as computed tomography (CT) or magnetic resonance imaging (MRI) allow measuring specific adipose tissue types and have established themselves as important tools for diagnosis. While CT and MRI are the current gold standard for adipose tissue quantification, high costs (and the radiation exposure of CT) restrict these modalities to large-scale studies, and ultrasound (US) is becoming an affordable, non-invasive alternative [deLucia2010]. In particular, echocardiography allows to measure epicardial adipose tissue (EAT) and has emerged as a novel approach to accurately estimate VAT [Iacobellis2003]. However, the quantification of adipose tissue from image data is still mostly performed manually; a tedious and time-consuming process prone to subjective bias. In particular, for the analysis of EAT from MRI, the adipose tissue has to be measured and contoured manually, which leads to noticeable discrepancies between different observers [Flüchter2007]. Measuring the thickness of EAT from US is even more challenging, which is why commonly several manual measurements are performed with electronic callipers and averaged [Iacobellis2008].

In order to decrease the manual workload of the operators, several methods have been proposed for semi- or completely automated image-based quantification of adiposity. The extraction of adipose tissue from MRI has been studied extensively, either for selected body regions [Poll et al. 2002, Peng et al. 2007, Positano et al. 2009] or for whole-body scans [Kullberg et al. 200985, Würslin et al. 201086, Wald et al. 201287]. Since adipose tissue features high intensities in MRI, many authors use thresholding to separate it

from the surrounding tissue. Although an automatic selection of thresholds has been proposed [Gronemeyer et al. 2000], different adipose tissue types (VAT and subcutaneous adipose tissue, SAT) still have to be separated manually. An automatic algorithm for this problem was developed [Positano et al. 2004] 88, based on an active contour algorithm. Liou et al. [2006] 89 proposed to use morphological operations, edge detection, and knowledge-based curvature fitting. In all these approaches, bone marrow is often misclassified as adipose tissue, because it features similar intensities in MRI. Thomas et al. [1998] 90 excluded bone marrow by user interaction, while Shen et al. [2003] eliminated the paravertebral adiposity tissue automatically. [Kullberg et al. 2007] used geometrical models of the pelvis and vertebra to exclude these structures and thresholding and morphological operations to automatically separate VAT and SAT. Zhou et al. [2011] employed fuzzy c-means clustering and thresholding to quantify VAT and SAT in both water-saturated and non-water saturated MR images. While automated ultrasound segmentation is feasible for a variety of anatomical structures [Noble et al. 2006], it has rarely been used on adipose tissue. One of the few approaches was proposed by Ng et al. [2009] who used US radiofrequency signals from different locations and beam angles and calculated the spectrum dispersion within the image. Pixels which represent adipose tissue change faster than other areas. To the best of our knowledge, there are no automatic algorithms quantifying intraabdominal fat from US.

In MD-Paedigree, we will re-use our proven anatomical organ models developed in Health-e-Child and Sim-e-Child to add prior knowledge to image analysis. This will enable us to assess different adipose tissue types automatically from image data and use this information in our further analysis. In addition to the fat distribution data from imaging, we will also use established biomarkers such as blood pressure, metabolic and haemodynamic data to estimate the CVD risk. Currently, most studies that analyse different factors of CVD risk employ univariate or, at best, multivariate but linear models, which represent a major limitation. Univariate models can only identify independent contributors to the risk, while they do not shed much light on the interplay between the factors. As demonstrated by [Colombet et al., 2000], cardiovascular risk can be modelled by multivariate machine learning models with only ten clinical variables (representing commonly acknowledged markers of CVD risk). In a similar study, Kurt et al. [2008] successfully modelled the risk of coronary artery disease with a multi-layer perceptron (MLP) and a comparable set of 8 clinical variables. Sumathi and Santhakumaran [2011] trained an Artificial Neural Network (ANN) on a set of 15 clinical variables and claimed to use it successfully for early diagnosis of hypertension. In MD-Paedigree, we will construct multivariate nonlinear models of CVD risk involving state-of-the-art statistical and machine learning techniques. This will not only help to build more accurate models of CVD risk, but also to better understand the mechanism of CVD development via the identification of important risk factors and understanding of their interrelation. Such personalised risk models may become a more reliable alternative or at least a useful complement to the CVD risk prediction charts of WHO [Prevention, 2007], especially since these charts are available for adults only.

A common drawback of the existing works of multivariate modelling is that the underlying techniques like Multi-layer-Perceptron (MLP) or Artificial Neuron Networks (ANN) are basically “black box” models, i.e. the reasons for their results cannot be conveyed to their human users, which leads to low acceptance rates among clinicians.

In our modelling, we will focus on case-based reasoning and discriminative distance learning instead Tsymbal et al, 2009; Zhou et al., 2006]. Since these systems base their decisions on concrete patient cases and are able to present the relevant cases (i.e. the ones utilised for decision making) to the user, they provide easy and intuitive decision support and a possibility for personalised therapy planning, based on the clinical history of retrieved similar patients.

Our work will be centred on the similarity search based decision support system HeC CaseReasoner [Manset et al., 2009] developed in the Health-e-Child project. It features recently suggested techniques for discriminative distance learning, including learning from equivalence constraints and the intrinsic random forest similarity.

The basic philosophy behind the design of CaseReasoner is to provide clinicians with a flexible and interactive tool to enable operations such as data filtering and similarity search over a grid of clinical centres, and to facilitate the exploration of the resulting data sets. The major aim is to let clinicians explore and compare the patients' records, regardless of geographical location, and to visualize their place in the distribution of both the whole population of patients, as well as in the distribution of its semantic subsets (Figure 8). The search platform can then be used for several tasks such as case-based retrieval [Depeursinge et al. 2010], support for curation [Ruch et. al. 2006] and ultimately decision support [Ruch et al. 2008; Pasche et al. 2011]. HeC CaseReasoner employs a domain-independent technology, and has been applied within Health-e-Child for decision support in three domains: cardiology, neurooncology, and rheumatology. With MD-Paedigree, HeC CaseReasoner will be further extended and applied to decision support in the domain of modelling cardiovascular risk in obese children and adolescents.

2 AIMS OF STUDY

2.1 MAIN GOALS

Our major objectives with modelling the cardiovascular risk in the obese child and adolescent are:

a) automated, objective quantification of different adipose tissue types and their distribution from MRI and ultrasound data;

b) collection of a large number of additional factors contributing to the risk, including metabolic and haemodynamic factors, clinical and family histories, and their interrelation;

c) construction of personalised multivariate retrieval-based models for the assessment of cardiovascular risk using state-of-the-art machine learning techniques, both for cross-sectional and longitudinal studies;

d) interpretation of the models with the purpose of better understanding the mechanism of cardiovascular dysfunction from childhood to adolescence and adulthood, and quantitative evaluation of their predictive performance with cross-validation and sensitivity analysis, and with evaluation on unseen subsequently acquired cases.

Main goal of the study is therefore to collect clinical, biochemical and imaging data to estimate cardiovascular risk associated with obesity in adolescents and to identify significant predictors of increased risk as estimated by changes in arterial stiffness over the time.

On the basis of the outcomes of the data collected and subsequent analysis MD-Paedigree will integrate the variety of known biomarkers for CVD risk assessment into one common framework, enhance body fat

distribution biomarker measurement, and analyse interdependencies between the biomarkers. In addition, MD-Paedigree will develop computational models with high predictive power to better understand the mechanism of CVD development. These models will also allow the simulation of interventions to make personalised predictions for the optimal therapy.

In a sub-sample of obese patients, the study will also assess the metabolic and cardiovascular response to a lipid reach liquid meal (ancillary study). Indeed, a mixed meal (carbohydrates and lipids) is able to elicit a powerful metabolic and cardiovascular response. The ancillary study may involve also offsprings of morbidly obese individuals and patients with type 2 diabetes since it has been firmly demonstrated that they have a significantly higher cardiovascular risk of age matched peers.

2.2 PRIMARY END POINT

The primary end point of the study is the successful collection of clinical, laboratory and diagnostic data to be subsequently modelled and simulated by the IT experts. The primary end point envisages estimation of the cardiovascular risk associated with obesity in 180 adolescents (60 for each clinical partner involved, 90 for Bambino Gesu' Children's Hospital) will include evaluation of insulin resistance based on modelling of glucose and insulin values following a high energy liquid mixed meal , lipid profile, liver function tests, white blood cell count, circulating adipokines and markers of low-grade inflammation; and estimation of glucose tolerance. Adiposity at the abdominal (visceral adipose tissue, VAT; subcutaneous adipose tissue, SAT; hepatic and pancreatic fat fractions, HFF & PFF, respectively) and epicardic (epicardic adipose tissue, EAT) sites will be estimated by ultrasonography (US) and magnetic resonance imaging (MRI). Stratification of the risk will include information of left ventricular (LV) morphology and haemodynamics by US and Cardiac Magnetic resonance (CMR) following the mixed meal..

3 STUDY DESCRIPTION

3.1 STUDY DESIGN:

MAIN STUDY

The study is designed as longitudinal observation of 180 obese adolescents (60 for each clinical partner involved, 90 males, 90 for BGCH). They will be evaluated at the baseline (month 1 to month 18) and re-evaluated between 19 and 36 months.

At ages 14 to 16 years (baseline study) and 15.5 to 17.5 years (follow-up study) , participants will visit a clinical research facility, where they will be asked to provide informed, written consent, to complete questionnaires and undergo the research investigations consisting of clinical and laboratory evaluations, ultrasound evaluation of heart morphology and function, ultrasound evaluation of liver brightness which are routinely performed in overweight and obese individuals [i.e. lipid profile, liver function tests, white blood cell count, circulating adipokines and markers of low-grade inflammation; and estimation of glucose tolerance]. Adiposity at the abdominal (visceral adipose tissue, VAT; subcutaneous adipose tissue, SAT; hepatic and pancreatic fat fractions, HFF & PFF, respectively) and epicardic (epicardic adipose tissue, EAT) sites will be estimated also by magnetic resonance imaging (MRI).

ANCILLARY STUDY

In a sub-sample of obese patients and eventually in offsprings of severely obese and diabetic patients an oral metabolic tolerance test, oMTT, containing 75 g glucose and 75g of fat per m^2 body surface area (prevalently saturated fatty acids) will be performed during RMI scanning (ancillary study). The lipid meal is expected to boost both the insulin and the cardiovascular response. Offsprings of obese and diabetic patients are suitable candidates to the study since they may present with metabolic responses not different from obese patients.

No sedation will be required for RMI studies of adolescent patients.

Hence, stratification of the risk will include evaluation of insulin resistance based on modelling of glucose and insulin values following the oral glucose tolerance test and/or the high energy liquid mixed meal (in patients undergoing the ancillary study), information of left ventricular (LV) morphology and haemodynamics by US and Cardiac Magnetic resonance (CMR). Reduced elasticity, distensibility, and stiffness at the follow-up will be used as end-point estimate of cardiovascular disease.

