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A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain

Argument: chronic/persistent low back pain

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1 Rational

Low back pain (LBP) is one of the most common medical problems encountered in daily life; it is related to disability and work absence and accounts for high economical costs in Western societies.

In North American and European studies, lifetime prevalence estimates range from 49% to 70%, with point prevalence from 12% to 30%[1,2].

In Europe the incidence of chronic/persistent low back pain (CLBP), defined simply as chronic/persistent pain between the costal margins and gluteal fold present for 3 or more months, varies from 5 to 11% with annual direct costs of more than 7000 Euros per person but with even higher indirect costs[3]. In fact, indirect costs, such as absenteeism, presenteism and lost work productivity, represent the majority of overall costs associated with LBP[1] accounting more than 55% of total costs. In Europe the economic burden of persistent CLBP is estimated to be 1-2% of GDP[4] making it a pressing clinical and socioeconomical problem that has to be addressed more in detail.

The major concerns about persistent CLBP syndrome are:

- lack of exact knowledge of its complex pathophysiology;
- lack of biomarkers able to predict risk of developing this syndrome;
- lack of biomarkers and imaging data that could help to interpret clinical symptoms and pain intensity;
- lack of biomarkers and clinical data that could help to predict response to interventions and to intervention's adverse effects.

Low-back pain is a diverse group of mixed pain syndromes (neuropathic and nociceptive) with different molecular pathologies at different structural levels displaying similar clinical manifestations. We know that all the structures of the lumbar column and around it may give rise to the pain sensation but it is still not well known why there is such a wide variability in pain sensation in patients with the same "macroscopic" pathology (i.e. disc herniation, arthrosis, etc). In principle, LBP could be divided according to the cause of the pain into: discogenic pain, facet joint pain, sacroiliac joint pain, widespread pain and spinal stenosis (congenital or acquired). One of the major determinants of pain in persistent CLBP syndrome is localised inflammation in epidural space (both following surgery and without previous surgery at this level)[5]. There is some evidence that there is a correlation of inflammatory cell type expression in the epidural space and severity of CBLP[5], but actually the correlation between different expression of proinflammatory cytokines and severity of CBLP and why there is such huge inter-personal variability in this expression is yet to be investigated.

Owing to its clinical and social impact, a clear diagnosis of this syndrome is needed in order to define besides pain intensity also the interference of pain with daily and work activity. Currently, there are limited biomarkers (mostly imaging) or clinical findings that can be used objectively to help the physician in precise anatomic diagnosis leading to the safest and most cost-effective treatment for the patient (reduction of direct and indirect costs and improvement of treatment efficacy). Both persistent CLBP and disc degeneration are known to be heritable[6,7]. A few genetic variants have

been identified and confirmed as associated with disc degeneration (reviewed by Eskola *et al*[8]) but little investigation has taken place for genetic variants in persistent CLBP – which might be those influencing personality, pain pathways, or local pathological processes such as inflammation.

Despite the high prevalence and cost of persistent CLBP, there are only two genome wide association studies (GWAS) reported to have been performed for chronic/persistent pain[9,10] and a single GWAS meta-analysis of intervertebral disc degeneration[11]. The individual effects of the identified loci are generally small and explain only a small fraction of the trait or disease variation[12]. As such, they do not substantially improve predictions over those based on known factors such as family history[13-15].

But they may be useful in identifying new biological pathways which are greatly needed in many conditions leading to persistent CLBP.

1.1 "OMIC" biomarkers

Genetics through GWA studies has already obtained really important results in pain research; however concerning low back pain, there is not yet suitable genotype-phenotype correlations helpful to stratify patients.

Glycomics is an emerging field that has recently been identified as a priority for the next decade by the US National Academies of Science[16,17]. Many common complex diseases will be associated with specific changes in glycan structures. In addition, common genetic polymorphisms influencing glycosylation and consequent differences in glycome composition could be important diagnostic and prognostic markers. The high level of structural complexity of glycans was a great obstacle to their analysis and until recently it was not possible to analyze glycans quantitatively in large samples. However, the recent development of high throughput methods for this using ultra performance liquid chromatography (UPLC), mass spectrometry and capillary electrophoresis represents a major technological advance which makes large-scale population studies possible for the first time. The first studies reporting protein glycosylation in large human population samples have been recently published by partners in the consortium[18-21]. Reliable identification of valid associations between specific glyco-phenotypes and predisposition for the development or progression of a specific disease requires analysis of thousands of patients.

Activomics combines data about enzymatic activity of numerous post-translational modification proteins in an integrated model which provides dynamic characterization of the current state of an organism. In this project information about numerous proteases, kinases, phosphatases and glycosidases will be collected and used to complement the existing phenotype information.

2 General objectives

The main aim of this trial is to identify **all "omics biomarkers"** associated with susceptibility to chronic/persistent LBP and its different pathophysiology.

To achieve it, we will compare "omic biomarkers" between patients with and without persistent CLBP.

Furthermore, we will link and relate clinical data (clinical and neurological signs leading to anatomical diagnosis plus a careful evaluation of inflammatory response of patient) to a multiple "omics" analysis in order to investigate promising biomarkers that could answer other unmet needs such as, diagnosis and an objective measure of pain intensity, correlation with its pathophysiology, and predictors of response to specific drugs/treatments.

2.1 Primary objective

The primary objective is to recognize genetic variants associated with persistent CLBP patients compared to patients without chronic/persistent pain. Through a Genetic Wide Association Study (GWAS) we will correlate genetic variants associated with persistent CLBP in a wide, international population of European ancestry.

2.2 Secondary objectives

1. Recognize Glycomic and Activomic data associated with persistent CLBP patients compared to patients without chronic/persistent pain. The sample size will better defined after the first interim analysis of first 400 patients.

Glycomic: Glycome composition is very variable between individuals and different glycosylation can significantly affect function of various proteins. Plasma glycome is expected to be relevant for generation and transmission of pain either as direct reflection of the immune cell function, or as a proxy for glycosylation of membrane proteins in different signalling pathways. By analysing composition of the plasma glycome in a large number of patients with low back pain, we will identify glyco-phenotypes which may be associated with individual variation in the way pain is generated, transmitted, or quenched.

Activomic: the objective will be to define a panel of putative biomarkers based on protein post-translational modification enzymatic activities present in patient samples that can be differentiated statistically from control samples in order to diagnose and stratify lower back pain and/or its different phenotypes

2. All omic data will be compared stratifying our population according to

- Pathophysiology: discogenic pain, spinal stenosis, facet joint pain, sacroiliac joint pain, low back pain with radicular pain (not predominant radicular pain) and widespread pain.
- pain intensity
- response to treatment
- duration of pain

3 Study design and planning

Retrospective observational multinational clinical study, with **a case control design.**

Cases and control definition is provided below (section 5.3)

The participation of six large clinical centres (point 10) of the PAIN-OMICS Consortium will enable the identification of novel individual and composite biomarkers.

The participating clinical Centres are leaders in treating patients with low-back pain, and are treating over 4,000 new patients with persistent CLBP each year, thus a large cohort of patients will be available for the collection of clinical data and biological samples.

We will apply "omics" biomarkers (genetic, glycomic and activomic biomarkers) for stratification of patients with persistent CLBP.

Clinical Centres involved will collect blood samples for "omics" determinations from a large cohort of patients already diagnosed with persistent CLBP.

The participating clinical centres will identify minimal diagnostic dataset available in all six clinical centres that will be sufficient to stratify persistent CLBP patients according to the origin of pain, progression and the response to therapy.

This harmonization of clinical definition and stratification of patients will create a framework for the correlation of well phenotyped subjects with "omics" results.

3.1 Study duration

Study duration: 30 months

Sample collection and use of clinical data will start only after the Ethical Approval of the present study protocol from the competent ethical bodies (Ethics Committees of the Institutions involved in patients enrolment), and after the Administrative Approval.

Copies of Ethical Approval will be provided to the European Commission before initiating the study.

All protocol, copies of Informed Consent and Information Sheets once approved by the competent ethical bodies, will be provided to the European Commission before initiating the study.

3.2 Study management

Investigator will delegate, in each participant centre, a clinical supervisor (in charge of ensuring that the study will be conducted according to the protocol, to the good clinical practices, and to national regulations) and a data monitor, to ensure accuracy, completeness and verification of patient's data.

Data monitoring committee: all data monitors, delegated at participating centres, will constitute the data monitoring committee.

Scientific and Steering committee: the External Project Advisory Committee (EPAC) of pain-OMICS FP7project will perform an overall scientific supervision of the trial and of emerging data.

