

**PROTOCOL modified by amendment n°2  
of 29/01/2013**

**Genetic & Environmental Determinants Of Immune Phenotype variance:  
Establishing A Path Towards Personalized Medicine**

**Institut Pasteur CoRC Number:** 2012.04

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## SYNOPSIS

1. Title	Genetic & Environmental Determinants Of Immune Phenotype Variance: Establishing A Path Towards Personalized Medicine
2. Short title	LABEX 2010 – Milieu Intérieur
3. Protocol number	2012.04
4. Study design	The study is a single center interventional study without investigational product
5. Number of centers / Country	1 center / France
6. Coordinating investigator	Dr Nicolas FAUCHOUX
7. Study rationale	<p>Susceptibility to infections, disease severity, and response to medical therapies and vaccines are highly variable from one individual to another. While the question of variance in human populations continues to be a focal point of scientific research, medical practices and public health policies typically take a 'one size fits all' model to disease management and drug development.</p> <p>Individual heterogeneity in the immune response can have an enormous impact on the likelihood to respond to therapy or the development of side effects secondary to vaccine administration. Because of the complexity of immune responses in the individual and within the population, it has not been possible thus far to define the parameters (genetic or environmental) that constitute a healthy immune system and its natural occurring variability.</p> <p>Efforts to restore the 'personal' in medical care are the current challenge, and the driving vision of the project, to which the current study belongs.</p> <p>In order to realize the promise of personalized medicine, an in-depth understanding of the determinants of heterogeneity in host response to stress is required.</p>
8. Principal objective	To assess the determinants of immunologic variance within the general healthy population.
9. Secondary objectives	To set-up a biobank of : <ul style="list-style-type: none"> <li>• EBV transformed B cell lines</li> <li>• iPS cells from fibroblasts of healthy human individuals</li> </ul>
10. Primary outcome	<p>To identify factors (genetic, immunological and environmental) that contributes to the observed heterogeneity in immune responses (individual and population levels).</p> <ul style="list-style-type: none"> <li>• To characterize the naturally occurring genetic variability of human response using whole genome sequencing and SNPs haplotyping.</li> <li>• To determine and measure cytokine/chemokine stimulated by 40</li> </ul>

	<p>pattern-recognition receptors agonists (PRR agonists) or immune stimulators.</p> <ul style="list-style-type: none"> <li>• To characterize commensal microbiota (nasal swab and stools samples) in the study population</li> <li>• To evaluate the metagenomic architecture of the population based on sequence analysis of bacterial, fungal and viral populations in fecal and nasal samples.</li> <li>• To associate immune response with nutrition data.</li> <li>• To associate immune phenotype variance with genetic polymorphisms and enterotype.</li> </ul>
11. Secondary outcomes	Determination of genotype-to-phenotype associations at a mechanistic level.
12. Subjects	Healthy adults subjects
13. Number of subjects	<p>Around 1200 subjects will be enrolled at V0 to include 1000 subjects at V1 among whom 500 will complete the visit V2 and, on these 500, up to 500 will perform a biopsy at V1.</p> <p>At V1, subject recruitment will be stratified by:</p> <ul style="list-style-type: none"> <li>• Gender (50%/50%): 500 males / 500 females</li> <li>• Age: 5 decade strata (20% in each): 200 subjects/decade</li> <li>• 100 subjects by gender by decade strata</li> <li>• Age (<math>\geq 20</math> and <math>\leq 69</math> years)</li> <li>• Biopsie: (10% in each decade and 50% by gender)</li> </ul> <p>In the event of a premature withdrawal, the subject will be replaced by a subject of the same gender and age decade, to ensure a number of 500 complete observations at V2 with 100 subjects in each decade strata and 50 subjects in each gender.</p>
14. Inclusion criteria	<ol style="list-style-type: none"> <li>1. Subjects considered as healthy by the investigator based on medical history, clinical examination, laboratory results and ECG (blood sampling for laboratory assessments and ECG should be done at V0 and only after signed informed consent).</li> <li>2. Subjects who, according to the investigator, can and will comply with the requirements of the protocol and are available for all scheduled visits at the investigational site.</li> <li>3. Healthy male or female aged between 20 and 69 (included) years</li> <li>4. Metropolitan French origin for 3 generations</li> <li>5. <math>18.5 \leq \text{BMI} \leq 32 \text{ kg/m}^2</math> (Appendix 18.6)</li> <li>6. Ability to give their informed consent in writing</li> <li>7. Must understand spoken and written French</li> <li>8. Affiliated to the French social security or assimilated regimens</li> <li>9. Registered on the French "Fichier des Volontaires se prêtant à la Recherche Biomédicale (VRB)"</li> </ol>

15. Exclusion criteria

1. Subjects who cannot participate according to their status on the registry mentioned at Art L. 1121-16 of the French Public Health Code
2. Participation in another clinical study in the last 3 months in which the subject has been exposed to an investigational product (pharmaceutical product or placebo or medical device) or concurrent participation in another clinical study during the study period
3. Relatedness to previously recruited individuals in the study cohort
4. Travel in (sub-)tropical countries within the last 3 months
5. For women: pregnant or breastfeeding or intending to become pregnant or peri-menopausal\*