MAIN STUDY

Questionnaires (attached to the protocol)

A paper copy of a basic questionnaire will be sent by post or given in person to the participants prior to their attendance at the clinical research facility. The full questionnaire will be completed at the facility. An accompanying letter will ask them to complete as much of the questionnaire as possible at home. Particular attention will be drawn to data that might require help from family members to obtain eg. Family history. A trained professional will then take the participants through their answers when they attend the clinical research facility to ensure complete and accurate responses and to address any questions or uncertainty that the participants may have. The questionnaires will address the following:

- ✓ Name, sex, date of birth, contact details (address, email, telephone)
- ✓ Ethnic group
- ✓ Educational attainment (grades)
- ✓ Maternal & paternal social class [The National Statistics Socio-Economic Classification 2001]

- ✓ Mother's age, weight & height
- ✓ Father's age, weight & height
- ✓ Birth weight and length of gestation
- ✓ Family history of medical conditions, including hypertension, diabetes, angina, myocardial infarction, stroke, peripheral vascular disease, and hypercholesterolaemia
- ✓ medical history including history of cardiac disease or cardiac surgery, any endocrine abnormality, including diabetes, familial hypercholesterolaemia, renal disease
- ✓ menstrual history& contraceptive use in the girls
- ✓ medication history, including steroid use
- ✓ smoking, alcohol, caffeine and recreational drug use history, including time of last consumption
- ✓ time of last consumption of food / drink
- ✓ activity / exercise [Baecke et al. 1982]

Self-assessment of pubertal status. Participants will be asked to compare themselves to a series of images representing Tanner stage to estimate their progress through puberty [Carel and Leger 2008]. This method has been shown to have good levels of agreement with physician examination in a large population of obese and non-obese boys and girls [Sun et al. 2012].

Perceived stress. Participants' sense of being under stress over the preceding 4 weeks will be assessed with the ten-item version of the Perceived Stress Scale [Cohen et al. 1983]. Each item (e.g., "Over the past week how often have you felt that you were unable to control the important things in your life?") is rated on a five-point scale. Total scores may range from 0 to 40, with higher scores indicating greater perceived stress. The Perceived Stress Scale has been widely used in research on stress and health, and has high internal consistency.

Emotional well-being. The 28-item version of the General Health Questionnaire (GHQ) [Richard et al. 2004] will be used to assess emotional wellbeing over the preceding 4 weeks. The GHQ is widely used in clinical and population studies, and its validity has been established against psychiatric interviews. It has high internal consistency. The hospital anxiety and depression scale (HADS) [Zigmond and Snaith 1983]will be used to augment this.

Eating style. Four scales of eating style will be assessed (routine restraint, compensatory restraint, susceptibility to external cues, and emotional eating) using the Weight-Related Eating Questionnaire (WREQ) [Schembre et al. 2009]. This 16-item questionnaire has been shown to have good psychometric properties and construct validity in an ethnically diverse population of young adult to elderly men and women [Schembre and Geller 2011].

Anthropometrics

Height will be measured to the nearest 1 mm, without shoes and with the Frankfurt plane of the participant's head aligned by eye to be parallel to the ground. A calibrated stadiometer or height board will be used. Weight will be determined to the nearest 10 grams, using calibrated scales, with the participant wearing only light clothing and no shoes. Waist and hip circumferences will be determined according to standard practice [Molarius et al. 1999], using a flexible measuring tape.

Routine laboratory tests will include evaluation of fasting glucose, insulin, c-peptide, lipid profile (total and HDL cholesterol, triglycerides), liver function tests (alanine-aminotransferase, aspartate amino transferase, γ-glutamyl transferase), glycated haemoglobin, white blood cell count; glucose tolerance by a standard OGTT (1.75 g/kg body weight up to a maximum of 75 g). Glucose, insulin and c-peptide will be measured at baseline and 30, 60, 90 and 120 min. Systolic (SBP) and diastolic blood pressure (DBP) will be measured three times while the subjects are seated, and the measurements will be averaged for the analysis.

All the examinations will not require withdraw of additional amount of blood respect to analyses routinely performed in obese patients (average 20 ml of blood).

Estimation of adipokines, low-grade inflammation and insulin resistance

Measurements of adipokines and markers of inflammation. Blood samples will be withdrawn to measure fasting plasma adipokines (leptin, adiponectin), circulating markers of inflammation (C-reactive protein, CRP; Tumor-Necrosis Factor-alpha, TNF-alpha; Interleukin 6, IL6) and endothelium dysfunction (e-Selectin, Intercellular Adhesion Molecule 1, ICAM-1).

Assessment of the renin-angiotensin-aldosterone axis. With the patient in the supine position, blood will be obtained for measuring plasma renin activity, aldosterone, cortisol, serum sodium and potassium. After being upright and ambulating for 2 hours, repeated blood samples will be obtained. Dietary sodium intake will be assessed by measuring 24 hour urinary sodium excretion.

Assays of adipokines will be centralized with anonymized samples sent to Department of Metabolic Diseases, University Medical Center Utrecht, Utrecht, the Netherlands, responsible person Dr. Hank Shipper.

On residual blood/plasma genetic analyses and assay of cytokines will be performed.

Body fat assessment

We will use a graphics processing unit (GPU) implementation of the T2*-IDEAL algorithm [Yu et al. 2007; Kowalik et al. 2011] to measure body fat content. This iteratively separates MR images into fat and water components, which can then be used to measure the proportion of fat in each 3x3x10 mm voxel. Data will be acquired in a continuous stack of 10 mm thick slices from the neck to the knees. To prevent motion artefact, we will use breath holding for the thorax and abdomen and cardiac gating for slices containing the heart. Fat quantification in the head, arms and below the knees is impractical due to the need for participant re-positioning or specialized coils. Due to their low fat content, we will exclude these body parts using anatomical landmarks to ensure consistency between participants.

Estimation of Insulin Resistance and secretion

Insulin resistance will be estimated in fasting condition, after the glucose load and patients undergoing the oMTT also after the mixed meal. It will be computed by means of the following methods: [QUICKI= 1/[log plasma fasting insulin(mIU/l) + log plasma fasting glucose (mg/dl)]; HOMA-IR = (insulin x glucose)/22.5); WBISI = (10,000/square root of [fasting glucose x fasting insulin] x [mean OGTT glucose x mean OGTT insulin]. Insulin secretion will be estimated by the deconvolution method. Beta-Cell ability to adapt insulin secretion to changes in insulin sensitivity (glucose disposition indexes) will be assessed.

Genetic Analysis

Sample collection, storage and DNA extraction

Blood samples will be collected from 180 obese patients at the baseline for DNA analysis. Faecal samples will be collected, at baseline and after 18 months for micorbioma/metagenome analyses. A database of patients, including name, age, disease, laboratory data but also specific indications about antibiotic, prebiotics, and probiotics administration will accomplish faecal sample datasheets for appropriate later description of gut microbiota enterotypes.

Blood and faecal samples will be stored at 4°C for at maximum of 24 hours or, alternatively, at -80°C until shipment to genomic/metagenomic facilities for automatic DNA extraction and targeted-sequencing. The samples will be sent every two months or when suitable for the laboratories included in the study in dry ice by express courier.

In detail, DNA will be extracted from faecal samples at the Bambino Gesù Children's Hospital, laboratory of mIcrobiology (responsible person: Dr. Lorenza Putignani).

DNA extracted from blood from genetic analysis will be sent to BMR Genetics s.r.l., via Redipuglia, 22 - 35131 PADOVA, Italia Codice fiscale e Partita IVA: 03888370289 (responsible person Dr. Barbara Simionati).

All samples will be anonymzed prior to be sent.

After DNA analysis, residual biological material will be destroyed within 12 months from the end of the follow up study. Indeed no future studies are foreseen.

Patients and legal representative will be informed of all results except for results of genetic analysis.

Genetic analysis

Genetic analysis will be performed on blood samples withdrawn at baseline, in order to build a genetic score of cardiovascular disease (CVD) risk. Genomic DNA will be extracted by GeneCatcher gDNA Blood Kit (Invitrogen). Analyses will be performed by using the Illumina technology. Candidate Single Nucleotide Polymorphisms (SNPs) for estimation of CVD risk in the MD-Paedigree study. DNA analysis. Analysis (DNA extraction and SNPs analysis) of a custom of SNPs in 180 patients plus the statistical analysis in order to build a genetic score of CVD risk. SNPs will be selected among SNPs identified in previous Genome Wide Association (GWAS studies). Selection will be based on either statistical significance threshold of the genetic association with the investigated variable (dyslipidemia, left ventricular hypertrophy, hypertension, type 2 diabetes, increased visceral adiposity and fatty liver) and/or clinical significance in a customized metabochip . Two genetic risk scores will be constructed on an a priori basis. Genetic risk scores will be the

sum of all cardiovascular risk alleles from all SNPs, both those associated with CVD (increased stiffness/IMT) and those associated with risk factors as done previously (Raynter NP; JAMA 2010; 303: 631-7; Peterson RE, Hum Genet 2011; 129: 221-30). The SNPs affecting more than one phenotype will be included once.

Associated variable/phenotype Single Nucleotide Polymorphisms (SNPs)

Associated variable/phenotype	**SNP**	**Reference**
Blood lipids	91 SNPS to be selected based upon significance	Teslovich TM Nature 2010; 466: 707-713 Aulchenko YS Nature genetics 2009; 41: 47-55
Blood pressure	rs3918226 NOS3 rs4846049 MTHFR-NPPB rs2004776 AGT rs661348 LSP1/TNNT3 rs11105354 ATP2B1 rs2014408 SOX6 rs1799945 HFE rs1421811 NPR3 rs9930333 FTO rs16933812 PAX5 rs7638110 MRPS22 rs17773430 MCR4	Johnson T, AJHG 2011 89: 688-700; Melka MG, JCEM 2012; 97:E145-E150
Type 2 diabetes/fasting glucose	rs560887 G6PC2 rs10830963 MTNR1B rs2191349 DGKB-TMEM195 rs780094 GCKR rs11708067 ADCY5 rs7944584 MADD rs10885122 ADRA2A rs174550 FADS1 rs11605924 CRY2 rs11558471 SLC30A8 rs4506565 TCF7L2 rs4607517 GCK rs7034200 GLIS3 rs340874 PROX1 rs11920090 SLC2A2	

	rs11071657 C2CD4B rs10923931 NOTCH2 rs11899863 THADA rs243021 BCL11A rs7578326 IRS1 rs13081389 PPARG rs6795735 ADAMTS9 rs1470579 IGF2BP2 rs1801214 WFS1 rs4457053 ZBED3 rs10440833 CDKAL1 rs849134 JAZF1 rs972283 KLF14 rs896854 TP53INP1 rs10965250 CDKN2A/B rs13292136 CHCHD9 rs12779790 CDC123/CAMK1D rs5015480 HHEX/IDE rs2334499 HCCA2 rs231362 KCNQ1 (a) rs163184 KCNQ1 (b) rs5215 KCNJ11 rs1552224 CENTD2 rs1531343 HMGA2 rs4760790 TSPAN8/LGR5 rs7957197 HNF1A rs11634397 ZFAND6	

	rs8042680 PRC1 rs11642841 FTO rs4430796 HNF1B (TCF2) rs5945326 DUSP9	
Left ventricular dimension	rs756529 KCNB1	Arnnet DK, BMC Medical Genetics 2009, 10:43 Vasan RS JAMA 2009; 302: 168-78
Fatty liver	rs738409 PNPLA3 rs2854116 APOC3 rs12979860 IL28B rs1260326 GCKR rs4986790 TLR4	
Visceral adiposity	CYP17A1 rs1004467 NT5C2 rs11191548 SH2B1 rs7498665	
Levels of adiponectin	ADIPOQ rs17366653	
Levels of CRP	rs2847281 rs6901250 rs4705952	Dehghan A, Circulation 2011; 123: 731-8
Genetic score (Hypertension+left ventricular wall thickness+stroke+CAD)	29SNPs	Ehret GB Nature 2011; 478:103-109

Microbioma/Metagenome analysis

Metagenome data analysis will be carried out on fecal samples from obese patients collected at baseline and at 18 months, and re-evaluated at the follow-up to investigate the risk to develop CVD associated with specific gut taxa at the baseline and during observational time-course (enterotype) (Vulevic et al., J Nutr. 2013 Mar;143(3):324-31. Epub 2013 Jan 9; Di Girolamo et al., 2012 Dec;6(6):759-73).