4 Subjects

4.1 Eligibility criteria of patients with persistent CLBP

- age: older than 18;
- chronic/persistent pain (pain lasting longer than 12 weeks) between the costal margins and gluteal fold, with or without symptoms into one or both legs
- written informed consent signed;
- Caucasian ancestry

4.2 Eligibility criteria of healthy volunteers

- age: older than 18;
- without any chronic/persistent pain (pain lasting longer than 12 weeks) in the last one year;
- written informed consent signed;
- Caucasian ancestry

4.3 Exclusion criteria of patients with persistent CLBP and healthy volunteer

- evidence of clinically unstable disease;
- severe psychiatric disorder (excluding mild depression) or mental impairment;
- recent history (< 1 year) of spinal fracture;
- pain in the back due to spinal tumor or infection;
- pregnancy

4.4 Withdrawal criteria

Patients will withdraw from the study in the following situations:

- Consent withdrawal (Subjects may always and without specification of reasons withdraw their informed consent)
- Loss of contact
- Any other condition that, upon clinical judgment of the investigator, will make unacceptable further study participation for that individual patient.

5 Detailed study procedures

5.1 Definitions of chronic/persistent low back pain

We will use the following definitions[34]:

Low back pain will be defined as "*pain and discomfort, localized below the costal margin and above the inferior gluteal folds, with or without leg pain*".

We will consider CHRONIC/PERSISTENT LBP: pain lasting longer than 12 weeks (at least 12 weeks).

5.2 Definition of minimal shared diagnostic datasets

The clinical Centres participating will define a minimal shared diagnostic dataset available in all six clinical centres.

Physicians involved will harmonize their approach and definition of patients with chronic/persistent low back pain: each of the participating Unit will stratify patients according to their clinical features, imaging data and applying a specific questionnaire (pain-DETECT, PD) to evaluate not only the type of pain and pain generator but also the possible pathophysiological (nociceptive and/or neuropathic) mechanism that sustains chronic/persistent pain and the functional impairment.

Considering patient's response to diagnostic procedures, patients will be sub-grouped (taking into account response to diagnostic blocks, clinical and radiological results) into 6 main categories:

- spinal stenosis,
- discogenic pain,
- facet joint pain,
- sacroiliac joint pain
- low back pain with radicular pain (not predominant radicular pain)
- wide-spread low back pain.

We will consider diagnostic the following procedures:

For sacroiliac joint pain: anesthetic block (two distinct procedures performed with two different anaesthetics, with different duration of action) of sacroiliac joint.

For facet joint pain: medial branch and dorsal ramus of L5 anesthetic block.

The rationale for controlled double anesthetic blocks is that anesthetic blockade of a painful joint will abolish pain arising from that joint for the duration of the anesthetic effect, while anesthetic blocks of a non-painful joint will not alter the pain report.

For discogenic pain: provocative discography done based on ISIS standards (low pressurization of the disc and manometry).

Discography continues to be the only diagnostic tool capable of establishing whether or not a particular disc is painful, irrespective of the presence or absence of degenerative pathology observed on other imaging modalities.

For spinal stenosis: MRI and patient's pain features.

For low back pain with radicular pain (not predominant radicular pain): low back pain without other causes with also not predominant radicular pain in the leg(s)

For widespread low back pain: exclusion diagnosis. Low back pain without a specific known diagnosis.

5.3 Observational nature of the study

All the diagnostic/therapeutic procedures are part of usual clinical practice at the clinical centers involved. None of the interventions performed can be considered experimental.

5.4 Persistent CLBP patients and control cohorts

Cases will be identified according to the definition above and collected at each participating centre. Every effort will be made to accumulate a well phenotyped cohort of patients with persistent CLBP, sub-grouped into 6 categories: discogenic pain, spinal stenosis (congenital or acquired), facet joint pain, sacroiliac joint pain, low back pain with radicular pain (not predominant radicular pain) and widespread low back pain.

Controls (patients without chronic/persistent pain as defined above) will be retrieved from 2 different sources:

Existing biobanks of healthy subjects which have collected information about chronic/persistent back pain

Subjects from the companion prospective study on acute LBP who will not have developed CLBP

Age (decades) and gender distribution of controls will be similar to cases.

The enrolment will be competitive among the participating clinical centres.

Patients and prospective controls selected for the participation into the study will receive detailed description of the study and be asked to sign an informed consent form prior to enter the study **(Enrolment Visit).**

Once enrolled in the trial, patients will be assigned a unique anonymous code and data will be collected in the designated ad-hoc CRF (12.2)

5.5 Data collection

Data collection will include:

- demographics (age, gender, race, BMI, workplace)
- clinical and pharmacological history
- pain characteristics (onset, duration, intensity, pain referral pattern, irradiation, sensory abnormalities, precipitating events, history of previous episodes)
- effectiveness/tolerability of pain treatments received, if applicable
- PD questionnaire.

5.6 "OMICS" determinations

All patients enrolled will undergo blood sample collection for "omics" determination; biological samples will be sent to analytical partners for omics analysis. The data will be centralized in a specific

database in order to continue supervise data and possible mistakes or new findings that could need to investigate new biological data.

5.7 Planned assessments

Patients enrolled will undergo blood samples collection for "omics" determination at enrolment once signed the Written Informed Consent.

5.8 Genetic methodology:

To perform GWAS studies, we will use genome-wide basis Illumina and Affymetrix platforms. Candidate gene approaches for the replication or validation will be done with the help of the MALDI TOF MS system (Sequenom) or a Taqman based assay (Applied Biosciences). The results of the separate GWAS will be meta-analyzed applying a standard model like inverse variance weighting. In addition we will look for independent SNPs in the same region as primary SNPs and we will identify additional associated SNPs by the implementation of functional association networks constructed from databases, mostly containing protein-protein interaction. To be able to assess the procedure we will estimate explained variances of separate SNPs as well as complete sets of markers.

5.9 Glycomics methodology:

Glycomics analyses will be performed on total serum proteins and on a single serum protein, immunoglobulin G (IgG). IgG will be isolated from serum samples by affinity chromatography using 96-well monolithic plates with Protein G as previously described (Pucic et al, MCP, 2011). N-glycans will be released from total serum proteins and IgG by overnight deglycosylation with N-glycosidase F (PNGase F). Released N-glycans will be fluorescently labeled with 2-aminobenzamide (2-AB) fluorescent tag and purified by hydrophilic interaction liquid chromatography (HILIC) solid phase extraction (SPE). Labeled N-glycans will be analyzed by hydrophilic interaction chromatography on a Waters Acquity UPLC instrument using Waters BEH Glycan chromatography column, 100 mM ammonium formate, pH 4.4, as solvent A and acetonitrile as solvent B. The system will be calibrated using an external standard of hydrolyzed and 2-AB labeled glucose oligomers from which the retention times for the individual glycans will be converted to glucose units. Data processing will be performed with an automatic processing method after which each chromatogram will be manually corrected to maintain the same intervals of integration for all the samples. The chromatograms obtained will all be separated in the same manner into peaks and the amount of glycans in each peak will be expressed as % of total integrated area.

5.10 Activomics methodology:

Activomics analyses will be performed on retrospective serum samples from anonymous but well characterised patients. Samples will be collected, handled and analysed in a way that minimises

freeze-thaw cycles. For each enzymatic reaction tested, 1 – 20 microliters of serum from patients and healthy controls will be incubated with the appropriate Activomics® substrate under controlled conditions (time, temperature, optimised buffer conditions, etc). Enzymatic modification of the substrate will be monitored quantitatively by proprietary charge-based microfluidic assays for subsequent statistical analysis. The assays will be repeated for a panel of different substrates in order to provide a wide view of disease-related changes to post-translational modification activities for multivariate statistical analysis. Data will be stored in an annotated database for cross reference with other 'omics' determinants.

6 Statistical issues

6.1 Design

The study will follow a two-phase 1:2 case-control study: discovery & validation [35-38].