\* *Peri-menopausal women as defined by menstrual irregularity: either a change in the menstrual cycle length of more than seven days (early perimenopause) or two or more missed periods with an interval of 60 days or more between periods (late perimenopause) (Stages of Reproductive Aging Workshop, STRAW)(11)*

6. Any physical exercise within the last 8 hours before inclusion (V1) and before (V2)
7. Subjects following a special diet for medical reasons as prescribed by a GP or dietician (e.g. calorie restricted or weight-loss diet for significant overweight, cholesterol lowering diet or subjects suffering from any clinically diagnosed food allergy or intolerance)
8. Alcohol abuse (more than 50 g of pure ethanol per day: for example, more than 4 x 150 mL glasses of wine, more than 4 x 250 mL glasses of beer, more than 4 x 40 mL glasses of high alcohol content drinks)
9. Illicit drug use or substance abuse within 3 months prior to inclusion
10. Presence of evidence of neurological or psychiatric diagnoses which, although stable, are deemed by the investigator to render the potential subject unable/unlikely to participate in the study satisfactorily.
11. Severe/chronic/recurrent pathological conditions, among them:
  - 11.1. Past or present diagnosed cancer, lymphoma, leukemia to the exception of:
    - Persons with a history of cancer who are disease-free without treatment for 5 years or more
    - Women who are disease free for 3 years or more after treatment for breast cancer and receiving long-term prophylactic tamoxifen
    - Cutaneous or cervical basal cell carcinoma
  - 11.2. Personal history of organ transplant
  - 11.3. Congenital or acquired immune deficiency (any confirmed or suspected immunosuppressive or immunodeficient condition, including history of HIV infection)
  - 11.4. Personal history of auto-immune diseases requiring or having previously required treatment (e.g. Rheumatoid Arthritis, Systemic Lupus Erythematosus, Sarcoidosis, Ankylosing Spondylitis, Autoimmune Hemolytic Anemia, Autoimmune Thrombocytopenic Purpura, Crohn's Disease, Psoriasis, Scleroderma, Wegener's Granulomatosis, Type I Diabetes, Thyroiditis,....)
  - 11.5. Splenectomy
  - 11.6. Acute or chronic, clinically significant, as determined by the investigator, pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests
  - 11.7. History of clinically significant, as determined by the investigator,

neurological disorder or seizures

11.8. Infectious diseases:

- Chronic infection (e.g. HIV, HBV, HCV...) or current acute infection or past acute infection based on investigator's judgment within the last 3 months,
- Presence of a rectal temperature  $\geq 38.4^{\circ}\text{C}$ , or axillary temperature  $\geq 37.5^{\circ}\text{C}$ , or intra-auricular temperature  $\geq 38.4^{\circ}\text{C}$ , or buccal temperature  $\geq 38^{\circ}\text{C}$  on the scheduled date of inclusion,
- Subject receiving (currently or in the last 3 months) antibiotics, intestinal, nasal or respiratory antiseptics.

11.9. Severe High Blood Pressure defined as systolic BP  $\geq 160$  mmHg and/or diastolic BP  $\geq 100$  mmHg (AHA stage 2 HBP). Treated and controlled HBP is allowed.

11.10. Type II diabetes mellitus requiring treatment with any medication. Diabetes mellitus treated by exercise and diet control only is permitted.

11.11. Chronic renal impairment as defined by Renal Insufficiency: GFR  $< 60$  mL/min/1.73 m<sup>2</sup> (National Kidney Foundation (2002) (8))

11.12. Chronic bone disease as treated by biphosphonates

11.13. Treated depression or any evidence of overt depressive episode during medical examination and interview

11.14. Any significant disorder of coagulation or treatment with warfarin derivatives or heparin or antiplatelet medications within 2 months preceding inclusion.

11.15. Dermatologic conditions: any current dermatological disorder that is severe enough to prevent the skin biopsy (e.g. eczema, psoriasis, acute or chronic dermatitis)

11.16. Severe acute/chronic allergy

- Severe Asthma defined as asthma requiring a combination of two or more controller therapies (e.g. medium or high dose inhaled glucocorticosteroid and long-acting inhaled beta-2 agonist) or requiring oral glucocorticosteroids (GINA(3)),
- Severe food allergy, as defined by history of giant urticaria, Quincke edema or anaphylactic shock,
- Severe insect bite allergy with history of giant urticaria, Quincke edema or anaphylactic shock,
- Atopic dermatitis treated with medication.

12. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within the 6 months prior to the inclusion. For corticosteroids, this will mean a dose equivalent to 20 mg/day of prednisone or equivalent for > 2 weeks (inhaled and topical steroids allowed)

13. Chronic administration of NSAIDs, including aspirin: prolonged intake (> 2 weeks) within 6 months before study or any intake within the 7 days preceding skin biopsy [exception for low dose aspirin: maximum 250mg/daily, see 8.1]

14. Receipt of any vaccination 3 months before the inclusion or planning to receive any vaccination during the study

15. Receipt of blood products or immunoglobulins within 3 months prior the inclusion or planning to receive blood products or immunoglobulins during the study