Microbioma analysis will provide an opportunity to understand how gut microbiota taxa distribution may possibly correlate with CVD risk (Vulevic et al., J Nutr. 2013 Mar;143(3):324-31. Epub 2013 Jan 9; Di Girolamo et al., 2012 Dec;6(6):759-73). Stool samples which will be collected at baseline and after 18 months. The results of gut microbiota analysis will be integrated with clinical data to assess how they correlate with obesity indexes, and in particular to explore the prognostic value of the presence of major gut taxa patterns in conditioning disease susceptibility as well as the immune response.

In order to analyse the taxonomic gut content of obese patients, a targeted approach based on sequencing of the variable regions V1 and V3 of 16S rRNA locus will be used (Aagaard *et al*., 2012. PLoS One 7(6):e36466. Epub Jun 13). Fecal samples will be collected and analysed at onset of disease, at time of clinical remission, and during disease flares, with a prediction of approximately 400 samples.

Analysis of microbiome of fecal samples will be carried out following DNA extraction (automatic EZ1 Biorobot, Qiagen), and further pyrosequencing using a 454 Junior apparatus and sequence analysis; comparison will be performed with the recently developed MEGAN 4 software (available at http://www-ab.informatik.unituebingen.de/software/megan) (Mitra et al., BMC Genomics 2011), or with the PhylOTU software (https://github.com/sharpton/PhylOTU) (Wylie et al., 2012. PLoS One 7(6):e35294. Epub Jun 13.), in order to identify the microbiota operational taxonomic units (OTUs).

ANCILLARY STUDY

MD-paedigree gives us the opportunity to provide addition information on the cardiovascular risk elicited by a mixed meal which resembles daily diet by performing an ancillary study. Such as not all the patients will undergo the study, but 60 patients are sufficient to achieve statistically significant results. Ideally, each centre will contribute with 20 patients, but one centre can replace an other in case of failure in the recruitment.

The amount of blood required for the ancillary study cannot exceed 100 ml per patient. By considering a medium body weight for each patient of 60 kilos, such amount is below the threshold suggested by the WHO as a safe (WHO guidelines).

oMTT

The oMTT will be performed simultaneously to an MRI scan. While the complete metabolic test will last 240 min, the MRI scan lasting up to 1 hour 30 minutes.

Prior to the MRI scan, an intravenous cannula will be placed in a peripheral vein and blood for a baseline metabolic profile will be obtained. To obtain useful basal metabolic measures, participants will be asked to fast overnight and drink nothing but water on the day of their assessment. They will be asked to consume their last meal prior to fasting at 9pm. They will also be asked to abstain from smoking, alcohol, recreational drugs or caffeine consumption and from formal physical exercise for the preceding 24 hours.

After resting in the MRI scanner for 15 minutes, during which time planning (scout) scans will be carried out, resting haemodynamic parameters will be measured. The participants will then be asked to ingest a

lipid and glucose rich meal as describe din detail below. The haemodynamic and metabolic responses to this meal will then be measured with repeated MR assessments, blood samples and saliva samples. Prior to completion of this assessment, a complete scan of the body will be carried out to accurately quantify body fat and its distribution.

Intravenous cannulation and venous blood sampling

This will be carried out according to standard hospital practice, using the largest suitable cannula up to gauge 18 in a peripheral vein. The following protocol will be used:

Hand hygiene:

- ✓ Decontaminate hands before and after each participant contact and before applying and after removing gloves.
- ✓ Use correct hand hygiene procedure.

Personal protective equipment:

8 Wear gloves.
9 Remove and discard gloves immediately after the exposure-prone activity.

Skin preparation:

6 Use 2% chlorhexidine/70% alcohol applicator (ChloraprepSepp®) and **allow to dry** (NB – this is important to avoid contamination of sample as well as for hygiene reasons).
7 Do not re-palpate the vein after the skin preparation.

Dressing:

- ✓ Use a sterile, semi-permeable, transparent dressing to allow observation of insertion site.

Safe maximum total blood draw will be defined according to body weight (3 mL/kg per 24 hours), in accordance with safe practice guidelines (WHO guidelines) and this limit will not be exceeded. In any case, the amount of blood withdrawn will not exceed 16 ml.

High energy liquid mixed meal

Studies will be carried out after a minimum 12h overnight fast. After fasting blood has been drawn, participants will consume a standard liquid meal (oral metabolic tolerance test, oMTT). This will contain heavy whipping cream and 75g glucose. The quantity of cream will be varied to deliver 75g of fat per m^2 body surface area. The drink will have a volume of approximately 500 mL and will be consumed within 10 minutes. This regimen has been shown to stimulate significant responses in vascular inflammatory markers [Ceriello et al. 2004] but also it can also boost the glucose induced insulin response to a different degree in normal-weight and obese individuals [Manco M et al 2004].

Assessments of insulin resistance using a similar meal were found to correlate well with standard oral glucose tolerance testing in children [Chandler-Laney et al. 2013].

Haemodynamic response to meal (MR)

To control for the effects of acute stress, participants will be asked to rate their level of anxiety or stress on a visual analog scale (1-100) just prior to entry to the MR scanner, just prior to the meal, 30 mins after the meal, and just prior to completion of the scan. Room temperature, which is known to affect the vasculature, will be recorded to the nearest 0.1°C.

Oscillometric BP will be measured in the non-dominant arm at one-minute intervals. All imaging will be performed on a 1.5T MR scanner (Avanto or Aera, Siemens Medical Solutions, Erlangen, Germany).

Flow quantification will be performed through-plane in a cross-section of the ascending aorta as it passes the bifurcation of the pulmonary arteries using an ECG-gated spiral phase-contrast MR sequence, as described previously [Steeden et al. 2011]. This technique will allow images to be acquired within a short breath-hold (~5 seconds) with a spatial resolution of 1.6x1.6 mm and a temporal resolution of 30 milliseconds.

Flow images will be processed to derive stroke volume (SV) and CO. Total peripheral resistance (TPR) will be calculated by dividing the mean BP (MBP) by CO. Total arterial compliance (TAC) will be calculated by optimization of a two-element windkessel model, as previously described [Stergiopulos et al. 1994].

Peak mesenteric artery flow assessed with phase-contrast MRI occurs 20 minutes following meal ingestion [Masui et al. 1994] as do changes in internal carotid and vertebral artery flow [Totman et al. 2009]. Cardiac index peaks 30-60 minutes after a meal in healthy young people and at 30 minutes for the elderly or individuals with dysautonomia [Lipsitz et al. 1993]. Forearm vascular resistance peaks at 45 minutes in all groups. There is minimal change in mean arterial blood pressure (MABP) in healthy young subjects but substantial reductions in MABP at 30 minutes in dysautonomic patients. Heart rate (HR) peaks at 30 minutes in elderly or dysautonomic patients, with a concomitant peak in noradrenaline levels in these subjects, but HR rises gently to a maximum at 1 hour for the healthy young. Taken together, these data suggest that the peak vascular load after meal ingestion is at some time between 20 and 40 minutes but that this load is well regulated in healthy young patients, where precise autonomic regulation of the circulation is preserved.

To achieve optimal characterisation of the cardiovascular response to a meal, HR will be monitored continuously, blood pressure (BP) will be measured every 1 minute, stroke volume will be assessed every 5 minutes for the first 40 minutes and then at 50 minutes and 1 hour after the meal. Flow into the carotids, vertebral arteries, superior mesenteric artery and flow at the descending aorta just proximal to the junction of the iliac arteries will be assessed at the same time to assess proportional vascular changes in the head,

lower limbs and mesentery. These data and the vascular parameters derived from them will be compared to the same measures taken just prior to meal ingestion after participants had rested for 15 minutes.

Participants will spend a total of 1 hour and 30 minutes in MRI scanner, during which time, they will be asked to watch a restful and un-stimulating movie (Winged Migration).

Appetite / satiety response to meal

Prior to the meal and at every blood draw, participants will be asked to rate their appetite / satiety on four visual analog scales (scored from 0 to 100 mm)[Blundell et al. 1993]:

2 "How hungry do you feel right now?" (0 = not at all, 100 = extremely)
3 "Rate your desire to eat at this moment." (0 = none, 100 = very strong)
4 "How full do you feel right now?" (0 = not at all, 100 = completely)
5 "How much food will you eat at your next opportunity?" (0 = none, 100 = a very large amount)

Blood and saliva measures of metabolic response to meal

Blood will be drawn just prior to consumption of the meal (T0). Further samples will be taken at 15, 30, 45, 60, 90, 120, 180, and 240 minutes following the meal (T15-T240). Saliva samples for cortisol assay will be taken at T0 and then at T15, T30, T45, T60, T120, T180 and T240 minutes following the meal to assess differences in hypothalamic-pituitary-adrenal axis response, which are known to differ according to fat distribution (Vicennati et al. 2002).Saliva samples will be collected using standard equipment (Salivette® Cortisol - Sarstedt, Nümbrecht, Germany). Concentration of salivary free cortisol will be measured using a commercially available chemiluminescence-immuno-assay (IBL, Hamburg, Germany).

The schedule for the measurement of each compound from blood will be as follows:

Glucose metabolism / insulin resistance will be assessed with these measures:

HbA_1C [van 't Riet et al. 2012]: T0

Glucose: T0, T15, T30, T45, T60, T90, T120

Insulin: T0, T15, T30, T45, T60, T90, T120, T240

C-peptide: T0, T15, T30, T60, T120

Hormonal response to meal:

GLP-1[Carroll et al. 2007; Baggio and Drucker 2007]: T0, T15, T30, T60, T90

GIP[Baggio and Drucker 2007]: T0, T15, T30, T60, T90

Leptin[Carroll et al. 2007]: T0, T240

Adiponectin: T0, T30, T60, T120, T240

Acylation-stimulating protein (ASP)[van Oostrom et al. 2004]: T0

Complement 3 (C3)[van Oostrom et al. 2004]: T0, T120

Ghrelin [Carroll et al. 2007]: T0, T15, T30, T45, T90, T180

ACTH[Vicennati et al. 2002]: T0, T15, T30, T45, T60, T120

Noradrenaline: T0, T15, T30, T60, T120

Adrenaline: T0, T15, T30, T60, T120

Lipid response to meal:

TG: T0, T60, T120, T240

FFA: T0, 60, 120, 240

HDL: T0, 60, 120, 240

Total cholesterol: T0, 60, 120, 240

Liver function

ALT: T0

AST: T0

GGT: T0

Inflammatory response to meal:

FBC (WBC, differential WCC, platelets)[Raz et al. 2013]: T0 T240

hsCRP[Raz et al. 2013]:T0 T120T240

TNF alpha[Nappo et al. 2002]:T0 T240

IL6[Nappo et al. 2002]:T0 T240

Chemerin:T0 T120 T240

Cathepsin-S:T0 T60 T120 T240

Nitrotyrosine[Ceriello et al. 2004]: T0 T30 T60 T90 T120 T240

sICAM-1[Ceriello et al. 2004; Nappo et al. 2002]: T0 T30 T60 T90 T120 T240

sVCAM-1[Ceriello et al. 2004; Nappo et al. 2002]: T0 T30 T60 T90 T120 T240

3.2 SUBJECTS SELECTION

For the main study, three clinical units will be involved for the enrollment of 180 individuals, 60 at each center.

University College London – Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust - Centre for Cardiovascular Imaging, UCL Institute of Cardiovascular Sciences. Responsible for the coordination of activities under WP4 and for the study coordination in UCL will be Prof. Andrew Taylor, Professor of Cardiovascular Imaging, Head – Centre for Cardiovascular Imaging, UCL Institute of Cardiovascular Sciences; Director – Centre for Cardiovascular MR.

Ospedale Pediatrico Bambino Gesù: Scientific Directorate, Research Area for Preventive and Predictive Medicine; Unit for Multifactorial Diseases, Bambino Gesù Pediatric Hospital, Rome. Responsible for the study coordination in OPBG will be Dr. Melania Manco, MD PhD.