Discovery population: random sample (2/3) of the entire population of cases. Validation population: the remaining one third. After the GWAS phase, the discovered genes (ranked based on discrimination ROC, for instance) will be assessed for biological plausibility and entered into the validating phase

6.2 Elements for sample size calculations or study power

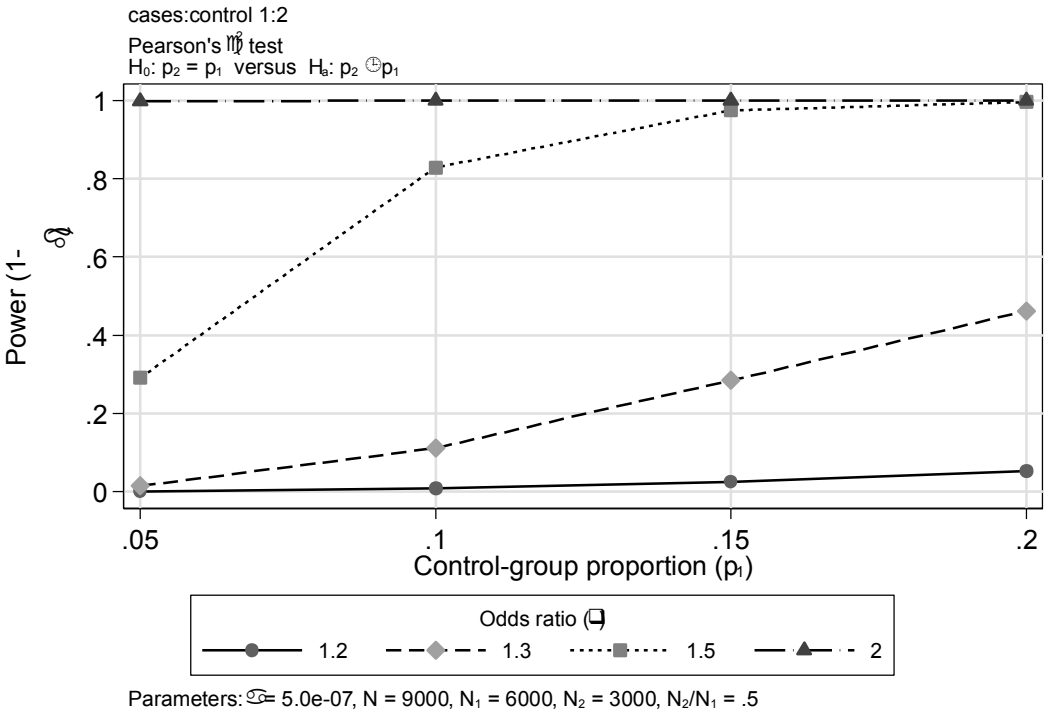
Given no data are available in the literature in the field of OMICS and variants are associated with most common diseases with modest ORs, the following scenarios will be considered [35-38]:

- Choice of OR based on literature for common diseases (effect generally modest): 1.2, 1.3, 1.5, 2.0.
- GWAS alpha 5*10-7
- candidate gene alpha from 5*10-3 [10 genes] to 5*10-4 [100 genes]
- Prevalence of risk variant from rare (5%-10%) to common (15-20%)
- Cases to controls ratios 1:2
- Stata 13 (Stata Corp, College Station, TX, USA) is used for computation.

6.3 Sample size/power calculation

With the above stated hypotheses, with 3000 cases and 6000 controls, the power curves for the discovery phase are shown in figure 1. Consistent with the literature, rare variants will not be discovered unless the association is sufficiently strong (OR=2). Variants with a prevalence of 10 to 20% will be detected with 80% power for ORs of 1.5 and above.

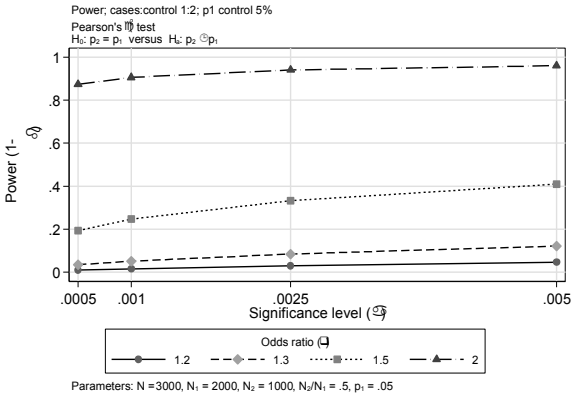
Figure 1: power curves for the discovery phase. The x-axis reports the proportion of the variant (p1) in the control group, the y axis the power.



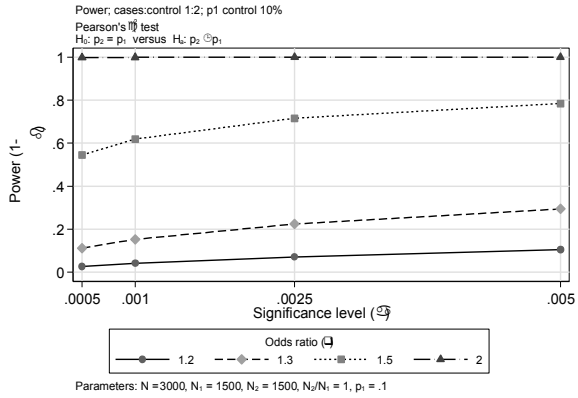
For the validation phase, with 1000 cases and 2000 controls, the power curves are reported in figure 2a, 2b, 2c and 2d, with prevalences of 5, 10 15 and 20%, respectively. As in the discovery phase, when the variant is rare, only ORs of 2 ore more will be detected with sufficient power. For more common variants, ORs of 1.5 and more will be detected with sufficient power.

Figure 2: Power curves for the validation phase (a) 5% prevalence, (b) 10% prevalence; (c) 15% prevalence; (d) 20% prevalence. The x-axis reports the p-value for experiment wise significance of 5% (depending on the number of genes, see 6.2), the y axis the power.

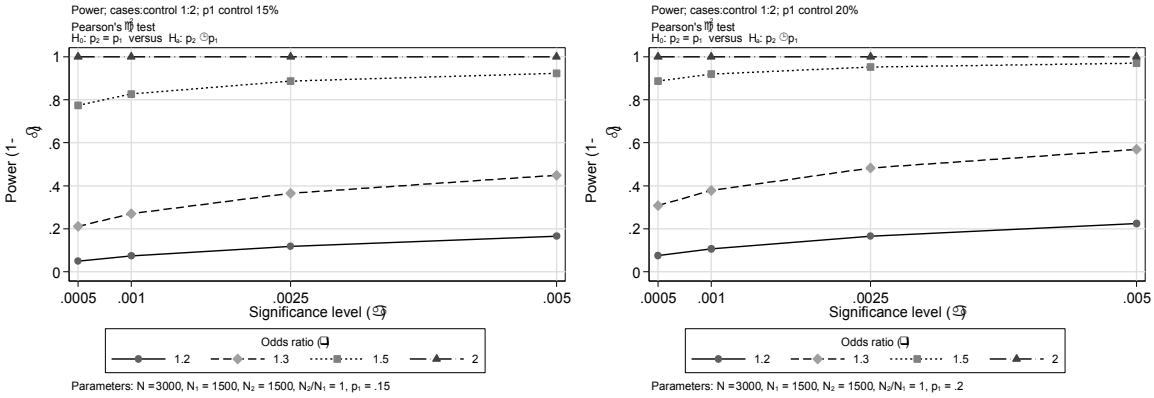
a



b



c



6.4 Study population/analysis strategy

All the enrolled patients and controls will be analysed.

6.4.1 Analysis plan primary objective

Discovery phase: For analyses investigating highly dimensional "omics" spaces we will use a range of approaches. The first approach to be used will extend classical sequential framework. Predictor screening will be performed by logistic or Cox regression models traversing through the omic space and incorporating few predictors at a time. Statistically significant (at experiment-wise level) predictors will be included in the model, and next iteration through the omic space will be performed, etc. Classical example of this approach includes genome-wide association analysis, followed by conditional analyses for identification of secondary signals. One of the major advantages of this approach is its ability rigorously to address statistical hypotheses testing. However, it is not suited for investigation of large numbers of predictors simultaneously. The second approach will deal with the latter concern: we will use modern regularization/shrinkage and machine learning methods allowing analysis of (relatively) large numbers of predictors jointly. While this type of approaches does not address the question of statistical testing in the same way as "classical" approaches do, it is widely used in the context of biomarker discovery, where prediction and not the *p*-values are of primary interest. Finally, we will explore the potential of an approach which will generate all possible pair-wise interactions between the investigated components of omics space. Low-dimension signatures will be extracted and tested as potentially predictive variables; specific predictive combinations will be identified by use of regularization/shrinkage and machine learning methods similar to the second approach. For all methods aimed towards biomarker discovery, the accuracy of prediction will be accessed by cross-validation, and optimal solutions will be analyzed to identify potential biomarkers, which will be selected on their discriminative value in a receiver operator characteristic (ROC) analysis.

Validating phase: The discovered genes will be examined for biological plausibility before entering the validating phase. The association of the candidate genes with the outcome (being a case) will be assessed with logistic regression. The following strategies will be used: single genes assessment /

genetic score (sum of candidate genes) /multiple genes. Adjustment for confounding (age, gender, clinical features) will be performed.

Detail of the statistical analyses will be provided in the final statistical analysis plan (SAP).

6.5 Analysis plan secondary objectives

The analysis of the secondary endpoints will be detailed in SAP. In the discovery phase, similar techniques as for the primary endpoint might be used.

6.6 Study termination

No reasons for study termination are identified. Patients and controls enrolled will be free to withdraw their consent (see 8.2)

7 Data management and confidentiality issues

All study material will be maintained in a safe place site in each Pain Unit participating in the study, for 5 years.