	<p>16. Hemoglobin measurement less than 10.0 g/dL for women and less than 11.5 g/dL for men</p> <p>17. Platelet count less than 120.000/mm<sup>3</sup></p> <p>18. ALAT and/or ASAT &gt; 3 times the upper limit of the norm (ULN)</p> <p>19. Allergy to lidocaine</p>
<p>16. Subject compensation</p>	<ul style="list-style-type: none"> <li>- 300 euros for subject performing the biopsy at V1 (V0: 50, V1: 150 euros, V2: 100 euros)</li> <li>- 250 euros for subject performing V1 and V2 without biopsy at V1 (V0: 50, V1: 100 euros, V2: 100 euros)</li> <li>- 150 euros for subject not performing the biopsy at V1 (V0: 50, V1: 100 euros)</li> <li>- 50 euros for subject performing only V0</li> </ul>
<p>17. Estimated calendar</p>	<ul style="list-style-type: none"> <li>- First enrolment (FSFV: first subject first visit V0): September 2012</li> <li>- Duration of the enrolment: about 12 months</li> <li>- Last enrolment: around August 2013</li> <li>- Total duration of the study follow-up (FSFV–LSLV): 14 months</li> <li>- Last visit (LSLV: last subject last visit V2): October 2013</li> <li>- Duration of participation for one subject: max 8 weeks (56 days) for subject performing V2 with or without biopsy at V1 and max 2 weeks (14 days) for subject performing V1 only</li> <li>- Total duration of the study (results analysis): 5.5 years</li> <li>- Results analysis: 2018</li> </ul>

## **PROTOCOL SIGNATURE PAGE**

Signing of this page of the protocol indicates that the protocol format and content has been agreed and approved by the parties, and that it is agreed that no further changes to the protocol are required at the time of signing.

### **Sponsor:**

**Cécile DELVAL, MD**

**Clinical Manager**

Date :

Signature:

### **Scientific Leaders:**

**Matthew ALBERT, MD, PhD**

Date :

Signature:

**Lluís QUINTANA-MURCI, PhD**

Date :

Signature:

### **Coordinating Investigator**

**Dr Nicolas FAUCHOUX, MD**

Date :

Signature:

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## **INVESTIGATOR PROTOCOL SIGNATURE PAGE**

I have read the protocol document and, on behalf of my institution, agree to comply with the protocol, with Good Clinical Practices (GCP), and all applicable regulations.

**Principal Investigator**

Date : 

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Signature:



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## **ABBREVIATIONS**

AE	Adverse Event
ALAT	ALanine AminoTransferase
AFSSAPS	Agence Française de Sécurité Sanitaire des Produits de Santé
aPTT	(activated) Partial Thromboplastin Time (=TCA in French)
ASAT	ASpartate AminoTransférase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CCTIRS	Comité Consultatif du Traitement de l'Information en matière de Recherche dans le domaine de la Santé
CIH	Centre d'Immunologie Humaine
CMV	CytoMégaloVirus
CNIL	Commission Nationale de l'Informatique et des Libertés
CPP	Comité de Protection des Personnes
CRP	C-Reactive Protein
CRF	Case Report Form
CSP	Code de la Santé Publique
DNA	DeoxiriboNucleic Acid
EBV	Epstein Barr Virus
ECG	ElectroCardioGram
eCRF	Electronic Case Report Form
eQTL	Expression Quantitative Trait Loci
GCP	Good Clinical Practices
GFR	Glomerular Filtration Rate
GP	General Practitioner
GWA	Genome Wide Association
HAV	Hepatitis A Virus
HBP	High Blood Pressure
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRT	Hormone Replacement Therapy
HTLV	Human T-Lymphotropic Virus
ICF	Informed Consent Form
iPS	Induced Pluripotent Stem
ITS	Internal Transcribed Spacer
MCV	Mean Corpuscular Volume
MR-001	Méthodologie de référence MR-001 pour les traitements de données

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personnelles opérés dans le cadre des recherches biomédicales

NSAIDs	NonSteroidal AntiInflammatory Drugs
PT	Prothrombin Time
QTL	Quantitative Trait Loci
RBC	Red Blood Count
RNA	RiboNucleic Acid
SAE	Serious Adverse Event
SNP	Single Nucleotide Polymorphism
TCA	Temps de Céphaline Activée (see aPTT)
VLP	Viral Like Particles
WBC	White Blood Count



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# 1. STUDY JUSTIFICATION

## 1.1. Background and rationale

In the context of an ambitious French program of research, financed through the *Investissement d'Avenir* as part of a *Laboratoire d'Excellence* (LabEx) research program, we have developed a protocol to ascertain the determinants of human immune variance. Indeed, susceptibility to infections, disease severity, and response to medical therapies and vaccines are highly variable from one individual to another. While the question of variance in human populations continues to be a focal point of scientific research, medical practices and public health policies typically take a 'one size fits all' model to disease management and drug development. Efforts to restore the 'personal' in medical care are the current challenge, and the driving vision of the project, to which the current study belongs. In order to realize the promise of personalized medicine, we require an in-depth understanding of the determinants of heterogeneity in host response to stress. From a patient's perspective, our ability to achieve the goals outlined herein will provide the needed insight to develop tailor-made treatments – a revolution for healthcare management.

Given the role of inflammation and host immunity in disease pathogenesis, our project is poised to impact: vaccination of healthy subjects (especially with respect to adjuvant choice for an aging population); selection of patients responding to treatment for chronic infectious disease (e.g HCV); management of individuals at risk for autoimmune or inflammatory disease (e.g Crohn's Disease); and the optimization of cancer treatment in order to minimize adverse effects (e.g. breast cancer).