Johns Hopkins University Hospital: Paediatric Cardiology - Helen Taussig Congenital Heart Center. Responsible for the study coordination in JHU: Dr. Allen Everett, paediatric cardiologist.

At each center, 90 obese adolescents (30 males; age 14-16.5) will be enrolled among patients admitted to clinic units. Patients will be selected from amongst those consecutively referred from November 2013 to October 2014. Inclusion criteria will be obesity (percentile of Body Mass Index ≥95° which equals to 1.645 SDS according to Kuczmarski RJ) with no systemic, endocrine and genetic disease. Exclusion criteria will be use of medication; alcohol and recreational drug. Enrolled patients will be restudied between November 2014 and April 2015.

For the ancillary study 20 offsprings of morbidly obese or patients affected by type 2 diabetes or family history of severe obesity or type 2 diabetes (at least one parent with a BMI >40 kg/m2 or type 2 diabetes under medication) per each centre can be enrolled instead of obese patients.

4 WITHDRAWAL FROM THE STUDY

Participation in this study is entirely voluntary. The parents may decide, at any time, that they do not wish their child to take part in the study without altering current or future treatment in any way.

If at any stage of the project the parents wish to withdraw their child from the study or the adolescent to retire the absent, the researcher responsible for the study will arrange for their child's information to be immediately removed from computer records and for any remaining samples we hold belonging to him/her to be destroyed.

5 PATIENT'S STUDY

5.1 STUDY TO BE PERFORMED

History will be recalled and clinical data collected as described in the paragraph 3.1. Patients will undergo routine laboratory tests (assay of fasting glucose, insulin, lipid profile, liver function tests, blood cell count and oral glucose tolerance test).

In addition, as required by the research protocol, they will undergo ultrasound and RMI estimation of abdominal and heart adiposity, ultrasound estimation of heart morphology and function, ultrasound estimation of intima media thickness.

Biological sample residual from routine laboratory assays will be used for the assay of circulating levels of adipokines and DNA analysis. Hence, no extra blood will be withdrawn for the purposes of this research protocol.

A database of patients, including name, age, disease, laboratory data but also specific indications about antibiotic, prebiotics, and probiotics administration will accomplish faecal sample datasheets for appropriate later description of gut microbiota enterotypes. Samples will be stored at 4°C for at maximum of 24 hours or, alternatively, at -80°C until shipment to metagenomic facilities for automatic DNA extraction and targeted-sequencing. In the latter case, the samples will be sent every two months or when suitable for the laboratories included in the study in dry ice by express courier.

6 STUDY PLANNING

6.1 EFFICACY PARAMETERS

Patients will be enrolled in the first 18 months of the study and re-evaluated 18 months later.

Reduced elasticity, distensibility, and stiffness at the follow-up will be used as end-point estimate of cardiovascular disease. Indeed, arterials stiffness is a marker of early atherosclerosis with good sensitivity and specificity.

Stiffness, Ultrasound and RMI assessment will be performed by trained project personnel to reduce inter-individual variability.

6.2 EXPERIMENTAL DESIGN

The study will last 4 years. It is designed as a prospective longitudinal study. The timeframe for patient recruitment spans the first 28 months. Follow up data for each data (clinical, imaging, immunologial etc) will be collected at follow-up visit as indicated in details in patient study session.

6.3 DATA PROTECTION

All collected data will be anonymised. Clinical data and biological samples will be coded and stored as such. The code will be generated by software using a system of 128-bit encryption. The code will be stored in a close drawer by Prof. Andrew Taylor. At the end of the study, the key code will be destroyed and, hence, data anonymised. From this moment on, it will not be possible for anyone to discover the patient's identity.

All clinical data will be communicated to participants and/or legal representative except for genetic testing.

7 SECURITY EVALUATION

7.1 DEFINITIONS

No adverse effects are foreseen as consequence of the clinical study.

8 SAMPLE DIMENSION AND STATISTIC METHODOLOGY

8.1 STATISTIC DESIGN

Main study

Main goals of this Study are the creation of a data repository for the development of biomechanical models and of workflows.

For these purposes no inferential statistical analysis is foreseen, nor the application of formal criteria for the definition of the sample size.

However, descriptive data analysis will be performer, both in absolute and in normalized way.

To guarantee quality and usability of the collected data the following procedures will be applied:

- data *pre-processing*: data validation, discretization, null & outlier removal will be performed using the system "Data Curator & Validator" (DCV) developed in the "*An integrated platform for European paediatrics based on a Grid-enabled network of leading clinical centres*" (Health-e-Child) project;
- mappi*ng: descriptor*s will be cross-mapped to standard data dictionaries (e.g. epSOS value sets, ICD-10 diagnosis, LOINC labs...);
- normalization: source numeric dat*a will be normalized to generate z-values;*
- *data enrichment: normal values will be added from legacy guidel*ines;
- calculation of new variables: aggregated scores will be computed out of various fields (e.g. Body Mass Index out of weight/size);
- data description:
  - standard descriptive statistical tests will be applied for categoric and for continuous variables;
  - distance measures will be applied to generate case-based retrieval application;
  - clustering of instances using statistical & visualization algorithms;
- for imaging data, visual features will be extracted from the images that describe image regions (local image content); data will be aggregated across cases in terms of visual data but also textual or structured data to be able to differentiate normal form abnormal visual data;
- simulations will be performed based on data-mining tecniques, using the AITION (*Scalable Platform for Interactive Data Mining)* system, developed in the "*An integrated platform for European paediatrics based on a Grid-enabled network of leading clinical centres*" (Health-e-Child) project.

Ancillary study

Table 1. Power calculations at the 80% level for samples sizes from N=60 to N=180.

Metabolic / vascular parameter	SD	Mean response to mixed meal in normals	Sample Size (subjects)			
			60	90	120	180
Fasting glucose (mmol/L) [Ceriello 2004]	0.89	-	0.65	0.53	0.46	0.38
HbA_1C (%)	0.89	-	0.65	0.53	0.46	0.38
Resting systolic BP (mmHg) [Gray L, 2011]	12.7	-	9.2	7.6	6.5	5.4
Heart rate response to meal (bpm) [Lipsitz LA 1993]	4.2	4.5	3.1	2.5	2.2	1.8
Change in peak systolic flow velocity in superior mesenteric artery in response to meal (cm/s) [Masui 1994]	19.0	29.6	13.8	11.3	9.8	8.0

Glucose response to meal (mmol/L) [Ceriello, 2004]	3.3	4.7	2.4	2.0	1.7	1.4
Triglyceride response to meal (mmol/L) [Ceriello, 2004]	0.59	1.03	0.43	0.35	0.31	0.25

Calculations were performed assuming two-sample comparisons of means between two groups (eg. Obese versus offspring of obese and diabetic patients) that are equally sized (50% of N) at a significance level of 0.05. Values are differences between the means in two groups that could be detected for each parameter, at each sample size. Estimates of variance (SD) and typical mean response to a mixed meal, where appropriate, are given as drawn from the literature, which is referenced.

8.2 MANAGEMENT OF MISSING DATA

Patients whose information is missing or incorrectly transcribed will not be used for the analysis, but will be marked appropriately in the electronic database by inserting a variable "Flag".

9. AMINISTRATIVE AND ETHICAL PROCEDURES

Confidentiality

Clinical data will be acquired as required by each partner's national law.

At each clinical center patient's data will be collected and stored as electronic files and will be accessible by the responsible research personnel. Access to data will be granted using their personal credentials. Access to the file will be protected and the log of the user who performed the operation will be required at regular intervals. The data manager will perform regularly a data backup.

Data publication and final report

The ownership of scientific data will be shared between all the partners involved in the Project. The WP leader, Dr. Andrew Taylor, and researchers who will conduct the study, will endeavor to promote the dissemination of the results through the project website, communications in national and international scientific meetings, publication in international journals of high scientific profile. The dissemination and publication of the results by the experimenters will be promoted in accordance with the provisions in force concerning the confidentiality of sensitive data.
In all scientific publications the efforts of all researchers will be recognized.

All health professionals involved in the project will seek to minimize the physical and psychological discomfort caused to patients and parents from participating in this study. In order to ensure the well-

being, they will not be notified in any way about the personal results of genetic investigations.

9.1 AUTORIZATIONS

Study implementation and patient enrollment will undergo prior approval of the present Protocol by the Local Ethical Committee of each partner involved in patient enrollment.

9.2 INFORMED CONSENT

Each parent/patient who will be asked for his/her enrollment in the study will be informed on every aspect concerning the study itself and, before the recruitment begins, he/she must sign the informed consent model. The date of the signature of the consent will be recorded. A Copy of the informed consent model must be handed to the parent/patient.

9.3 INSURANCE COVERAGE

Insurance coverage used is as foreseen by each research structure for clinical and research activities.

9.4 USE OF THE INFORMATION AND DATA PUBBLICATION

Researchers must have the right to disseminate/publish the results of the study, with respect to the current legislation on sensible data protection and Intellectual property Rights. European Commission does not have any right in the publication/dissemination of study results

9.5 CLINICAL PROTOCOL AMENDMENTS

Clinical protocol amendment is intended to be any modifications to the final version of the clinical protocol that, after being approved by the Researcher and by the Ethical Committee, cannot be informally modified.

9.6 DATA MANAGEMENT AND DOCUMENT PRESERVATION

Researchers will manage the preservation of patients identification codes for at least 15 years after the end of the study. Patients clinical data and other original data will be kept for the maximum period allowed by the Hospital (min. 15 years).

9.7 BUDGET

The project costs are covered by the specific Grant by the European Commission. Such grant will cover 75% of staff costs, of consumables and equipment used for the project, and of all other expenses involved. Annex 1 (GPF) contains the project's entire budget, including details of the Institution's budget.

10. RESEARCHER RESPONSIBILITY

The researcher, as principle investigator of the study declares to be aware of all the duties committed to him/her and his/her collaborators for the conduction of the study. Except where explicitly edited, the term "researcher" on this Protocol and on the CRF is referred to the researcher or to any other person appointed by him/her, who will have power to sign documents instead of him/her.

The researcher will conduct the study according to the principles of the Helsinki declaration (1964) and Amendments (Appendix 1), and to the European Guidelines on Clinical Good Practices .

11. Annexes

Annex 1: GPF

Annex 2: DOW

Annex 3: Questionnaires

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Children assent form

SCHEDA INFORMATIVA E DICHIARAZIONE DI ASSENSO

ai fini della richiesta di partecipazione ad uno studio clinico

per i bambini

1. OSPEDALE PEDIATRICO BAMBINO GESÙ

SCHEDA INFORMATIVA RELATIVA ALLO STUDIO:

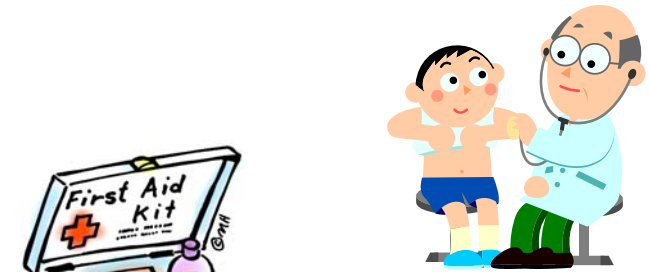
MD-Paedigree - Acquisizione dati e processi sulle Cardiomiopatie

Gli studi di ricerca mirano ad ottenere nuove conoscenze che possano aiutare altre persone in futuro.

Perché stiamo facendo questo studio di ricerca?