7.1 Patient's lists

The following lists will be maintained:

Screened patients, with reason for exclusion (anonymous)

Eligible patients, with reason for not consent (anonymous)

Enrolled patients (encoded)

Data will be coded as follow:

PO - RT - acronym of center - progressive number of enrolment.

Each centre will keep separately the list of codes assigned to their patients.

7.2 Data recording

A standard case report form (CRF) has been designed, to record in written all study details, and will be completed by the designated personnel.

Any written change or correction must be dated, initialled and explained (if necessary) and must not obscure the original entry.

In the patient's medical records, study participation, date of consent, assigned code and any other relevant information will be recorded.

A check for data completeness and consistency will be performed weekly.

7.3 Data quality control

The investigator must ensure the accuracy, completeness, legibility and timeliness of data reported in the CRF and all required reports. Any written change or correction to a source document must be

dated, initialled and explained (if necessary) and must not obscure the original entry. Data reported on the electronic database, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

7.4 Data monitoring

The Coordinating Investigator is the responsible for validity, completeness, exactness and plausibility of data recorded in the CRFs, and for their correspondence to data recorded in subject's medical records.

The Coordinating Investigator will delegate, in each participant centre, a clinical supervisor (in charge of ensuring that the study will be conducted according to the protocol, to the good clinical practices, and to national regulations) and a data monitor, to ensure accuracy, completeness and verification of patient's data.

7.5 Blood sample labelling

Each sample will be coded as follow:

PO – RT - acronym of center - progressive number of enrolment - GEN (for GWAS study);

PO – RT - acronym of center - progressive number of enrolment - GLY (for Glycomic study);

PO – RT - acronym of center - progressive number of enrolment - ACT (for Activomic study);

Each centre will keep separately the list of codes assigned to their patients.

8 Ethical issues

The trial will be conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, adopted by the General Assembly of the World Medical Association (1996), as well as with the valid national law(s) of the participating countries, with the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) (E6) issued in June 1996, and with the Commission Directives 2001/20/EC and 2005/28/EC.

The trial will be executed in strict compliance with local, national, and international legislation and guidelines; the EC-GCP Guidelines (European Community – Good Clinical Practice), Note for Guidance, III/3976/88-EN, 11.7.1990; the Directive 95/46/EC of the European Parliament and of the Council of 24th October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data. This project has been devised in accordance with the specific ethical rules of the Seventh Framework Programme (EC) No 1906/2006 of the European Parliament and of the Council of 18th December 2006 laying down the rules for the participation of undertakings, research centres and universities in actions under the Seventh Framework Programme and for the dissemination of research results (2007-2013).

8.1 Informed consent and Information Sheets

The Information Sheets (12.1) will specify, in an understandable language for patients, each aspect concerning their participation in the study (including protection, encoding and usage of data collected, moments for biological samples collection and biosamples uses). Moreover the Information Sheets and Informed Consent Form will specify that the participation in the study will be voluntary, will provide patients with staff contact numbers (office number, e-mail or other) to be used in case of any doubts or question before the signature of Informed Consent or throughout the study, will detail any aspects of the possible findings.

Eligible patients will receive Information Sheets and to be included in the study patients have to sign an Informed Consent Form (12.1)

Patients enrolled may always and without specification of reasons withdraw their informed consent.

Informed Consent Form and Information Sheets are in language and terms understandable to the participants. Participants have the right:

- To know that participation is voluntary;
- To ask questions and receive understandable answers before making a decision;
- To know the degree of risk and burden involved in participation;
- To know if there are any benefits involved in participation;
- To withdraw themselves and their biosamples from the project at any time;
- To know how their data will be collected, protected during the project;

Patients will receive a detailed description of the study purposes and planning during the enrolment visit by the local investigator (or a delegate), the visit will last about 30 minutes and all participation details and rights will be extensively described to the patient. Patients will have enough time to decide about their participation and any his/her question will be answered.

8.2 Consent withdrawal

Subjects may always and without specification of reasons withdraw their informed consent.

8.3 Amendments

The Coordinating Investigator and all participant Principal Investigators will act according to Article 10(a) of the Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001.

Only amendments that are substantial will be notified to the Competent Authority (CA) and ethics committee concerned. In addition when an investigator must take urgent safety measures to protect the trial subjects from immediate hazard Article 10(b) allows them to do so before notifying the CA, but they must notify them as soon as possible.

Non-substantial amendments

The investigator does not have to notify non-substantial amendments to the documentation provided to the competent authority or the ethics committee. However, they should be recorded

and if appropriate included in the next update of the Investigator Brochure and be available on request for inspection at the trial site.

Substantial amendments

Substantial amendments to the conduct of the clinical trial may arise from changes to the protocol or from new information relating to the scientific documents in support of the trial.

Amendments to the trial will be regarded as "substantial" where they are likely to have a significant impact on:

- the safety or physical or mental integrity of the subjects;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any IMP used in the trial.

In all cases, an amendment is only to be regarded as "substantial" when one or more of the above criteria are met.

In the case the Coordinating Investigator will intend to make a substantial amendment to the protocol he will notify the concerned CA and relevant ethics committee, Substantial Amendment Form will be applied (Annex 2, "Detailed guidance for the request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities, notification of substantial amendments and declaration of the end of the trial" October 2005) and procedures detailed in Article 10(a) of the Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 will be respected.

8.4 Potential conflicts of interest

All the researchers participating to the study declare that they have no potential conflict of interest.

8.5 Other ethical issues

The study design is not complex, does not interfere with routine clinical management and does not require additional human resources in the participating centres.

9 Costs and financing

The present study is not funded by Industry or any other commercial sponsors.

This trial is a funded academic research; it is supported by founding from the European Commission in the context of the Seventh Framework Program of the European Community for Research, Technological Development and Demonstration Activities.

Project's costs will be covered by founds from EC to Pain-OMICS project, already allocated for trial's execution.

10 Staff and duties

10.1 Fondazione IRCCS Policlinico San Matteo (OSM)

UNIT	NAME	ROLE
Pain Therapy Unit	Dr Massimo Allegri Dr Cristina E Minella Dr Manuela De Gregori	Coordinating Investigator Clinical Supervisor Data Monitor
Scientific Direction	Dr Catherine Klersy	Statistical Issues

10.2 Clinical Centers Participating

UNIT	Name of principal investigator
Multidisciplinary Pain Centre, Hospital Oost-Limburg (ZOL), Genk , Belgium	Dr Jan Van Zundert
"St.Catharine" Orthopedics, Surgery, Neurology and Physical Medicine and Rehabilitation Specialty Hospital (St-Cat), Zabok, Croatia	Prof Dragan Primorac
Anesthesia and Pain Therapy Department, Università degli Studi di Parma (UNIPR), Parma, Italy	Prof Guido Fanelli
The Center for Clinical Research (CPI), Winston-Salem, USA	Dr Leonardo Kapural
Edith Cowan University (ECU), Perth, Australia	Prof Wei Wang

10.3 Participating laboratories

UNIT	NAME of Principal Investigator
Pain Therapy Unit	Dr Allegri
GENOS DOO	Prof Lauc
IP RESEARCH CONSULTING SASU	Prof Pemberton
HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH	Prof Geiger Prof M. Waldenberger
KING'S COLLEGE LONDON	Prof Spector Dr Williams

10.4 Bioinformatics centers

UNIT	NAME
YURIIA	Prof YURII AULCHENKO

11 Data property, publications and further studies

All details regarding data property, transfer and dissemination of foreground will be regulated by the Consortium Agreement signed by all the Parties involved in the Pain-OMICS project.

The proposing group will manage patients' data and publications. The authors of the publications will be decided on the basis of indications contained in the Uniform Requirements for Manuscripts (http://www.icmje.org/urm_full.pdf).

Once approved the study will be registered on clinicaltrials.gov, and results will be posted as soon as available.

12. Annexes

12.1 Informed consent and Information Sheets

INFORMATIVE SHEET

Protocol Code: …….. Patient Code: PO-RT-____-____

Dear Mr/Mrs……..

This Hospital is running a medical-scientific research entitled:

A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain

For this research we need the collaboration and availability of people who meet the scientific requirement suitable to the evaluation that will be performed. People with chronic/persistent low back pain will be enrolled as well as people without chronic/persistent low back pain. However, before you make your decision to accept or reject your participation, we invite you to read these pages carefully, taking all the time that you need, and to ask for explanation whenever you need further clarification. Moreover, if you wish so, you could request an opinion to your family or your doctor, before deciding.
This consent form describes in detail the research with an invitation to take part.
The present study is not funded by industry or any other commercial sponsors.
This trial is a funded by the European Commission in the context of the Seventh Framework Programme of the European Community for Research, Technological Development and Demonstration Activities.
Project's costs will be covered by funds from EC to Pain-OMICS project, already allocated for the trial.
The study will involve about 9000 subjects: 3000 people with chronic/persistent low back pain and 6000 without chronic/persistent low back pain.
The maximum duration of the study will be 30 months.