The immune system is responsible for maintaining a healthy state and preventing infection, in most cases. For some people, however, immune system dysfunction results in increased susceptibility to infections, inflammation, autoimmunity or even development of cancer. Moreover, individual heterogeneity in the immune response can have an enormous impact on the likelihood to respond to therapy or the development of side effects secondary to vaccine administration. Because of the complexity of immune responses in the individual and within the population, it has not been possible thus far to define the parameters (genetic or environmental) that constitute a healthy immune system and its natural occurring variability.

### **Phenotype, genotype, enterotype determination - Genome-annotated cell lines generation**

To define the genetic architecture of the study-group, next generation sequencing technologies will be used and combined with whole genome genotyping (e.g whole genome sequencing and genome-wide SNP typing). Defined Immune Phenotypes (measures of the diversity of commensal flora) will be then assayed and correlations between genomewide genetic variation and heterogeneity in immune responses to different stimuli (e.g expression Quantitative Trait Loci or eQTLs) will be established. eQTLs, and more generally QTLs, are stretches of DNA that are closely linked to the genes that underlie the phenotype trait. In addition, the role of the commensal microbiota (bacteria, fungi and viruses) will be examined by the study of samples taken from the nasal mucosa (nasal swab) and from faeces (stools sample). Finally, the integration of these data in a population genetics analytical framework will allow distinguishing those genotype-to-phenotype and enterotype-to-phenotype correlations that have conferred a major selective advantage in host survival.

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The definition of genotype -to- phenotype correlations obtained in this study will serve as an investment for fundamental research into immune regulation. Based on the results from genomewide association studies, new information will be available regarding the defined Immune Phenotypes. Mechanistic studies (e.g. biochemistry, studies in relevant experimental models, cell biology) will be required to exploit these rich data sets.

## **1.2. Aims of the study**

The aim of the project is to define how a healthy immune system responds to defined immune stimuli. We aim to establish a deep understanding of how variable the immune system response is; and establish how this phenotypic variation is genetically controlled. In addition, through the investigation of the commensal microbiota, we will ascertain the role of environmental factors in regulating immune programs. We will focus on nasal and gut flora as these mucosae represent important surfaces of inter-connectedness with the outside-world. Importantly, these microbial populations are relatively stable despite exposure to different microbial populations during food intake; and strikingly, data suggest an important association between our commensal microbiota and host immune responses. This association appears essential in maintaining homeostasis in health, and drives immunopathology in situations when homeostasis is broken.

These efforts will establish, for the first time, parameters for stratifying individuals within a population, thus making it possible to glean meaningful interpretation from measurements of stress-induced host response. In achieving this goal, we will provide a foundation for defining perturbations in an individual's immune system responses.

The study design has been developed to allow investigations of gender-dependent and age-dependent immunologic changes, while limiting genetic variability related to ethnic background.

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### **1.3. Study plan**

#### **Subjects and procedures**

The study will recruit a group of 1,000 healthy individuals, aged [20 – 69] and stratified across five-decades of life, with a 1:1 sex ratio within each age group. All donors will be metropolitan French origin for 3 generations. To avoid the influence of hormonal fluctuations in women during the peri-menopausal phase, only pre- and post-menopausal women will be recruited. Women in the peri-menopausal phase (see “non-inclusion criteria n°5\*\*”) will not be recruited.

This study involves the recruitment of healthy subject, which will provide a better understanding of what is a healthy immune system, a necessary step in establishing a reference population for personalized medicine.

As in any genetic association study, it is imperative to minimize the presence of population variability in the study cohort (i.e. genetic heterogeneity due to basic population genetic differences). It is thus essential to restrict the recruitment to individuals belonging to the same ethnic background. As the study will be conducted in France, it will be limited to Metropolitan French origin individuals for 3 generations. Consequently, the subjects’ ancestry has to be metropolitan French’s for 3 generations (i.e. the subject, his/her parents and his/her grandparents).

Subjects will undergo two or three consecutive visits, depending on the randomization that will occur at visit 1 (inclusion), during which detailed medical histories and questionnaires collecting lifestyle and family health history will be taken.

Overall, whole blood, nasal swab, stools sample and punch biopsy of the skin will be obtained. Blood for immune assays and genetic analysis, nasal swab and stools sample will be collected at two time intervals (V1 and V2) – separated by 4 weeks +/- 2 weeks (28 days +/- 14 days) – thus providing a validation sample. Biopsy of the skin will be performed once at V1 and only from up to 500 subjects among 1000 subjects.

### **1.4. Expected Results**

The integration of the genetic, epigenetic, metagenomic and phenotypic data generated by this study will shed light on the extent to which immune responses are naturally variable in the general healthy human population, providing insight as to how this variation is under genetic/epigenetic and environmental control.

Importantly, these studies will also shed light on human adaptation to changing microbial pressures. We expect to identify differences in the naturally-occurring variability of immune responses to different immune stimuli. These data will reveal the degree of population heterogeneity in immune responses and provide novel insights into host responses to products of bacterial, fungal and viral origin, using an unbiased study-design of transcriptional and proteomic profiling.

We also expect to identify genes (*i.e.* exons or miRNAs) showing differences in expression after activation with immune stimuli that (i) show enrichment for particular pathways or processes or (ii) are part of the same regulatory processes (co-expressed regulatory networks).

In addition, we expect to identify the range of naturally-occurring variation in human microbiota.