Lo scopo di questo studio è di creare un sistema per prevedere l'andamento di alcune malattie e delle loro terapie. Ti è stato chiesto di partecipare allo studio perché i tuoi genitori ed il tuo medico ritengono

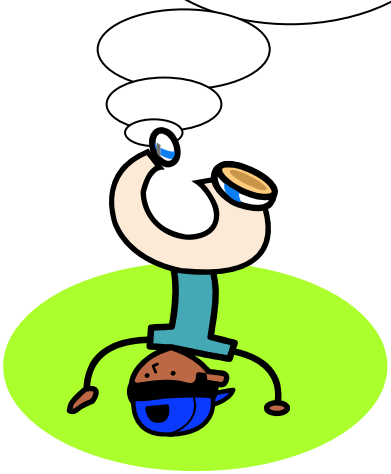
Cosa accadrà durante lo studio?



1. Sarai esaminato dal tuo medico all'inizio dello studio;
2. Quando tornerai per i controlli per la tua malattia, il tuo medico ti esaminerà anche per il progetto.
3. 1 sola volta, il tuo medico userà un poco del sangue rimasto dopo un prelievo di sangue, per fare alcune analisi genetiche. Non saranno fatti prelievi in più per il progetto.
4. Dovrai fare una Risonanza Magnetica Nucleare.
5. Riteniamo che tu possa prendere parte a questo studio che durerà 4 anni.

Cosa può accadere di buono?

Dalla partecipazione allo studio non verrà nessun beneficio diretto per te. Contribuirai però a costruire dei modelli che in futuro potranno aiutare te ed altri bambini con la tua stessa malattia .



Che fastidi puoi avere?

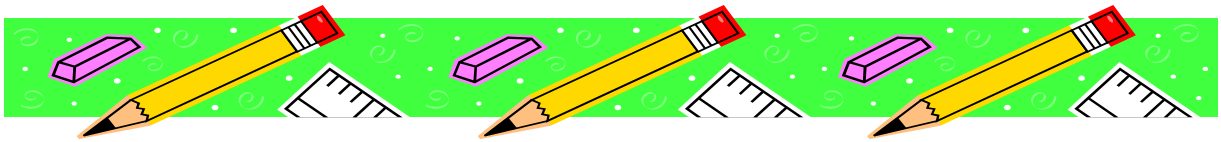
Da questo studio non avrai alcun fastidio. Ti chiediamo comunque di raccontare al tuo medico se ti senti poco bene.

Non ti preoccupare, se dovessi avere dei fastidi ti aiuteremo a stare meglio.

Domande?

Se vuoi puoi chiedere al tuo medico dell'ospedale di spiegarti qualcosa che non hai capito.

Insieme ai tuoi genitori puoi decidere se partecipare a questo



_____ _____

Data/ora Scrivi il tuo nome in stampatello qui se desideri partecipare allo studio

_____ _____

Data/ora Firma del medico che ha informato il paziente

Adolescents assent form

INFORMATION SHEET AND DECLARATION OF CONSENT

for the purposes of the request to take part in a paediatric clinical study

for ADOLESCENT patients

STUDY INFORMATION SHEET:

MD-Paedigree (Risk of cardiovascular disease in obese children and adolescents)

Dear ……..,

As you know, you are being treated at our facility for your obesity.

This hospital is conducting a medical and scientific research programme entitled MD-Paedigree – Risk of cardiovascular disease in obese adolescents. This research is a multicentre project, which means that it involves various hospitals and treatment centres in Italy and abroad, and is being funded by the European Commission. It is a non-profit academic research project.

What the research is proposing

The general objective of the MD – Paedigree project is to create computerised models of various diseases that make it possible to determine forecasts of the development of those diseases and the impact of various treatments and therapies.

With regard to obesity, the study proposes to create this type of model, incorporating all available information and knowledge about the risk of cardiovascular disease in obese children and adolescents. This will therefore involve the incorporation of clinical, imaging, biological and genetic information as part of a multidisciplinary and multilayered patient

approach. All of the information collected during patient treatment will be analysed and interpreted using innovative electronic tools and the use of software developed on an *ad hoc* basis, in order to obtain a more detailed diagnosis and a more realistic picture of the future course of the disease. This will provide doctors with access to a tool that is able to provide a more detailed, predictive and personalised diagnosis for each patient, and therefore to provide better quality care.

In particular, the study will involve the collection and processing of clinical data on obese patients, so that corresponding cardiovascular risk can be assessed, i.e. the risk of exhibiting or developing, even subsequently, cardiovascular conditions such as hypertension, and of developing dyslipidaemia, diabetes mellitus, steatosis (fatty liver), etc.

What your participation in the research will involve

If you decide to take part in the study, the clinical data and laboratory tests performed in relation to your condition (assessment of fasting blood sugar, blood insulin, lipid profile, liver function, blood count, glucose tolerance), as envisaged in your treatment plan, will be used for the purposes of this research.

Furthermore, for the purposes of this research, you will undergo additional, non-invasive tests, that will be valuable in enabling a more detailed and specific determination of your cardiovascular risk: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging.

Furthermore, we will use residual blood samples to test for certain molecules responsible for inflammation and to study the profile of certain genes that could influence cardiovascular risk.

Changes in cardiovascular risk will be reassessed after 18 months, irrespective of the treatment provided and the associated success in terms of weight loss.

The study will last 36 months, and will involve the participation at this hospital of XX patients, to be chosen from among all obese patients of the same age, and a further XX patients in two other international clinics (Ospedale Pediatrico Bambino Gesù and...).

If you consent to take part in this study, you will undergo an initial visit to verify that your condition meets the criteria required for the study. All of the additional blood chemistry tests required by the protocol will be performed on residual biological material from the tests already performed in relation to your condition, while the non-invasive diagnostic tests you will need to undergo will use ultrasound or magnetic waves.

Your participation in the trial will not involve any additional cost for you and your family.

Procedures you will undergo during the research

You will be asked to undergo an evaluation of fasting blood sugar, blood insulin, lipid profile, liver function, blood count and glucose tolerance in relation to your obesity, according to standard practice.

In addition to the above evaluations, the study envisages the performance of the following procedures, in addition to the evaluations and procedures already planned for you in relation to your condition: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging. You will also be asked to provide two faecal samples for analysis of intestinal bacterial flora.

Your residual plasma/serum, usually approximately 1 cc, from the blood chemistry tests you will be asked to undergo, in relation to your condition and as envisaged in your treatment plan, will be used to test circulating levels of certain molecules that cause inflammation and modulate the risk of cardiovascular diseases (for example, C-reactive protein, some interleukins, tumour necrosis factor-alpha, etc).

Still using residual bacterial material, we will assess whether you are a carrier of certain genes that have been associated with a greater or lesser cardiovascular risk.

For the purposes of this research, it is not necessary for additional blood samples to be taken, other than those already required from you in relation to your condition.

Genetic tests and storage of samples

At the end of the study, the data and corresponding samples relating to your child will be anonymised. The samples will be kept for 15 years following sampling, and may be used in the future for research in the same field. Because it will not be possible to trace your identity, approval from the Ethics Committee in relation to the objectives of any subsequent research will, in any case, be obtained.

For the duration of the study, the samples will be stored in a safe place, in a dedicated refrigerator at -80^{0}C in the research laboratories of the Ospedale Pediatrico Bambino Gesù, with access limited solely to the research personnel. Only the study manager, Dr Melania Manco, and/or the laboratory personnel may work on personal samples.

The faecal samples will be stored and analysed at the Microbiology Laboratory in OPBG hospital (Manager: Dr Lorenza Putignani).

With reference to the blood samples, these will be anonymised and sent periodically to:

- the laboratory at the company BMR Genomics, for genetic analysis;
- the Metabolic Medicine Department of the University of Utrecht, Holland, for analysis of inflammatory cytokines.

What benefits might you obtain from taking part in the research

It is not expected that you will obtain any direct benefit from participation in this research, including the genetic analysis, but your participation will help in determining whether all of the information gathered in the study could be helpful, in the future, in taking the most appropriate therapeutic decisions about your health and that of other adolescents suffering from the same condition.

What are the risks associated with participation in the research

Participation in the research, including the genetic tests, will not entail any risk for you.

What will happen if you decide not to consent to the research

You are free to decide not to consent to take part in the study. In such a case, you will not undergo the additional tests such as the echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging; the residual blood from the sampling envisaged in your treatment plan will not be used for the additional analysis, your personal data will not be processed and you will still receive all of the treatments envisaged for your condition, without restriction, and the doctors will continue to monitor the course of your condition with the appropriate degree of attention.

Suspension of the research

Your involvement in this research programme is completely voluntary and you may withdraw your consent at any time in the course of the study by providing written notice to the doctor responsible for the study at the hospital (Dr .....), who will provide notification accordingly to the study coordinator Dr Andrew Taylor.

If you decide to withdraw your consent to participate, Dr ....., manager for the study for ....., will decode the data identifying the information about you. Your data and the

images relating to your tests will be deleted from the file and the biological material will be destroyed.

Six months after the end of the study, your codified data will be anonymised and the codification keys used to match the code to your data will be destroyed. From that point, it will no longer be possible to identify your data and samples.

Confidentiality of personal data

Please note that the ..... and the European Commission, which have commissioned the study being described to you here, each within its own specific remit and in accordance with the responsibilities set down in the standards of good clinical practice and under Legislative Decree No 196 of 30 June 2003 on the protection of personal data, will process your data, in particular those on your health and, solely to the extent absolutely necessary in relation to the objective of the study, other data relating to your origin, lifestyle and sex life, solely for the purposes of implementation of the study.

For this purpose, the data will be collected by the ..... and sent to the European Commission and to the external individuals or companies acting on its behalf, including in countries that are not members of the European Union.

Please note, furthermore, that processing of the personal data collected in the course of the study by the doctor monitoring your condition is essential for performance of the study: refusal to provide that information will mean that you cannot participate.

The doctor monitoring your condition in the study will identify you using a code generated by a computer program: the data about you collected in the course of the study, except for your name, will be sent to the European Commission, and recorded, processed and stored with that code, date of birth, sex, weight and height. Only the doctor and the individuals authorised may match this code to your name.

At the end of the study, the codification/decodification keys will be destroyed and the data will be anonymised, and no one will be able to determine your identity.

The data, which will be processed using electronic and non-electronic tools, will be distributed only in strictly anonymous form, such as through scientific publications, statistics and scientific conferences. Your participation in the study means that, in accordance with the legislative requirements on clinical studies, the personnel from the

European Commission or external companies that perform study monitoring and verification on its behalf, the Ethics Committee and the Italian and foreign health authorities may gain information about your data, including where contained in the original clinical documentation, using procedures designed to guarantee that your identity remains confidential.

You may exercise the rights set down in Article 7 of the Personal Data Protection Code (e.g. accessing your personal data, supplementing, updating and correcting those data, and opposing processing of those data for legitimate reasons, etc.) by approaching the study manager directly.

You may discontinue your participation in the study at any time without providing any justification: in such a case, the associated biological samples will be destroyed. No further data about you will be collected, notwithstanding the use of those data already collected, without alteration, for the purposes of determining the results of the research.

Information about the results of the research

If you wish, at the end of the study, you may be advised of the results obtained in general and, in particular, those relating to you, except for the results of the genetic tests. Many genetic polymorphisms, including those we will be studying in this research, only provide minimal predictive information about cardiovascular risk for the subject, and cannot therefore be used for diagnostic or therapeutic purposes. You will not therefore be provided with the results for the individual tests.

Additional information

For further information and reporting during the research, the following individuals will be available: Dr ....., Scientific Management, .

The study protocol proposed to you has been drafted in accordance with the European Union Standards for Good Clinical Practice and the Helsinki Declaration, and has been approved by the Ethics Committee for Drug Trials associated with this hospital.

You can report any fact that you believe should be notified, in relation to the trial and relating to your child, to the Ethical Committee associated with this hospital. Such information should be reported to the Chairman of the Ethical Committee .....