RATIONALE AND OBJECTIVE OF THE STUDY

Low back pain is one of the most common reasons for adults to consult a family physician and the majority of people will experience back pain at some point in their life. Although most patients recover quickly with minimal treatment, about 35% of these patients develop chronic symptoms (defined as pain that persists 3 months or more).
In Europe, more than 40% of adults suffer from at least one episode of low back pain (LBP), with temporary inability to work. Furthermore in Europe the incidence of chronic/persistent low back pain (CLBP), defined simply as chronic/persistent pain between the costal margins and gluteal fold present for 3 or more months, varies with annual direct costs of more than 7000 euros per person but with an

even higher indirect costs.

Low-back pain is a diverse group of mixed pain syndromes (neuropathic and nociceptive) with different molecular pathologies at different structural levels displaying similar clinical manifestations. We know that all the structures of the lumbar column and around it may give rise to the pain sensation but it is still not well known why there is such a wide variability in pain sensation in patients with the same "macroscopic" pathology (i.e. disc herniation, arthrosis, etc).

Owing to its clinical and social impact, a clear diagnosis of this syndrome is needed in order to define besides pain intensity also the interference of pain with daily and work activity. Currently, there are limited biomarkers (mostly imaging) or clinical findings that can be used objectively to help the physician in precise anatomic diagnosis leading to the safest and most cost-effective treatment for the patient (reduction of direct and indirect costs and improvement of treatment efficacy).

The main aim of this trial is to identify "**omics biomarkers**" associated with susceptibility to chronic LBP and its different pathophysiologies.

We will compare "omic biomarkers" between patients with and without chronic/persistent LBP. We will link and relate clinical data (clinical and neurological signs leading to anatomical diagnosis plus a careful evaluation of inflammatory response of patient) to a multiple "omics" analysis in order to investigate promising biomarkers that could answer other unmet needs, such as, diagnosis and an objective measure of pain intensity, correlation with its pathophysiology, and predictors of response to specific treatments.

"Omics" analysis will include genetics, glycomics and activomics studies, all of them will analyse biological biomarkers possibly related with the predisposition to chronic/persistent low back pain.

These biomarkers will be analysed through a simple blood sample collection from participants.

Genetics is the science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment.

Glycomics is an emerging field. Many common complex diseases will be associated with specific changes in glycan structures. In addition, common genetic polymorphisms influencing glycosylation and consequent differences in glycome composition could be important diagnostic and prognostic markers.

Activomics: combines data about enzymatic activity of numerous post-translational modification proteins in an integrated model which provides dynamic characterization of the current state of an organism. In this project information about numerous proteases, kinases, phosphatases and glycosidases will be collected and used to complement the existing phenotype information.

WHAT WILL YOUR PARTICIPATION IN THE STUDY DO?

If you decide to participate, you will be asked to sign a written informed consent and you will be assigned a code.

The participation in the study involves the collection of clinical documentation that we already have, on

a dedicated data collection form, with data concerning your pain symptoms, clinical background, with special attention to the pathology that caused pain and to analgesic and not analgesic drugs that you take. You will also be asked to fill out a specific questionnaire regarding your pain.

You will be subjected to a peripheral blood sample (10 ml), for the determination of the biomarkers above.

All diagnostic/therapeutic procedures, that might be performed, are part of usual clinical practice at the clinical centre you are being treated.

NONE of the interventions could be considered experimental and you will receive standard medical care for your pain.

WHAT ARE THE RISKS AND BENEFITS ARISING FROM PARTICIPATION IN THE STUDY

You WILL NOT have any immediate clinical benefit from participate in this study, but the collected data will be useful for the advancement of scientific research on pain.

This study does not provide therapeutic conduct different from the normal daily routine performed in our hospital.

Potential risks related to your participation are in connection to the execution of the peripheral blood sample, that usually are:

- pain due to venipuncture ;
- formation of a small hematoma when taking the sample;
- dizziness/faint.

Any possible damage related to your participation in the study are covered by the insurance policy signed by the Foundation for the coverage of civil liability of their employees.

OTHER IMPORTANT INFORMATION

We invite you to ask any questions you may consider appropriate to the physician responsible for the research, who will then provide all the requested clarifications.

You will be asked to sign and date page 6 (Written Informed Consent) of this document, to confirm that you have read all the information have understood the aims of the research, the relative risks and future benefits that may arise from it, and that, finally, that you freely gave consent to participate.

The original of the written informed consent signed by you will be preserved and you will be provided of a copy.

The purposes and procedures of this research, described in the protocol of the main clinical study were evaluated and approved by the Ethics Committee of the Foundation.

YOUR RIGHTS

At any time you may exercise the right to terminate your participation in the research, without explanation. The withdrawal from the study will not affect in any way the care of your pathology. At the same time, will not contribute additional data relating to your condition and your sample will be destroyed, unless you give written signed consent that authorize its storage.

The information gathered for this study will be recorded and associated, about the documents of the study, with the number assigned to you, not linked with personal details.

Only the staff responsible for the study will be able, therefore, to revisit, when necessary the identity of the various participants to research. Unless required by regulatory authorities, or by the regulations, with the aim to verify the information obtained from the study and the proper conduct of it, only qualified staff and duly authorized by the Unit who organized the research will have access to the original documents (medical records of hospitalization, outpatient documentation), on which are the personal identification data.

CONFIDENTIALITY OF PERSONAL DATA

Pursuant to Legislative Decree of the Italian law 196/2003. "Code regarding the protection of personal data" and the Guidelines for the processing of personal data in the context of clinical trials of drugs - July 24, 2008, we inform you that your personal data will be collected and stored, and will be used only for purposes of scientific research.

On the form collecting the clinical data, you will be identified by a code, assigned to you, and not with your name or any other personal identifier. Only the investigator in charge of the centre home of the study will have the list of participants and their codes through which you can trace the identity of each patient.

The documentation and coded data from the study are kept at the service of Pain Therapy Foundation IRCCS Policlinico San Matteo of Pavia; the decoding file is stored in password-protected computer. Data and samples will be kept for a period of five years from the conclusion of the study and then destroyed.

Dr. Massimo Allegri is responsible for the coding of clinical data and the management of the code list.

You have the right to know what information will be stored and to update and modify erroneous data.

Access to such data will be protected by the investigator; only the investigator promoter of the study and the people who work in the research will use Your data.

Regulatory authorities and medical personnel responsible for monitoring and verifying procedures will be able to inspect the documents of the study and hospital records. By signing the informed consent form you consent to the processing of Your data as described.

The results of the study may be subject to publication but your identity will remain secret.

The results and other information that will result from this research could be presented to national health agencies and other nations ones.

In any case, the data presented will be anonymous and aggregate. Your identity as a participant in a clinical trial will NEVER be revealed in any report or any publication concerning this research.
The research team will remain the only owner of all data collected and of any discoveries that will originate. Any results will be made available to the scientific community to validate the result and for further studies.

INFORMATION ABOUT THE STUDY RESULTS

If you would like to be notified of the results of the study please let us know

FURTHER INFORMATION

The following stuff will be available for further information and communications during the study

- Dr. Massimo Allegri
- _____
- _____

If you have questions about further features of the research, participant's rights in this research, requests for any damages arising from research, withdrawal from the research itself, contact at any time:
Dr Massimo Allegri at the phone number 0382502627
The study protocol that has been proposed to you has been prepared in accordance with the requirements of Good Clinical Practice of the European Union and the current revision of the Declaration of Helsinki, and was approved by the Ethics Committee of this property.
You can report any fact You deem appropriate to point out, regarding tests that concern you to the Ethics
Committee of this structure, c/o the Scientific Director.

WHAT HAPPENS IF YOU DECIDE NOT TO PARTICIPATE IN THE STUDY

You are free to not participate in the study. The doctors will continue to follow you anyway with due care assistance.

INTERRUPTION OF THE STUDY

Your participation in this research program is completely voluntary and you may withdraw from the study at any time, without explanation. You will still receive all the treatment and assistance you may need, at the same time, will not be collected for the study further data relating to You.

DECLARATION OF CONSENT (written informed consent form)

A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain

The undersigned _____ declare that I have received from Dr. _____ and explanations about participation in the study.