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The combination of these phenotypic data with detailed genetic and epigenetic profiling in the same individuals will allow us to map how this phenotypic variation is controlled by variation (e.g. SNPs) at specific host loci.

Finally, and perhaps most importantly, use of established population genetics analyses will help integrate genotype and phenotype variation, narrowing the hits to those genetic loci that changed during human evolution. Indeed, loci that are found to present molecular signatures of selection in addition to immunological (e)QTLs will most likely correspond to targets of selection, and therefore should pinpoint functions and mechanisms that have conferred a selective advantage on the human host.

Finally, based on the results of available GWA studies, we expect that some of the loci that our project will reveal as controlling variation in immune responses and displaying signals of selection will be found to be associated with infectious, inflammatory or autoimmune disorders. This should help us to delineate genes that are important for host defence and should inform the relationship between adaptive immunological phenotypes and present-day increased resistance or susceptibility to disease.

The project will advance systems approaches to biology and predictive, preventive and personalized medicine. Inexpensive DNA sequencing for personal genomes, low cost immune profiling and information about the role of our commensal microbiota in maintaining health will ultimately help to reduce the cost of medical care. Presymptomatic diagnosis, stratification of disease, more precise follow-up of disease progression, insight into medical use of beneficial bacteria and source material for regenerative medicine are only some of the mechanisms that will emerge from this new generation of scientific and translational research.

## **2. STUDY OBJECTIVES**

### **2.1. Primary objective**

The main objective of the study is to assess the determinants of immunologic variance within the general healthy population.

### **2.2. Secondary objectives**

To set-up a biobank of:

- EBV transformed B cell lines
- iPS cells from fibroblasts of healthy human individuals

## **3. METHODOLOGY**

### **3.1. Study design**

- Type of study: Clinical interventional study.
  - Number of subjects included: 1000 healthy volunteers.
  - Investigational product: Not applicable, no investigational product
  - Experimental design: single center study (1 center) in France
  - Visit/contact schedules: 3 scheduled visits
    - V0: 1200 subjects
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- V1: 1000 subjects (500 performing V2 with or without biopsy at V1 on the draw by lot)
  - V2: 500 subjects having or not performed biopsy at V1
  - Data collection: data collected by investigator on an electronic CRF
  - Biological sample: blood, stools, nasal swab and skin biopsy will be collected for the evaluation of the trial objectives
  - Duration of the study (FSFV – LSLV): Approximately 1 year. All subjects will be enrolled within 10 months after study set-up. For each individual subject performing the biopsy and for each individual performing V2 without biopsy at V1, the duration of the study will be around 8 weeks (56 days) from V0 to V2. For each individual subject only performing V1, the duration of the study will be around 2 weeks (14 days) from V0 to V1.
  - Statistical analysis: data handling and statistical analysis will be performed at the Institut Pasteur.

## **3.2. Outcomes**

### **3.2.1. Primary outcome**

The primary outcome is to identify factors (genetic, immunological and environmental) that contributes to the observed heterogeneity in immune responses (individual and population levels).

- To characterize the naturally occurring genetic and variability of human response using whole genome sequencing and SNPs haplotyping.
- To determine and measure cytokine/chemokine stimulated by 40 pattern-recognition receptors agonists (PRR agonists) or immune stimulators
- To characterize commensal microbiota (nasal swab and stools samples) in the study population
- To evaluate the metagenomic architecture of the population based on sequence analysis of bacterial, fungal and viral populations in fecal and nasal samples.
- To associate immune response with nutrition data.
- To associate immune phenotype variance with genetic polymorphisms and enterotype

### **3.2.2. Secondary outcomes**

The secondary outcome is the determination of genotype-to-phenotype associations at a mechanistic level.

### **3.2.3. Estimated calendar**

- First enrolment (FSFV: first subject first visit V0): September 2012
  - Duration of the enrolment : about 12 months
  - Last enrolment: around August 2013
  - Total duration of the study follow-up (FSFV–LSLV): 14 months
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- Last visit (LSLV: last subject last visit V2): October 2013
- Duration of participation for one subject: max 8 weeks (56 days) for subject performing V2 with or without biopsy at V1 and max 2 weeks (14 days) for subject performing V1 only
- Total duration of the study (results analysis): 5.5 years
- Results analysis: 2018

## 4. STUDY POPULATION

### 4.1. Description of study population

1000 healthy donors will be included in the study by the following center:

- Biotrial Rennes

Subjects will be stratified according to gender with a 1:1 ratio (with 500 subjects by gender); age (5 decades of age: [20, 29], [30, 39], [40, 49], [50, 59] and [60, 69] years, with 200 subjects per strata), and V2 (100 subjects performing V2 per decade with 50% female and 50% male) to ensure 100 subjects per gender per decade strata..

Subjects with biopsy (up to 500 from subjects performing V2) will be selected after draw by lot at visit V1.