2. DECLARATION OF CONSENT

I, the undersigned: _____

declare that I have been given, by Dr _____,

exhaustive explanations about the request for participation in the above-mentioned research project, as shown in the information sheet attached to this form, a copy of which I have been given (indicate the date and time when this was provided) Date……time…….

I also declare that I have had an opportunity to discuss those explanations and to ask all questions that I felt necessary and that I have been given satisfactory answers, and that I have had the opportunity to discuss the specific aspects of the study with people I trust.

I therefore freely consent to take part in the research, having fully understood the meaning of the request and the risks and benefits that may derive from participation.

I have been informed, furthermore, of my right to have free access to the documentation (insurance, clinical, scientific, pharmacotherapeutic) relating to the research and the express evaluation by the Ethics Committee for Drug Trials.

I consent to take part in the study	**yes** □ **no** □
I consent to performance of the additional tests	**yes** □ **no** □

_____ _____

Date/Time Signature of

_____ _____

Date/Time

Parents/guardian consent form

INFORMATION SHEET AND DECLARATION OF CONSENT

for the purposes of the request to take part in a paediatric clinical study

<u>for parents/legal guardian</u>

STUDY INFORMATION SHEET:

MD-Paedigree (Risk of cardiovascular disease in obese children and adolescents)

Dear Parents of ……..,

As you know, your child is being treated at our facility for obesity.

This hospital is conducting a medical and scientific research programme entitled MD-Paedigree – Risk of cardiovascular disease in obese adolescents. This research is a multicentre project, which means that it involves various hospitals and treatment centres in Italy and abroad, and is being funded by the European Commission. It is a non-profit academic research project.

What the research is proposing

The general objective of the MD – Paedigree project is to create computerised models of various diseases that make it possible to determine forecasts of the development of those diseases and the impact of various treatments and therapies.

With regard to obesity, the study proposes to create this type of model, incorporating all available information and knowledge about the risk of cardiovascular disease in obese children and adolescents. This will therefore involve the incorporation of clinical, imaging, biological and genetic information as part of a multidisciplinary and multilayered patient approach. All of the information collected during patient treatment will be analysed and interpreted using innovative electronic tools and the use of software developed on an *ad*

hoc basis, in order to obtain a more detailed diagnosis and a more realistic picture of the future course of the disease. This will provide doctors with access to a tool that is able to provide a more detailed, predictive and personalised diagnosis for each patient, and therefore to provide better quality care.

In particular, the study will involve the collection and processing of clinical data on obese patients, so that corresponding cardiovascular risk can be assessed, i.e. the risk of exhibiting or developing, even subsequently, cardiovascular conditions such as hypertension, and of developing dyslipidaemia, diabetes mellitus, steatosis (fatty liver), etc.

What participation in the research will involve for your child

If you decide to have your child take part in the study, the clinical data and laboratory tests performed in relation to your child's condition (assessment of fasting blood sugar, blood insulin, lipid profile, liver function, blood count, glucose tolerance), as envisaged in the treatment plan, will be used for the purposes of this research.

Furthermore, for the purposes of this research, your child will undergo additional, non-invasive tests, that will be valuable in enabling a more detailed and specific determination of your child's cardiovascular risk: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging.

Furthermore, we will use residual blood samples to test for certain molecules responsible for inflammation and to study the profile of certain genes that could influence cardiovascular risk.

Changes in cardiovascular risk will be reassessed after 18 months, irrespective of the treatment provided and the associated success in terms of weight loss.

The research project will last 36 months, and will involve the participation at this hospital of 90 patients, to be chosen from among all obese patients like your child and of the same age, and a further 120 patients in two other international clinics (Ospedale Pediatrico Bambino Gesù).

If you consent to have your child take part in this study, he/she will undergo an initial visit to verify that his/her condition meets the criteria required for the study. All of the blood chemistry tests will be performed on residual biological material from the tests already performed in relation to your child's condition, while the non-invasive diagnostic tests he/she will need to undergo will use ultrasound or magnetic waves.

Participation in the trial will not involve any additional cost for you.

Procedures your child will undergo during the research

Your child will be asked to undergo an evaluation of fasting blood sugar, blood insulin, lipid profile, liver function, blood count and glucose tolerance in relation to his/her obesity, according to standard practice.

In addition to the above evaluations, the study envisages the performance of the following procedures, in addition to the evaluations and procedures already planned for your child in relation to his/her condition: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging. Your child will also be asked to provide two faecal samples for analysis of intestinal bacterial flora.

Your child's residual plasma/serum, usually approximately 1 cc, from the blood chemistry tests he/she will be asked to undergo, in relation to his/her condition and as envisaged in his/her treatment plan, will be used to test circulating levels of certain molecules that cause inflammation and modulate the risk of cardiovascular diseases (for example, C-reactive protein, some interleukins, tumour necrosis factor-alpha, etc).

Still using residual bacterial material, we will assess whether your child is a carrier of certain genes that have been associated with a greater or lesser cardiovascular risk.

For the purposes of this research, it is not necessary for additional blood samples to be taken, other than those already required from your child in relation to his/her condition.

Genetic tests and storage of samples

At the end of the study, the data and corresponding samples relating to your child will be anonymised. The samples will be kept for **15 years** following sampling, and may be used in the future for research in the same field. **Because it will not be possible to trace the identity of your child, approval from the Ethics Committee in relation to the objectives of any subsequent research will, in any case, be obtained.**

For the duration of the study, the samples will be stored in a safe place, in a dedicated refrigerator at -80^{0}C in the research laboratories of the Ospedale Pediatrico Bambino Gesù, with access limited solely to the research personnel. Only the study manager, Dr Melania Manco, and/or the laboratory personnel may work on personal samples.

The faecal samples will be stored and analysed at the Microbiology Laboratory in our hospital (Manager: Dr Lorenza Putignani).

With reference to the blood samples, these will be anonymised and sent periodically to:

- the laboratory at the company BMR Genomics (Manager: Dr Barbara Simionati), for genetic analysis;
- the Metabolic Medicine Department of the University of Utrecht, Holland (Manager: Dr Hank Shipper), for analysis of inflammatory cytokines.

What benefits might your child obtain from taking part in the research

It is not expected that your child will obtain any direct benefit from participation in this research, including the genetic analysis, but participation will help in determining whether all of the information gathered in that study could be helpful, in the future, in taking the most appropriate therapeutic decisions about your child's health and that of other adolescents suffering from the same condition.

What are the risks associated with participation in the research

Participation in the research, including the genetic tests, will not entail any risk for your child.

What happens if you decide not to allow your child to take part in the ancillary study

You are free to decide not to have your child take part in the study. In such a case, he/she will not undergo the additional tests such as the echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging; the residual blood from the sampling envisaged in his/her treatment plan will not be used for the additional analysis, his/her personal data will not be processed and he/she will still receive all of the treatments envisaged for his/her condition, without restriction, and the doctors will continue to monitor the course of his/her condition with the appropriate degree of attention.

Suspension of the research

Your involvement in this research programme is completely voluntary and you may withdraw your consent at any time in the course of the study by providing written notice

to the doctor responsible for the study at the hospital (Dr .....), who will provide notification accordingly to the study coordinator Dr Andrew Taylor.

If you decide to withdraw your consent for your child to participate, Dr Melania Manco, manager for the study for OPBG, will decode the data identifying the information about your child. The data about your child and the images relating to his/her tests will be deleted from the file and the biological material will be destroyed.

Six months after the end of the study, the codified data about your child will be anonymised and the codification keys used to match the code to that data will be destroyed. From that point, it will no longer be possible to identify the data and samples relating to your child.

Confidentiality of personal data

Please note that the UCL and the European Commission, which have commissioned the study being described to you here, each within its own specific remit and in accordance with the responsibilities set down in the standards of good clinical practice and under Legislative Decree No 196 of 30 June 2003 on the protection of personal data, will process the data about child, in particular those on his/her health and, solely to the extent absolutely necessary in relation to the objective of the study, other data relating to your child's origin, lifestyle and sex life, solely for the purposes of implementation of the study.

For this purpose, the data will be collected by the OPBG and sent to the European Commission and to the external individuals or companies acting on its behalf, including in countries that are not members of the European Union.

Please note, furthermore, that processing of the personal data collected in the course of the study by the doctor monitoring your child's condition is essential for performance of the study: refusal to provide that information will mean that your child cannot participate.

The doctor monitoring your child's condition in the study will identify him/her using a code generated by a computer program: the data about your child collected in the course of the study, except for his/her name, will be sent to the European Commission, and recorded, processed and stored with that code, date of birth, sex, weight and height. Only the doctor and the individuals authorised may match this code to your child's name.

At the end of the study, the codification/decodification keys will be destroyed and the data will be anonymised, and no one will be able to determine your child's identity.

The data, which will be processed using electronic and non-electronic tools, will be distributed only in strictly anonymous form, such as through scientific publications,

statistics and scientific conferences. The participation of your child in the study means that, in accordance with the legislative requirements on clinical studies, the personnel from the European Commission or external companies that perform study monitoring and verification on its behalf, the Ethics Committee and the Italian and foreign health authorities may gain information about your child's data, including where contained in the original clinical documentation, using procedures designed to guarantee that his/her identity remains confidential.

You may exercise the rights set down in Article 7 of the Personal Data Protection Code (e.g. accessing your child's personal data, supplementing, updating and correcting those data, and opposing processing of those data for legitimate reasons, etc.) by approaching the study manager directly.

You may discontinue your child's participation in the study at any time without providing any justification: in such a case, the associated biological samples will be destroyed. No further data about your child will be collected, notwithstanding the use of those data already collected, without alteration, for the purposes of determining the results of the research.

Information about the results of the research

If you wish, at the end of the study, you may be advised of the results obtained in general and, in particular, those relating to your child, except for the results of the genetic tests. Many genetic polymorphisms, including those we will be studying in this research, only provide minimal predictive information about cardiovascular risk for the subject, and cannot therefore be used for diagnostic or therapeutic purposes. You will not therefore be provided with the results for the individual tests.

Additional information

For further information and reporting during the research, the following individuals will be available: Dr .....,; e-mail:

The study protocol proposed to you has been drafted in accordance with the European Union Standards for Good Clinical Practice and the Helsinki Declaration, and has been approved by the Ethical Committee associated with this hospital.

You can report any fact that you believe should be notified, in relation to the trial and relating to your child, to the Ethical Committee associated with this hospital. Such information should be reported to the Chairman of the Ethical Committee .....

2. DECLARATION OF CONSENT

I, the undersigned: _____

parent/legal guardian of

_____

declare that I have been given, by Dr _____,

exhaustive explanations about the request for participation in the above-mentioned research project, as shown in the information sheet attached to this form, a copy of which I have been given (indicate the date and time when this was provided) Date……time…….

I also declare that I have had an opportunity to discuss those explanations and to ask all questions that I felt necessary and that I have been given satisfactory answers, and that I have had the opportunity to discuss the specific aspects of the study with people I trust.

I therefore freely consent to allow my child to take part in the research, having fully understood the meaning of the request and the risks and benefits that may derive from participation.

I have been informed, furthermore, of my right to have free access to the documentation (insurance, clinical, scientific, pharmacotherapeutic) relating to the research and the express evaluation by the Ethics Committee for Drug Trials.

I consent to the enrolment of my child in the study	**yes** □ **no** □
I consent to performance of the additional tests	**yes** □ **no** □
I consent to the sampling of my child's genetic material	**yes** □ **no** □
I authorise the performance of genetic analysis as described in the information sheet	**yes** □ **no** □
I authorise the storage of anonymised samples	**yes** □ **no** □
I authorise the storage of anonymised samples beyond six months following collection	**yes** □ **no** □
I authorise storage of biological material	**yes** □ **no** □
I consent to be contacted again in the future for any further studies	**yes** □ **no** □
I wish to be contacted again for notification of the results of the	**yes** □ **no** □

procedures performed on my child	
I authorise the use of the data, in anonymised form, for scientific purposes	**yes** □ **no** □
	yes □ **no** □
	yes □ **no** □

_____ _____

Date/Time Signature of the doctor providing information to the parent/legal guardian

Date/Time _____

Signature of the parent/legal guardian

Date/Time

_____

Signature of the parent/legal guardian

[*In the event that the parent/legal guardian is not able to sign*]

I, the undersigned: _____

confirm that I was present at the information interview between Dr _____ and Mr _____ and

I confirm that it was not possible for the latter individual to state his consent in writing.