The undersigned declare to agree to the proposal to participate at the study described in this document. My consent is an expression of a free decision, not influenced by promises of economic benefits or otherwise, nor by obligations towards the doctor in charge of the study. I was given the opportunity to read the information contained in the informative session of this document and ask questions about the purposes and methods of the study, the benefits and the possible risks involved and my rights as explanations that were given to me and I had sufficient time to consider my participation in this study. I give my consent, also pursuant to Italian Law 196 of 30.06.2003, code regarding the protection of personal data, at the processing of my personal data, including sensitive data, in order to conduct this study. I understand that my medical records may be made available to the Health Authority and the Ethics Committee, to be examined in order to check the proper conduct of the trial, in full consideration of all my rights, clarified to me in the informative part of this document. I confirm that I have been given a copy of this document for information and consent.

By signing this consent I don't renounce at any of my legal rights.

The signature certifies that:

1 . I have read this form and I received information about the study;

2 . I discussed the research with questions and I am satisfied with the responses received;

3 . I've had time to evaluate the proposal to participate at the study or not

Patient's signature _____ Date ____ / ____ / ____

Doctor's signature _____ Date ____ / ____ / ____

(In case the patient is unable to sign) [(1)]

The undersigned _____ testify that Dr._____has thoroughly explained to Mr./Mrs_____the features of the study in question, as reported in the information sheet attached, and that he/she freely accepted to join the study, after having had the opportunity to ask all the necessary questions:

Signature of independent witness _____ Date ____ / ____ / ____

(1) If the patient is unable to read, a witness independent by the investigator must be present during the entire discussion about the informed consent, after that the module itself and any other written information will have been read and explained to the patient, as well as at the expression of verbal consent to participation at the study.

INFORMATION SHEET

(Healthy Volunteer)

Protocol Code: ……..... Patient Code: PO-RT-____-____

Dear Mr/Mrs……..

This Hospital is running a medical-scientific research entitled:

A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain

For this research we need the collaboration and availability of people who meet the scientific requirement suitable to the evaluation that will be performed. People with chronic/persistent low back pain will be enrolled as well as people without chronic/persistent low back pain. However, before you make your decision to accept or reject your participation, we invite you to read these pages carefully, taking all the time that you need, and to ask for explanation whenever you need further clarification. Moreover, if you wish so, you could request an opinion to your family or your doctor, before deciding.
This consent form describes in detail the research with an invitation to take part.
The present study is not funded by industry or any other commercial sponsors.
This trial is a funded by the European Commission in the context of the Seventh Framework Programme of the European Community for Research, Technological Development and Demonstration Activities.
Project's costs will be covered by funds from EC to Pain-OMICS project, already allocated for the trial.
The study will involve about 9000 subjects: 3000 people with chronic/persistent low back pain and 6000 without chronic/persistent low back pain.
The maximum duration of the study will be 30 months.

RATIONALE AND OBJECTIVE OF THE STUDY

Low back pain is one of the most common reasons for adults to consult with a family physician and the majority of people will experience back pain at some point in their life. Although most patients recover quickly with minimal treatment, about 35% of these patients develop chronic symptoms (defined as pain that persists 3 months or more).

In Europe, more than 40% of adults suffer from at least one episode of low back pain (LBP), with temporary inability to work. Furthermore in Europe chronic/persistent low back pain (CLBP), defined simply as chronic/persistent pain between the costal margins and gluteal fold present for 3 or more months, with annual direct costs of more than 7000 euros per person but with an even higher indirect costs.

Low-back pain is a diverse group of mixed pain syndromes (neuropathic and nociceptive) with different molecular pathologies at different structural levels displaying similar clinical manifestations. We know that all the structures of the lumbar column and around it may give rise to the pain sensation but it is still not well known why there is such a wide variability in pain sensation in patients with the same "macroscopic" pathology (i.e. disc herniation, arthrosis, etc).

Owing to its clinical and social impact, a clear diagnosis of this syndrome is needed in order to define besides pain intensity also the interference of pain with daily and work activity. Currently, there are limited biomarkers (mostly imaging) or clinical findings that can be used objectively to help the physician in precise anatomic diagnosis leading to the safest and most cost-effective treatment for the patient (reduction of

direct and indirect costs and improvement of treatment efficacy).

The main aim of this trial is to identify "**omics biomarkers**" associated with susceptibility to chronic LBP and its different pathophysiologies.

We will compare "omic biomarkers" between patients with and without chronic/persistent LBP. We will link and relate clinical data (clinical and neurological signs leading to anatomical diagnosis plus a careful evaluation of inflammatory response of patient) to a multiple "omics" analysis in order to investigate promising biomarkers that could answer other unmet needs, such as, diagnosis and an objective measure of pain intensity, correlation with its pathophysiology, and predictors of response to specific treatments.

"Omics" analysis will include genetics, glycomics and activomics studies, all of them will analyse biological biomarkers possibly related with the predisposition to chronic/persistent low back pain.

These biomarkers will be analysed through a simple blood sample collection from participants.

Genetics is the science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment.

Glycomics is an emerging field. Many common complex diseases will be associated with specific changes in glycan structures. In addition, common genetic polymorphisms influencing glycosylation and consequent differences in glycome composition could be important diagnostic and prognostic markers.

Activomics: combines data about enzymatic activity of numerous post-translational modification

proteins in an integrated model which provides dynamic characterization of the current state of an organism. In this project information about numerous proteases, kinases, phosphatases and glycosidases will be collected and used to complement the existing phenotype information.

WHAT WILL YOUR PARTICIPATION IN THE STUDY DO?

As your clinical conditions meet the criteria required for the admission to the study, if you decide to participate, you will be asked to sign a written informed consent and you will be assigned an alphanumeric code.

In case you will accept the participation in the study, only your therapy and pathologies will be recorded. You will also be subjected to a peripheral blood sample (10 ml), for the determination of the biomarkers above.

All diagnostic/therapeutic procedures, that might be performed, are part of usual clinical practice at the clinical centre you are being treated, none of the interventions could be considered experimental.

WHAT ARE THE RISKS AND BENEFITS ARISING FROM PARTICIPATION IN THE STUDY

You WILL NOT have any immediate clinical benefit from participate in this study, but the collected data will be useful for the advancement of scientific research on pain.

This study does not provide therapeutic conduct different from the normal daily routine performed in our hospital.

Potential risks related to your participation are in connection to the execution of the peripheral blood sample, that usually are:

- pain due to venipuncture ;
- formation of a small hematoma when taking the sample;
- dizziness/faint.

Any possible damage related to your participation in the study are covered by the insurance policy signed by the Foundation for the coverage of civil liability of their employees.

OTHER IMPORTANT INFORMATION

We invite You to ask any questions You may consider appropriate to the Physician responsible for the research, who will then provide all the requested clarifications.

The medical doctor also will ask you to sign and date page 6 (Written Informed Consent) of this document, to confirm that You have read all the information contained herein, that you have understood the aims of the research, the relative risks and future benefits that may arise from it, and that, finally, you freely gave consent to participate.

The original of the written informed consent signed by you will be preserved in the archives of the Centre, while you will be provided of a copy.
The purposes and procedures of this research, described in the protocol of the main clinical study were evaluated and approved by the Ethics Committee of the Foundation.

YOUR RIGHTS

At any time you may exercise the right to terminate your participation in the research, without explanation. The withdrawal from the study will not affect in any way the care of your pathology. At the same time, will not be collected additional data relating Your condition and Your sample will be destroyed, unless you give your written signed consent that authorize the physician to store it.
The information gathered for this study will be recorded and associated, about the documents of the study, with the number assigned to You by the doctor leading the study, not with personal details.
Only the physician responsible for the study will be able, therefore, to revisit, when necessary the identity of the various participants to research. Unless required by regulatory authorities, or by the regulations, with the aim to verify the information obtained from the study and the proper conduct of it, only qualified staff and duly authorized by the Unit who organized the research will have access to the original documents (medical records of hospitalization, outpatient documentation), on which are the personal identification data.

CONFIDENTIALITY OF PERSONAL DATA

Pursuant to Legislative Decree of the Italian law 196/2003. "Code regarding the protection of personal data" and the Guidelines for the processing of personal data in the context of clinical trials of drugs - July 24, 2008, we inform You that Your personal data will be collected and stored, and will be used only for purposes of scientific research.
On the form collecting the clinical data, you will be identified by a code, assigned to You, and not with your name. Only the investigator in charge of the centre home of the study will have the list of participants and their codes through which you can trace the identity of each patient.
The documentation and coded data from the study are kept at the service of Pain Therapy Foundation IRCCS Policlinico San Matteo of Pavia; the decoding file is stored in password-protected computer. Data and samples will be kept for a period of five years from the conclusion of the study and then destroyed.
Dr. Massimo Allegri is responsible for the coding of clinical data and the management of the code list.
You have the right to know what information will be stored and to update and modify erroneous data.
Access to such data will be protected by the investigator; only the investigator promoter of the study and the people who work in the research will use Your data.
Regulatory authorities and medical personnel responsible for monitoring and verifying procedures will

be able to inspect the documents of the study and hospital records. By signing the informed consent form you consent to the processing of your data as described.