E.g.: decade strata [20, 29]: 100 males and 100 females

Decade	Age	Gender	Biopsy	V1	V2
1	20 - 29 (200)	males (100)	Yes*	50	50
			No	50	0
		females (100)	Yes*	50	50
			No	50	0
2	30 - 39 (200)	males (100)	Yes*	50	50
			No	50	0
		females (100)	Yes*	50	50
			No	50	0
3	40 - 49 (200)	males (100)	Yes*	50	50
			No	50	0
		females (100)	Yes*	50	50
			No	50	0
4	50 - 59 (200)	males (100)	Yes*	50	50
			No	50	0
		females (100)	Yes*	50	50
			No	50	0
5	60 - 69 (200)	males (100)	Yes*	50	50
			No	50	0
		females (100)	Yes*	50	50
			No	50	0
<b>TOTAL SUBJECTS</b>				<b>1000</b>	<b>500</b>

\* Up to 500 biopsies will be performed. In case less biopsy is performed, 500 subjects will even though performed V2 without biopsy at V1.

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As in any genetic association study, it is imperative to minimize the presence of population variability in the study cohort (i.e. genetic heterogeneity due to basic population genetic differences). It is thus essential to restrict the recruitment to individuals belonging to the same ethnic background. As the study will be conducted in France, it will be limited to Metropolitan French origin individuals for 3 generations. Consequently, the subjects' ancestry has to be metropolitan French's for 3 generations (i.e. the subject, his/her parents and his/her grandparents).

To avoid hormonal cyclic fluctuations observed in females during the peri-menopausal period, only pre- and post-menopausal women will be included.

#### **4.2. Inclusion criteria**

1. Subjects considered as healthy by the investigator based on medical history, clinical examination, laboratory results and ECG (blood sampling for laboratory assessments and ECG should be done at V0 and only after signed informed consent).
2. Subjects who, according to the investigator, can and will comply with the requirements of the protocol and are available for all scheduled visits at the investigational site.
3. Healthy male or female aged between 20 and 69 (included) years
4. Metropolitan French origin for 3 generations
5.  $18.5 \leq \text{BMI} \leq 32 \text{ kg/m}^2$  (Appendix 18.6)
6. Ability to give their informed consent in writing
7. Must understand spoken and written French
8. Affiliated to the French social security or assimilated regimens
9. Registered on the French "Fichier des Volontaires se prêtant à la Recherche Biomédicale (VRB)"

#### **4.3. Non-inclusion criteria**

1. Subjects who cannot participate according to their status on the registry mentioned at Art L. 1121-16 of the French Public Health Code
2. Participation in another clinical study in the last 3 months in which the subject has been exposed to an investigational product (pharmaceutical product or placebo or medical device) or concurrent participation in another clinical study during the study period
3. Relatedness to previously recruited individuals in the study cohort
4. Travel in (sub-)tropical countries within the last 3 months
5. For women: pregnant or breastfeeding or intending to become pregnant or peri-menopausal\*

*\*Peri-menopausal women as defined by menstrual irregularity: either a change in the menstrual cycle length of more than seven days (early perimenopause) or two or more missed periods with an interval of 60 days or more between periods (late perimenopause) (Stages of Reproductive Aging Workshop, STRAW)(11)*