_____ _____

Date

Signature of the independent witness

Patient over 18 yrs consent form

INFORMATION SHEET AND DECLARATION OF CONSENT

for the purposes of the request to take part in a paediatric clinical study

<u>for ADULT patients (of legal age)</u>

STUDY INFORMATION SHEET:

MD-Paedigree (Risk of cardiovascular disease in obese children and adolescents)

Dear ……..,

As you know, you are being treated at our facility for your obesity.

This hospital is conducting a medical and scientific research programme entitled MD-Paedigree – Risk of cardiovascular disease in obese adolescents. This research is a multicentre project, which means that it involves various hospitals and treatment centres in Italy and abroad, and is being funded by the European Commission. It is a non-profit academic research project.

What the research is proposing

The general objective of the MD – Paedigree project is to create computerised models of various diseases that make it possible to determine forecasts of the development of those diseases and the impact of various treatments and therapies.

With regard to obesity, the study proposes to create this type of model, incorporating all available information and knowledge about the risk of cardiovascular disease in obese children and adolescents. This will therefore involve the incorporation of clinical, imaging, biological and genetic information as part of a multidisciplinary and multilayered patient approach. All of the information collected during patient treatment will be analysed and interpreted using innovative electronic tools and the use of software developed on an *ad hoc* basis, in order to obtain a more detailed diagnosis and a more realistic picture of the future course of the disease. This will provide doctors with access to a tool that is able to

provide a more detailed, predictive and personalised diagnosis for each patient, and therefore to provide better quality care.

In particular, the study will involve the collection and processing of clinical data on obese patients, so that corresponding cardiovascular risk can be assessed, i.e. the risk of exhibiting or developing, even subsequently, cardiovascular conditions such as hypertension, and of developing dyslipidaemia, diabetes mellitus, steatosis (fatty liver), etc.

What your participation in the research will involve

If you decide to take part in the study, the clinical data and laboratory tests performed in relation to your condition (assessment of fasting blood sugar, blood insulin, lipid profile, liver function, blood count, glucose tolerance), as envisaged in your treatment plan, will be used for the purposes of this research.

Furthermore, for the purposes of this research, you will undergo additional, non-invasive tests, that will be valuable in enabling a more detailed and specific determination of your cardiovascular risk: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging.

Furthermore, we will use residual blood samples to test for certain molecules responsible for inflammation and to study the profile of certain genes that could influence cardiovascular risk.

Changes in cardiovascular risk will be reassessed after 18 months, irrespective of the treatment provided and the associated success in terms of weight loss.

The research project will last 36 months, and will involve the participation at this hospital of 90 patients, to be chosen from among all obese patients of the same age, and a further 120 patients in two other international clinics (Ospedale Pediatrico Bambino Gesù and.....).

If you consent to take part in this study, you will undergo an initial visit to verify that your condition meets the criteria required for the study. All of the additional blood chemistry tests required by the protocol will be performed on residual biological material from the tests already performed in relation to your condition, while the non-invasive diagnostic tests you will need to undergo will use ultrasound or magnetic waves.

Your participation in the trial will not involve any additional cost for you and your family.

Procedures you will undergo during the research

You will be asked to undergo an evaluation of fasting blood sugar, blood insulin, lipid profile, liver function, blood count and glucose tolerance in relation to your obesity, according to standard practice.

In addition to the above evaluations, the study envisages the performance of the following procedures, in addition to the evaluations and procedures already planned for you in relation to your condition: echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging. You will also be asked to provide two faecal samples for analysis of intestinal bacterial flora.

Your residual plasma/serum, usually approximately 1 cc or more, from the blood chemistry tests you will be asked to undergo, in relation to your condition and as envisaged in your treatment plan, will be used to test circulating levels of certain molecules that cause inflammation and modulate the risk of cardiovascular diseases (for example, C-reactive protein, some interleukins, tumour necrosis factor-alpha, etc).

Still using residual bacterial material, we will assess whether you are a carrier of certain genes that have been associated with a greater or lesser cardiovascular risk.

For the purposes of this research, it is not necessary for additional blood samples to be taken, other than those already required from you in relation to your condition.

Genetic tests and storage of samples

At the end of the study, the data and corresponding samples relating to your child will be anonymised. The samples will be kept for **15 years** following sampling, and may be used in the future for research in the same field. **Because it will not be possible to trace your identity, approval from the Ethics Committee in relation to the objectives of any subsequent research will, in any case, be obtained.**

For the duration of the study, the samples will be stored in a safe place, in a dedicated refrigerator at -80^{0}C in the research laboratories of the Ospedale Pediatrico Bambino Gesù, with access limited solely to the research personnel. Only the study manager, Dr Melania Manco, and/or the laboratory personnel may work on personal samples.

The faecal samples will be stored and analysed at the Microbiology Laboratory in our hospital (Manager: Dr Lorenza Putignani).

With reference to the blood samples, these will be anonymised and sent periodically to:

- the laboratory at the company BMR Genomics (Manager: Dr Barbara Simionati), for genetic analysis;
- the Metabolic Medicine Department of the University of Utrecht, Holland (Manager: Dr Hank Shipper), for analysis of inflammatory cytokines.

What benefits might you obtain from taking part in the research

It is not expected that you will obtain any direct benefit from participation in this research, including the genetic analysis, but your participation will help in determining whether all of the information gathered in the study could be helpful, in the future, in taking the most appropriate therapeutic decisions about your health and that of other adolescents suffering from the same condition.

What are the risks associated with participation in the research

Participation in the research, including the genetic tests, will not entail any risk for you.

What will happen if you decide not to take part in the research

You are free to decide not to take part in the study. In such a case, you will not undergo the additional tests such as the echocardiograph, ultrasound assessment of carotid intima-media thickness, tonometric analysis of arterial stiffness, evaluation of myocardial morphology and function and of the accumulation of adipose tissue on the abdomen, liver and heart through ultrasound and magnetic resonance imaging; the residual blood from the sampling envisaged in your treatment plan will not be used for the additional analysis, your personal data will not be processed and you will still receive all of the treatments envisaged for your condition, without restriction, and the doctors will continue to monitor the course of your condition with the appropriate degree of attention.

Suspension of the research

Your involvement in this research programme is completely voluntary and you may withdraw your consent at any time in the course of the study by providing written notice to the doctor responsible for the study at the hospital (Dr .....), who will provide notification accordingly to the study coordinator Dr Andrew Taylor.

If you decide to withdraw your consent to participate, Dr ....., manager for the study for UCL, will decode the data identifying the information about you. Your data and the images relating to your tests will be deleted from the file and the biological material will be destroyed.

Six months after the end of the study, your codified data will be anonymised and the codification keys used to match the code to your data will be destroyed. From that point, it will no longer be possible to identify your data and samples.

Confidentiality of personal data

Please note that the UCL and the European Commission, which have commissioned the study being described to you here, each within its own specific remit and in accordance with the responsibilities set down in the standards of good clinical practice and under Legislative Decree No 196 of 30 June 2003 on the protection of personal data, will process your data, in particular those on your health and, solely to the extent absolutely necessary in relation to the objective of the study, other data relating to your origin, lifestyle and sex life, solely for the purposes of implementation of the study.

For this purpose, the data will be collected by the UCL and sent to the European Commission and to the external individuals or companies acting on its behalf, including in countries that are not members of the European Union.

Please note, furthermore, that processing of the personal data collected in the course of the study by the doctor monitoring your condition is essential for performance of the study: refusal to provide that information will mean that you cannot participate.

The doctor monitoring your condition in the study will identify you using a code generated by a computer program: the data about you collected in the course of the study, except for your name, will be sent to the European Commission, and recorded, processed and stored with that code, date of birth, sex, weight and height. Only the doctor and the individuals authorised may match this code to your name.

At the end of the study, the codification/decodification keys will be destroyed and the data will be anonymised, and no one will be able to determine your identity.

The data, which will be processed using electronic and non-electronic tools, will be distributed only in strictly anonymous form, such as through scientific publications, statistics and scientific conferences. Your participation in the study means that, in accordance with the legislative requirements on clinical studies, the personnel from the European Commission or external companies that perform study monitoring and verification on its behalf, the Ethics Committee and the Italian and foreign health authorities may gain information about your data, including where contained in the

original clinical documentation, using procedures designed to guarantee that your identity remains confidential.

You may exercise the rights set down in Article 7 of the Personal Data Protection Code (e.g. accessing your personal data, supplementing, updating and correcting those data, and opposing processing of those data for legitimate reasons, etc.) by approaching the study manager directly.

You may discontinue your participation in the study at any time without providing any justification: in such a case, the associated biological samples will be destroyed. No further data about you will be collected, notwithstanding the use of those data already collected, without alteration, for the purposes of determining the results of the research.

Information about the results of the research

If you wish, at the end of the study, you may be advised of the results obtained in general and, in particular, those relating to you, except for the results of the genetic tests. Many genetic polymorphisms, including those we will be studying in this research, only provide minimal predictive information about cardiovascular risk for the subject, and cannot therefore be used for diagnostic or therapeutic purposes. You will not therefore be provided with the results for the individual tests.

Additional information

For further information and reporting during the research, the following individuals will be available: Dr .....,; e-mail:

The study protocol proposed to you has been drafted in accordance with the European Union Standards for Good Clinical Practice and the Helsinki Declaration, and has been approved by the Ethical Committee.

You can report any fact that you believe should be notified, in relation to the trial and relating to your child, to the Ethical Committee associated with this hospital. Such information should be reported to the Chairman of the Ethical Committee .....

2. DECLARATION OF CONSENT

I, the undersigned: _____

declare that I have been given, by Dr _____,

exhaustive explanations about the request for participation in the above-mentioned research project, as shown in the information sheet attached to this form, a copy of which I have been given (indicate the date and time when this was provided) Date……time…….

I also declare that I have had an opportunity to discuss those explanations and to ask all questions that I felt necessary and that I have been given satisfactory answers, and that I have had the opportunity to discuss the specific aspects of the study with people I trust.

I therefore freely consent to take part in the research, having fully understood the meaning of the request and the risks and benefits that may derive from participation.

I have been informed, furthermore, of my right to have free access to the documentation (insurance, clinical, scientific, pharmacotherapeutic) relating to the research and the express evaluation by the Ethics Committee for Drug Trials.

I consent to take part in the study	**yes** □ **no** □
I consent to performance of the additional tests	**yes** □ **no** □
I consent to the sampling of genetic material	**yes** □ **no** □
I authorise the performance of genetic analysis as described in the information sheet	**yes** □ **no** □
I authorise the storage of anonymised samples	**yes** □ **no** □
I authorise the storage of anonymised samples beyond six months following collection	**yes** □ **no** □
I authorise storage of biological material	**yes** □ **no** □
I consent to be contacted again in the future for any further studies	**yes** □ **no** □
I wish to be contacted again for notification of the results of the procedures performed	**yes** □ **no** □
I authorise the use of the data, in anonymised form, for scientific purposes	**yes** □ **no** □

Date/Time _____

Signature of the minor patient

[*In the event that the minor patient is not able to sign*]

I, the undersigned: _____

confirm that I was present at the information interview between Dr _____ and Mr _____ and

I confirm that it was not possible for the latter individual to state his consent in writing.