The results of the study may be subject to publication but your identity will remain secret.

The results and other information that will result from this research could be presented to national health agencies and other nations ones.

In any case, the data presented will be anonymous and aggregate. Your identity as a participant in a clinical trial will NEVER be revealed in any report or any publication concerning this research.

The research team will remain the only owner of all data collected and of any discoveries that will originate. Any results will be made available to the scientific community to validate the result and for further studies.

INFORMATION ABOUT THE STUDY RESULTS

If you require it, at the end of the study you could be of notified of the results of the study, in general and, in particular, those concerning You.

FURTHER INFORMATION

The following stuff will be available for further information and communications during the study

- Dr. Massimo Allegri
- _____
- _____

If you have questions about further features of the research, participant's rights in this research, requests for any damages arising from research, withdrawal from the research itself, contact at any time:

Dr Massimo Allegri at the phone number 0382502627

The study protocol that has been proposed to you has been prepared in accordance with the requirements of Good Clinical Practice of the European Union and the current revision of the Declaration of Helsinki, and was approved by the Ethics Committee of this property.

You can report any fact you deem appropriate to point out, regarding tests that concern you to the Ethics

Committee of this structure, c/o the Scientific Director.

WHAT HAPPENS IF YOU DECIDE NOT TO PARTICIPATE IN THE STUDY

You are free to not participate in the study. The doctors will continue to follow you anyway with due care assistance.

INTERRUPTION OF THE STUDY

Your participation in this research program is completely voluntary and you may withdraw from the study at any time, without explanation. You will still receive all the treatment and assistance you may need, at the same time, will not be collected for the study further data relating to you.

DECLARATION OF CONSENT (written informed consent form)

A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain

The undersigned _____ declare that I have received from Dr. _____ explanations about the request of participation at the study in question.

The undersigned declare to agree to the proposal to participate at the study described in this document. My consent is an expression of a free decision, not influenced by promises of economic benefits or otherwise, nor by obligations towards the doctor in charge of the study. I was given the opportunity to read the information contained in the informative session of this document and ask questions about the purposes and methods of the study, the benefits and the possible risks involved and my rights as explanations that were given to me and I had sufficient time to consider my participation in this study. I give my consent, also pursuant to Italian Law 196 of 30.06.2003, code regarding the protection of personal data, at the processing of my personal data, including sensitive data, in order to conduct this study. I understand that my medical records may be made available to the Health Authority and the Ethics Committee, to be examined in order to check the proper conduct of the trial, in full consideration of all my rights, clarified to me in the informative part of this document. I confirm that I have been given a copy of this document for information and consent.

By signing this consent I don't renounce at any of my legal rights.

The signature certifies that:

1 . I have read this form and I received information about the study;

2 . I discussed the research with questions and I am satisfied with the responses received;

3 . I've had time to evaluate the proposal to participate at the study or not

Patient's signature _____ Date ____ / ____ / ____

Doctor's signature _____ Date ____ / ____ / ____

(In case the patient is unable to sign) [(1)]

The undersigned _____ testify that Dr._____has thoroughly explained to Mr./Mrs_____the features of the study in question, as reported in the information sheet attached, and that he/she freely accepted to join the study, after having had the opportunity to ask all the necessary questions:

Signature of independent witness _____ Date ____ / ____ / ____

(1) If the patient is unable to read, a witness independent by the investigator must be present during the entire discussion about the informed consent, after that the module itself and any other written information will have been read and explained to the patient, as well as at the expression of verbal consent to participation at the study.

12.2 Case Report Forms

CASE REPORT FORM

"A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain"

pain-OMICS-RT

Chief Investigator: Dr Massimo Allegri

NCT:

Name of site (code):

CRF Version Number: I, 10/16/2013

Patient Code: PO – RT - _ _ _ _ - _ _ _ _

Date of Assessment: _ _ / _ _ / _ _ _ _

(DD / MM / YYYY)

Participant Informed Consent:		
Date participant signed written consent form:	_ _ / _ _ / _ _ _ _ (DD / MM / YYYY)	
Name of person taking informed consent: _____		

Demographic Data:		
Date of Birth:	_ _ / _ _ / _ _ _ _ (DD / MM / YYYY)	
Ethnicity:		
Sex:	☐ Male ☐ Female	
Weight:	**Height:**	**BMI:**
Smoker:	☐ Yes (_ _ _ cigarettes/day)	☐ No
Ex-smoker:	☐ Yes (_ _ _ cigarettes/day)	

Telephone number:

Medical History			
Condition / illness	**Start date** (DD/MM/YYYY)	**Stop date** (DD/MM/YYYY)	**Or tick if ongoing at Screening Visit?**
COPD (Chronic obstructive pulmonary disease)	___/___/___	___/___/___	☐
Obesity	___/___/___	___/___/___	☐
Diabetes Mellitus	___/___/___	___/___/___	☐
Coronary Artery Disease	___/___/___	___/___/___	☐
Cardiomyopathy	___/___/___	___/___/___	☐
Hypertensive Heart Disease	___/___/___	___/___/___	☐
Cardiac Dysrhythmias	___/___/___	___/___/___	☐
Valvular Heart Disease	___/___/___	___/___/___	☐
Cerebrovascular Disease	___/___/___	___/___/___	☐
Peripheral Arterial Disease	___/___/___	___/___/___	☐
Arterial Hypertension	___/___/___	___/___/___	☐
Chronic Kidney Disease	___/___/___	___/___/___	☐
Chronic Liver Disease	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐

Medications			
Medication (Record Generic or name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	Frequency
1. NSAIDs			

2. Aspirin			
3. Statins			
4. Antibiotics (please specify)			
5. Thiazide diuretics			
6. Beta Blockers			
7. Angiotensin-Converting Enzyme (ACE) Inhibitors			
8. Calcium Channel Blockers			
9. Alpha Blockers			
10. Insulin			
11. Anticonvulsants			
12. Antidepressant			
13.			
14.			

Pain Characteristics

Pain onset: __ __ / __ __ / __ __ __ __ (DD / MM / YYYY)

☐ Spontaneous

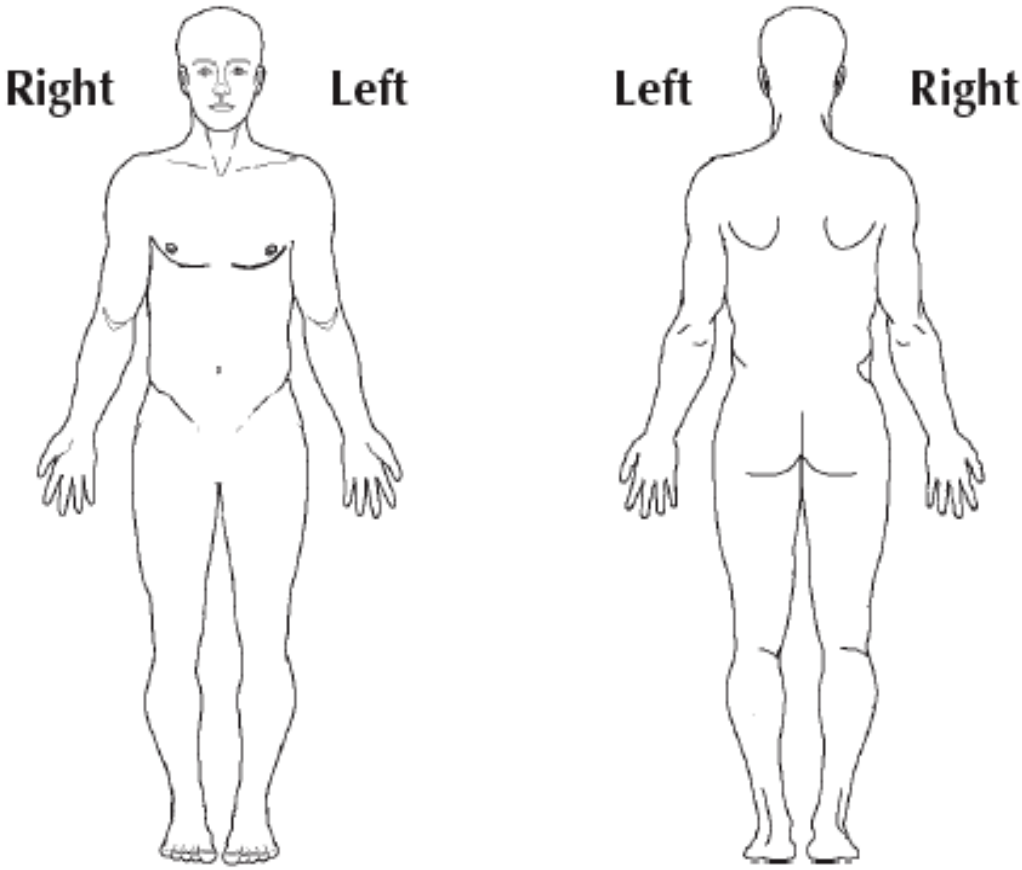
☐ Trauma/injury

Pain duration: ☐ Occasional/Episodic

☐ Constant

☐ Only at night

Pain distribution:



MEAN daily pain intensity:

0 1 2 3 4 5 6 7 8 9 10

No Pain	Moderate Pain	Worst Possible Pain

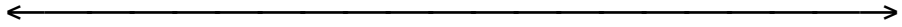
LOWEST daily pain intensity:

0 1 2 3 4 5 6 7 8 9 10

No Pain	Moderate Pain	Worst Possible Pain

WORST daily pain intensity:

0 1 2 3 4 5 6 7 8 9 10



No Pain	Moderate Pain	Worst Possible Pain

Physical Examination:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Radiological Findings:

_____
_____
_____

_____

_____

_____

_____

_____

_____

_____

_____

Pain medications:

☐ NSAIDs Which?_____
(specify dose and frequency)
Which?_____
(specify dose and frequency)

☐ PARACETAMOL _____
(specify dose and frequency)

☐ OPIOIDS Which?_____
(specify dose and frequency)
Which?_____
(specify dose and frequency)
Which?_____
(specify dose and frequency)

☐ other medications Which?_____
(specify dose and frequency)
Which?_____
(specify dose and frequency)

Interventions/Procedures

☐ Which?_____
Outcome:
☐ PAIN RELIEF<30% ☐ PAIN RELIEF: 30-50% ☐ PAIN RELIEF>50%

☐ Which?_____
Outcome:
☐ PAIN RELIEF<30% ☐ PAIN RELIEF: 30-50% ☐ PAIN RELIEF>50%

☐ Which?_____
Outcome:
☐ PAIN RELIEF<30% ☐ PAIN RELIEF: 30-50% ☐ PAIN RELIEF>50%

☐ Which?_____
Outcome:
☐ PAIN RELIEF<30% ☐ PAIN RELIEF: 30-50% ☐ PAIN RELIEF>50%

Side effects:

Nausea	☐ MILD	☐ MODERATE	☐ SEVERE
Vomiting	☐ MILD	☐ MODERATE	☐ SEVERE
Constipation	☐ MILD	☐ MODERATE	☐ SEVERE
Itching	☐ MILD	☐ MODERATE	☐ SEVERE
Headache/Dizziness	☐ MILD	☐ MODERATE	☐ SEVERE
Abdominal pain/Discomfort Cramping/Bloating	☐ MILD	☐ MODERATE	☐ SEVERE
Dry Mouth	☐ MILD	☐ MODERATE	☐ SEVERE
Fatigue, Sleepiness, Trouble Concentrating, Hallucination and/or Drowsiness, Somnolence	☐ MILD	☐ MODERATE	☐ SEVERE
Gastrointestinal cramp	☐ MILD	☐ MODERATE	☐ SEVERE

Pain relief with medications/interventions:

Patient Satisfaction with therapy

☐ COMPLETE

☐ PARTIALLY SATISFACTION

☐ POOR SATISFACTION

☐ UNSATISFIED

The following criteria MUST be answered YES for participant to be included in the trial: (enrolment ONLY)		**Yes**	**No**
1.	Age: older than 18	☐	☐
2.	chronic/persistent pain (pain lasting longer than 12 weeks) between the costal margins and gluteal fold, with or without symptoms into one or both legs	☐	☐
3.	Written informed consent signed	☐	☐
4.	Caucasian ancestry	☐	☐
If any of the above criteria is answered NO, the participant is NOT eligible for the trial and must not be included in the study.			

The following criteria MUST be answered NO for the participant to be included in the trial: (enrolment ONLY)		**Yes**	**No**
1.	Evidence of clinically unstable disease	☐	☐
2.	Severe psychiatric disorder (excluding mild depression) or mental impairment	☐	☐
3.	Recent history (< 1 year) of spinal fracture	☐	☐
4.	Pain in the back due to spinal tumor or infection	☐	☐
5.	Pregnancy	☐	☐
If any of the above criteria is answered YES, the participant is NOT eligible for the trial and must not be included in the study.			

End of Enrolment Visit Checklist:		
	Yes	**No**

1.	**Does the participant satisfy the inclusion and exclusion criteria to date?**	☐	☐
2.	**Have the PD questionnaire been completed?**	☐	☐
3.	**Have the Medical History and Concomitant Medication pages been completed?**	☐	☐
4.	**Has the blood sample for "omics" biomarkers been collected?**	☐	☐

Completed by: _____

Name Signature Date

CASE REPORT FORM
(Healty volunteer)

"A retrospective study to identify new "omics" biomarkers of chronic/persistent low back pain"

pain-OMICS-RT

Chief Investigator: Dr Massimo Allegri

NCT:

Name of site (code):

CRF Version Number: I, 10/16/2013

Patient Code: PO – RT -_ _ _ _ - _ _ _ _

Date of Assessment: _ _ / _ _ / _ _ _ _

(DD / MM / YYYY)

Participant Informed Consent:	
Date participant signed written consent form: _ _ / _ _ / _ _ _ _ (DD / MM / YYYY)	
Name of person taking informed consent: _____	

Demographic Data:		
Date of Birth:	_ _ /_ _ / _ _ _ _ (DD / MM / YYYY)	
Ethnicity:		
Sex:	☐ Male ☐ Female	
Weight:	**Height:**	**BMI:**
Smoker:	☐ Yes (_ _ _ cigarettes/day)	☐ No
Ex-smoker:	☐ Yes (_ _ _ cigarettes/day)	
Telephone number:		

Medical History	

Condition / illness	**Start date** (DD/MM/YYYY)	**Stop date** (DD/MM/YYYY)	**Or tick if ongoing at Screening Visit?**
COPD (Chronic obstructive pulmonary disease)	____/____/____	____/____/____	☐
Obesity	____/____/____	____/____/____	☐
Diabetes Mellitus	____/____/____	____/____/____	☐
Coronary Artery Disease	____/____/____	____/____/____	☐
Cardiomyopathy	____/____/____	____/____/____	☐
Hypertensive Heart Disease	____/____/____	____/____/____	☐
Cardiac Dysrhythmias	____/____/____	____/____/____	☐
Valvular Heart Disease	____/____/____	____/____/____	☐
Cerebrovascular Disease	____/____/____	____/____/____	☐
Peripheral Arterial Disease	____/____/____	____/____/____	☐
Arterial Hypertension	____/____/____	____/____/____	☐
Chronic Kidney Disease	____/____/____	____/____/____	☐
Chronic Liver Disease	____/____/____	____/____/____	☐
	____/____/____	____/____/____	☐
	____/____/____	____/____/____	☐

Medications

Medication (Record Generic or name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	Frequency
1. NSAIDs			
2. Aspirin			
3. Statins			
4. Antibiotics (please specify)			
5. Thiazide diuretics			
6. Beta Blockers			
7. Angiotensin-Converting Enzyme (ACE) Inhibitors			
8. Calcium Channel Blockers			
9. Alpha Blockers			
10. Insulin			
11. Anticonvulsants			
12. Antidepressant			
13.			
14.			

The following criteria MUST be answered YES for participant to be included in the trial: (enrolment ONLY)		Yes	No
1.	Age: older than 18	☐	☐
2.	Written informed consent signed	☐	☐
3.	Without any chronic/persistent pain (pain lasting longer than 12 weeks) in the last one year		
4.	Caucasian ancestry	☐	☐
If any of the above criteria is answered NO, the participant is NOT eligible for the trial and must not be included in the study.			

The following criteria MUST be answered NO for the participant to be included in the trial: (enrolment ONLY)		Yes	No
1.	Evidence of clinically unstable disease	☐	☐
2.	Severe psychiatric disorder (excluding mild depression) or mental impairment	☐	☐
3.	Recent history (< 1 year) of spinal fracture	☐	☐
4.	Pregnancy	☐	☐
If any of the above criteria is answered YES, the participant is NOT eligible for the trial and must not be included in the study.			

End of Enrolment Visit Checklist:			
		Yes	No
1.	**Does the participant satisfy the inclusion and exclusion criteria to date?**	☐	☐
2.	**Have the Medical History and Concomitant Medication pages been completed?**	☐	☐
3.	**Has the blood sample for "omics" biomarkers been collected?**	☐	☐

Completed by: _____

Name Signature Date

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