6. Any physical exercise within the last 8 hours before inclusion (V1) and before (V2)
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7. Subjects following a special diet for medical reasons as prescribed by a GP or dietician (e.g. calorie restricted or weight-loss diet for significant overweight, cholesterol lowering diet or subjects suffering from any clinically diagnosed food allergy or intolerance)
  8. Alcohol abuse (more than 50 g of pure ethanol per day: for example, more than 4 x 150 mL glasses of wine, more than 4 x 250 mL glasses of beer, more than 4 x 40 mL glasses of high alcohol content drinks)
  9. Illicit drug use or substance abuse within 3 months prior to inclusion
  10. Presence of evidence of neurological or psychiatric diagnoses which, although stable, are deemed by the investigator to render the potential subject unable/unlikely to participate in the study satisfactorily.
  11. Severe/chronic/recurrent pathological conditions, among them:
    - 11.1. Past or present diagnosed cancer, lymphoma, leukemia to the exception of:
      - Persons with a history of cancer who are disease-free without treatment for 5 years or more
      - Women who are disease free for 3 years or more after treatment for breast cancer and receiving long-term prophylactic tamoxifen
      - Cutaneous or cervical basal cell carcinoma
    - 11.2. Personal history of organ transplant
    - 11.3. Congenital or acquired immune deficiency (any confirmed or suspected immunosuppressive or immunodeficient condition, including history of HIV infection)
    - 11.4. Personal history of auto-immune diseases requiring or having previously required treatment (e.g. Rheumatoid Arthritis, Systemic Lupus Erythematosus, Sarcoidosis, Ankylosing Spondylitis, Autoimmune Hemolytic Anemia, Autoimmune Thrombocytopenic Purpura, Crohn's Disease, Psoriasis, Scleroderma, Wegener's Granulomatosis, Type I Diabetes, Thyroiditis,....)
    - 11.5. Splenectomy
    - 11.6. Acute or chronic, clinically significant, as determined by the investigator, pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests
    - 11.7. History of clinically significant, as determined by the investigator, neurological disorder or seizures
    - 11.8. Infectious diseases:
      - Chronic infection (e.g. HIV, HBV, HCV...) or current acute infection or past acute infection based on investigator's judgment within the last 3 months,
      - Presence of a rectal temperature  $\geq 38.4^{\circ}\text{C}$ , or axillary temperature  $\geq 37.5^{\circ}\text{C}$ , or intra-auricular temperature  $\geq 38.4^{\circ}\text{C}$ , or buccal temperature  $\geq 38^{\circ}\text{C}$  on the scheduled date of inclusion,
      - Subject receiving (currently or in the last 3 months) antibiotics, intestinal, nasal or respiratory antiseptics.
    - 11.9. Severe High Blood Pressure defined as systolic BP  $\geq 160$  mmHg and/or diastolic BP  $\geq 100$  mmHg (AHA stage 2 HBP). Treated and controlled HBP is allowed.
    - 11.10. Type II diabetes mellitus requiring treatment with any medication. Diabetes mellitus treated by exercise and diet control only is permitted.
    - 11.11. Chronic renal impairment as defined by Renal Insufficiency: GFR  $< 60$  mL/min/1.73 m<sup>2</sup> (National Kidney Foundation (2002) (8))
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- 11.12. Chronic bone disease as treated by biphophonates
  - 11.13. Treated depression or any evidence of overt depressive episode during medical examination and interview
  - 11.14. Any significant disorder of coagulation or treatment with warfarin derivatives or heparin or antiplatelet medications within 2 months preceding inclusion.
  - 11.15. Dermatologic conditions: any current dermatological disorder that is severe enough to prevent the skin biopsy (e.g. eczema, psoriasis, acute or chronic dermatitis)
  - 11.16. Severe acute/chronic allergy
    - Severe Asthma defined as asthma requiring a combination of two or more controller therapies (e.g. medium or high dose inhaled glucocorticosteroid and long-acting inhaled beta-2 agonist) or requiring oral glucocorticosteroids (GINA(3)),
    - Severe food allergy, as defined by history of giant urticaria, Quincke edema or anaphylactic shock,
    - Severe insect bite allergy with history of giant urticaria, Quincke edema or anaphylactic shock,
    - Atopic dermatitis treated with medication.
  12. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within the 6 months prior to the inclusion. For corticosteroids, this will mean a dose equivalent to 20 mg/day of prednisone or equivalent for > 2 weeks (inhaled and topical steroids allowed)
  13. Chronic administration of NSAIDs, including aspirin: prolonged intake (> 2 weeks) within 6 months before study or any intake within the 7 days preceding skin biopsy [exception for low dose aspirin: : maximum 250mg/daily, see 8.1]
  14. Receipt of any vaccination 3 months before the inclusion or planning to receive any vaccination during the study
  15. Receipt of blood products or immunoglobulins within 3 months prior the inclusion or planning to receive blood products or immunoglobulins during the study
  16. Hemoglobin measurement less than 10.0 g/dL for women and less than 11.5 g/dL for men
  17. Platelet count less than 120.000/mm<sup>3</sup>
  18. ALAT and/or ASAT > 3 times the upper limit of the norm (ULN)
  19. Allergy to lidocaine

## **5. INVESTIGATOR CENTERS**

The study will be conducted in Biotrial's clinical pharmacology unit (CPU) in Rennes:

Biotrial Rennes: 7 – 9, rue Jean Louis Bertrand – CS 34246, 35042 Rennes

The global capacities of 150 beds and the 250 employees across the 2 hospital based CPU's allows Biotrial to perform around 80 healthy volunteer studies per year since its creation in 1989 across a wide spectrum of therapeutic areas.

Studies are conducted according to GCP-ICH guidelines and common Standard Operating Procedures in the 2 CPUs certified by AFSSAPS (authorizations 05001M, 1001 OM, AFSSAPS). Medical supervision of the subjects is ensured by 8 full-time investigators supported by a full clinical team composed of study nurses and laboratory technicians. In

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addition, Phase I healthy volunteers studies are performed under the control of an experienced Medical Director and the collaboration of the Medical Scientific and Quality Affairs department.

Each site has access to a local laboratory which is compliant with GBEA standard (French law) and certified Cofrac (French Accreditation Committee) NF17025 and to state-of-the-art equipment for ECG recording or vital signs collection.

Due to its significant experience in healthy volunteer studies, Biotrial has elaborated a large volunteer database of more than 36 000 active volunteers. Existing processes for recruitment (advertising, information of the volunteers) are designed to allow efficient subject enrolment.

## **6. PRACTICAL ASPECTS OF THE RESEARCH**

### **6.1. Overall study organization**

The recruitment of the healthy donors will be done in the investigator center: Biotrial's clinical pharmacology unit (CPU) in Rennes.

The first step of the study will be the selection of the subjects based on study eligibility criteria then up to 3 visits will be planned for each included subject: enrolment visit (V0), inclusion visit (V1) and end of study visit (V2).

The Flow chart and the detailed of the study visit are detailed in appendix 18.3 and 18.4.

#### **6.1.1. Eligibility**

The eligibility of the subjects will be determined in accordance with the sites procedures and the following criteria will be checked by phone:

- 1/ Healthy male or female aged between 20 and 69 years ( $\geq 20$  and  $\leq 69$  years)
- 2/ Metropolitan French origin for 3 generations
- 3/ Covered by a social security regimen or beneficiary of a social security regimen
- 4/ For women, not pregnant or breastfeeding
- 5/ BMI  $\geq 18.5$  and  $\leq 32$  kg/m<sup>2</sup> (Appendix 18.6)
- 6/ Access to a consistent means of telephone contact, which may be either in the home or at the workplace, land line or mobile
- 7/ Must understand spoken and written French

The site will use their subjects' registries in order to identify those subjects who meet the main eligibility criteria. The subjects' eligibility will be assessed and confirmed through a telephone interview.