_____ _____

Date

Signature of the independent witness

CRF - CASE REPORT FORM

CASE REPORT FORM

Project: "Model-Driven European Paediatric Repository- MD–PAEDIGREE -

WP 4: Risk of cardiovascular disease in obese children and adolescents"

Date ___/___/___

1. ID_____
2. Name_____
3. Surname_____
4. CS_____
5. Date of birth___/___/___
6. Informed consent obtained □No □Yes
7. by Dr _____
8. on ___/___/___
9. Sex M□ F □
10. Race Caucasian □ Hispanic □ African-American □
11. Weight _____ (kg)
12. Height (cm) _____
13. BMI (kg/m2)_____

Physiological history:

14. Born at _____ weeks
15. Normal delivery □ Caesarean delivery □
16. Weight at birth (g) _____
17. Length at birth (g) _____
18. Allergies foods □ drugs □ other □

Family history

19. Mother in apparent good health □ deceased □ from _____
20. Mother's age _____
21. Mother's weight (kg) _____
22. Mother's height (cm)_____
23. Father in apparent good health □ deceased □ from _____
24. Father's age _____
25. Father's weight (kg) height (cm)
26. Father's weight (kg) _____
27. Father's height (cm)_____

28. Family history of cardiovascular conditions No □ Yes□ (father □ mother □ brother □ second-degree relatives □)
29. Family history of DM2 No □ Yes □ father □ mother □ brother □ second-degree relatives □
30. Family history of DM1 No □ Yes □ father □ mother □ brother □ second-degree relatives □
31. Patient can be enrolled YES □ NO □
32. Samples taken on an empty stomach: No □ Yes □
33. Systolic blood pressure (mmHg) _____
34. Diastolic blood pressure (mmHg) _____
35. Fasting blood sugar (mg/dl) _____
36. Fasting insulin (µUI/ml) _____
37. Blood sugar at 30 mins (mg/dl) _____
38. Insulin at 30 mins (µUI/ml) _____
39. Blood sugar at 120 mins (mg/dl) _____
40. Insulin at 120 mins (µUI/ml) _____
41. Cholesterol (mg/dl) _____
42. HDL cholesterol (mg/dl) _____
43. Triglycerides (mg/dl) _____
44. Alanine aminotransferase (µUI/ml) _____
45. Aspartate aminotransferase (µUI/ml) _____
46. GammaGT (µUI/ml) _____
47. Leukocytes (10^3/µl) _____

Intima-media thickening (IMT)

48. Right carotid, 10 cm from the bifurcation _____
49. Left carotid, 10 cm from the bifurcation _____
50. Common carotid at the point of maximum thickness_____

ADIPOSE DEPOSITS (ultrasound)

51. Visceral fat _____mm
52. Subcutaneous fat _____mm
53. Epicardial fat _____mm
54. Hepatic steatosis <>absent <> mild <> moderate <> severe

MORPHOLOGICAL & FUNCTIONAL ECHOCARDIOGRAPH

55. Left ventricular mass _____
56. Left ventricular mass normalised for height _____
57. Left ventricular systolic function (endocardial fractional shortening & midwall fractional shortening) _____&_____
58. Circumferential end-systolic stress _____
59. Relative diastolic wall thickness normalised for age (RWTn) _____
60. Ratio between observed and predicted left ventricular mass (SDS) _____
61. Transmitral pulsed Doppler _____
62. Pulsed Tissue Doppler _____
63. Peak systolic myocardial velocity (Sm) _____
64. Peak diastolic myocardial relaxation velocity (Em) _____
65. Ratio between peak early transmitral velocity (E) and mean Em (E/Em ratio) _____

66. Stroke volume (linear measurement of diastolic and systolic diameters) _____
67. Cardiac output o (stroke volume x heart rate) _____
68. Total peripheral resistance _____

TONOMETRIC TEST

69. Increase index (AIX) _____
70. Reflex wave form (Tr) _____
71. AIX @ 75 _____

ABDOMINAL MRI

72. Hepatic fat fraction (HFF%)_____
73. Visceral adipose tissue (VAT, cm3)_____
74. Subcutaneous adipose tissue (SAT, cm2)_____

CHEST MRI

75. Intrathoracic adipose tissue (cm3)_____
76. Epicardial adipose tissue (cm3)_____

MORPHOLOGICAL & FUNCTIONAL MRI

77. Left ventricular mass _____
78. Left ventricular mass normalised for height _____
79. Left ventricular systolic function (endocardial fractional shortening & midwall fractional shortening) _____
80. Circumferential end-systolic stress _____
81. Relative diastolic wall thickness normalised for age (RWTn) _____
82. Ratio between observed and predicted left ventricular mass (SDS) _____
83. Transmitral pulsed Doppler _____
84. Pulsed Tissue Doppler _____
85. Peak systolic myocardial velocity (Sm) _____
86. Peak diastolic myocardial relaxation velocity (Em) _____
87. Ratio between peak early transmitral velocity (E) and mean Em (E/Em ratio) _____
88. Stroke volume (linear measurement of diastolic and systolic diameters) _____
89. Cardiac output o (stroke volume x heart rate) _____

ANCILLARY STUDY

	T0	T15	T30	T60	T90	T120	T180	T240
Blood sugar								
Blood insulin								
C-peptide								

GIP								
GLP1								
Ghrelin								
Leptin								
Adiponectin								
ICAM								
IL6								
TNF-alpha								
ASP								
CRP								
ACTH								
Adrenalin								
Noradrenalin								
Chemerin								
Cathepsin S								
sVCAM								

Ethical clearance – OPBG

Bambino Gesù
OSPEDALE PEDIATRICO

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Il Comitato Etico

Gent.ma
Dr.ssa Melania Manco
Malattie Multifattoriali A.R.
SEDE

Roma, 31/05/2013
Prot. n. 452 RA

Oggetto:Model-Driven European Paediatric Repository- MD–PAEDIGREE - WP 4: Risk of cardiovascular disease in obese children and adolescents"

Si trasmette l'estratto del verbale della riunione del 15 maggio 2013 di questo Comitato, relativo alla valutazione per lo studio in oggetto.

IL SEGRETARIO del CE
(Dr.ssa Chiara Mennini)

Piazza Sant'Onofrio, 4
00165 Roma
Tel. +39 06 6859 <inserire numero>
Fax +39 06 6859 <inserire numero>
e-mail <inserire indirizzo>@opbg.net

Bambino Gesù
Ospedale Pediatrico
Istituto di Ricovero e Cura
a Carattere Scientifico
www.ospedalebambinogesu.it

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Il Comitato Etico

ESTRATTO VERBALE

Studio n° 615 / 2013 **Arrivato il** 04-03-2013

Titolo Model-Driven European Paediatric Repository- MD–PAEDIGREE - WP 4: Risk of cardiovascular disease in obese children and adolescents"

Promotore Ospedale Pediatrico Bambino Gesù
Piazza S. Onofrio, 4 00165 Roma

C. Coordinatore IRCCS Ospedale Pediatrico Bambino Gesù - Roma (Italy)
Responsible Work Package 4 : (University College London - Prof. Prof. Andrew Taylor)

Data Seduta **15-05-2013**

Il Comitato Etico, in osservanza a quanto previsto dal D.M. 15 luglio 1997; dalla Circolare n. 15 del 15 ottobre 2000 e dal D. Lgs. n. 211 del 24 giugno 2003, si è riunito per esaminare lo studio in oggetto ed in particolare la seguente documentazione:

Sperimentatori dello Studio

Nome	Cognome	Unità Operativa	Sede
Melania	**Manco**	**Malattie Multifattoriali A.R.**	**P.zza S. Onofrio, 4 - 00165 Roma**

DOCUMENTAZIONE

	Ver	Data ver.
Richiesta di parere		**27-02-2013**
Sinossi	**3**	**06-05-2013**
Protocollo	**3**	**06-05-2013**
Scheda Finanziaria		
Dichiarazione conflitto d'interesse		**27-02-2013**
Curriculum vitae PI		
Lettera di trasmissione		**16-04-2013**
Scheda Informativa Assenso adolescenti	**2**	**10-04-2013**
Scheda Informativa Consenso	**2**	**10-04-2013**

Piazza Sant'Onofrio, 4
00165 Roma
Tel. +39 06 68592572
Fax +39 06 68592301
e-mail chiara.mennini@opbg.net

Bambino Gesù
Ospedale Pediatrico
Istituto di Ricovero e Cura
a Carattere Scientifico
www.ospedalebambinogesu.it

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genitori/tutore legale					
Scheda maggiorenne	Informativa	Consenso		**2**	**10-04-2013**
Scheda Raccolta Dati CRF					**12-04-2013**
Scheda Informativa Assenso adolescenti			Studio ancillare	**2**	**14-04-2013**
Scheda genitori/tutore legale	Informativa	Consenso	Studio ancillare	**2**	**10-04-2013**
Scheda maggiorenne	Informativa	Consenso	Studio ancillare	**2**	**14-04-2013**

Esito PARERE FAVOREVOLE

DELIBERAZIONE

Il Comitato Etico **approva** lo studio.

COMPONENTI DEL COMITATO

Prof. Rocco Agostino — PRESENTE
Pediatra, Primario Pediatria, Neonatologia e T.I.N., Ospedale Fatebenefratelli – Isola Tiberina

Prof.ssa Maria Luisa Barbaccia — ASSENTE
Professore Ordinario di Farmacologia- Facoltà di Medicina e Chirurgia, Università "Tor Vergata"

Prof. Ignatio Carrasco De Paula — ASSENTE
Presidente della Pontificia Accademia Pro Vita

Dr.ssa Tiziana Corsetti — ASSENTE
Responsabile della Farmacia, Ospedale Pediatrico Bambino Gesù

Dott.ssa Sonya Jane Martin per il Prof Bruno Dallapiccola — ASSENTE
Direttore Scientifico dell'Ospedale Pediatrico Bambino Gesù

Prof.ssa Maria Luisa Di Pietro — PRESENTE
Professore Associato di Bioetica all'Università Cattolica del Sacro Cuore

Prof. P. Mastroiacovo — PRESENTE
Clinico Pediatra, Direttore International Center on Birth Defects

Dr.ssa C. Offidani — PRESENTE
Medico Legale Ospedale Pediatrico Bambino Gesù

Piazza Sant'Onofrio, 4
00165 Roma
Tel. +39 06 68592572
Fax +39 06 68592301
e-mail chiara.mennini@opbg.net

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Il Comitato Etico

Prof.ssa Laura Palazzani *Professore Ordinario Facoltà di Giurisprudenza, Università Lumsa*	PRESENTE
Dott.ssa Alessandra Querciati *Coordinatore Infermieristico di Dipartimento-DPUO, , Ospedale Pediatrico Bambino Gesù*	ASSENTE
Dr.ssa L. Reali *Pediatra di famiglia*	PRESENTE
Dr.ssa C. Offidani per il Dr. M. Raponi *Direttore Sanitario dell'Ospedale Pediatrico Bambino Gesù*	PRESENTE
Prof.ssa Carla Rossi *Professore Ordinario di Statistica Medica, Policlinico Universitario "Tor Vergata"*	PRESENTE
Dott.ssa Gianna Scarpelli *Membro Associazione Volontari, Ospedale Pediatrico Bambino Gesù*	PRESENTE

PRESIDENTE
Prof. P. Mastroiacovo
Clinico Pediatra, Direttore International Center on Birth Defects

SEGRETARIO
Dr.ssa C. Mennini
Segreteria tecnico-scientifica, Ospedale Pediatrico Bambino Gesù – Roma

IL SEGRETARIO del CE
(Dr.ssa Chiara Mennini)

Piazza Sant'Onofrio, 4
00165 Roma
Tel. +39 06 68592572
Fax +39 06 68592301
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