In addition, the subjects could be recruited by means of an advertising campaign.

Subjects will be stratified according to age and gender in a 1:1 ratio. The stratification will be performed through a centralized stratification system. The stratification per group should be stopped once 100 subjects per strata were included. In addition, a randomization will be done at V1 to select 500 subjects who will complete the V2 (100 in each decade with 50% male and 50% female) and up to 500 subjects from these V2 subjects to perform a biopsy at V1.

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A preliminary information meeting will take place upstream to the visit V0. At this visit the research will be explained by the investigator.

### 6.1.2. Enrolment visit (V0)

Subjects will attend visit V0 (enrolment visit) at the study centre to determine if they can be eligible for the study. The subject will have to be on an empty stomach for at least 6 hours.

Subject will receive an information consent form that will describe the research.

Subjects will be informed of the characteristics and the implications of the study both verbally and in writing by an investigator.

The subject will be informed and encouraged to participate in a non-interventional nutritional survey called “Etude Nutrinet-Santé” ([www.etude-nutrinet-sante.fr](http://www.etude-nutrinet-sante.fr)) at least during the Labex study participation (between V0 and V2). The participation in this study will be optional (See below).

#### **Nutrinet-Santé** (Herberg et al. BMC Public health 2010) <sup>(13)</sup>

Nutrinet study is a prospective cohort to study the relationship between nutrition and health and determinants of dietary patterns and nutritional status. It was launched in May 2009.

If subject agrees to participate in this study, they should register themselves via a web site ([www.etude-nutrinet-sante.fr](http://www.etude-nutrinet-sante.fr)). All questionnaires and forms are made to be completed online, using a secure HTML interface where all conditions of data security and confidentiality are assured.

In accordance to the law n°2004-801 on August 6<sup>th</sup> 2004, before the data entry and computerization of the collected study data, the approval of the “Comité Consultatif pour le traitement Informatique en matière de recherche dans le domaine de la santé” and the authorization of the “Commission Nationale de l’Informatique et des Libertés” have been obtained.

Participant will be asked to complete the initial dietary intake questionnaires, questionnaire on physical activity, anthropometric data, life style and health status. The total duration for completing these questionnaires will be about 2h over the course of 23 days as described below:

- 3 questionnaires for collecting dietary intake data: 1h30
- 1 questionnaire for collecting:
  - physical activity data: 5mns
  - anthropometric data: 5mns
  - life style: 10mns
  - health status: 10mns

The data collected from this first step will be sent to Institut Pasteur for data handling and analysis. The data will be sent under pseudo-anonymised code (Labex enrolment number).

The subject will have the option to continue or withdraw from the Nutrinet Study at any time.

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The subject will be informed that during the Labex-MI study they are not allowed to make any additional blood donation (e.g., EFS donation or other investigative studies).

The subject will be given time to discuss the information received before deciding to consent. Written informed consent will be obtained from the subject before any study procedure is performed.

The subject will be asked to sign the informed consent and a unique identification number (enrolment number) will be assigned and notified in the ICF and a complete pre-study evaluation will be conducted as described below:

- Demographic data;
- Clinical assessments;
  - Physical examination;
  - Vital signs measurements;
    - Blood pressure (2 supine measurements);
    - Heart rate;
    - Weight / Height;
    - Waist circumference;
    - Body temperature;
- Other assessments;
  - Past medical and surgical history;
  - Concomitant diseases;
  - Past (last 3 months) and current medications;
- Urine pregnancy test for women of childbearing potential;
- Electrocardiogram (12-lead electrocardiogram);
- Urinalysis;
- Blood sample collection for standard analysis (biochemistry, haematology, hemostasis, serologies): 20 mL (see appendix 18.5);
- Eligibility checks (inclusion / non-inclusion criteria).

The urine pregnancy test will be the first to be performed. In case of positive test, the subject is withdrawn from the study and no other tests will be performed.

The collected data will be recorded in the subject's e-CRF.

The investigator has to register the subjects into the French "Fichier des Volontaires se prêtant à la Recherche Biomédicale (VRB)" and check they have not reached the maximum amount of yearly cumulative compensation and that they are not in an exclusion period following a former participation in another research.

In case of any inclusion criterion not met or any non-inclusion criteria being present, the study must be stopped and the subject must be withdrawn from further study investigations.

At the end of the visit, the subject will be provided with material for stool collection that will be performed at home before or after returning at V1 (within 24 hours before the visit and up to 2 days after).

If subject agrees to participate in the Nutrinet-Santé Study, he/she will receive a card including the procedure and the enrolment number in order to register him/her to the study.

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After receiving the results of the standard biological analysis, all the inclusion and non-inclusion criteria could be checked in order to confirm the subject inclusion. In case of non-inclusion criteria (due to standard biology results), the subject will be contacted for cancelling the inclusion visit (V1).

### **6.1.3. Inclusion visit (V1)**

The inclusion visit (V1) will take place 4 to 14 days after (V0). The subject will have to be on an empty stomach for at least 6 hours. In addition the subject must not have made any physical exercise within 8 hours before the visit V1.

A unique inclusion number (randomization number) will be attributed to every included subject and the volunteer will be informed of the randomization outcome (i) to perform V2 with or without biopsy at V1 (ii) to perform only V1 without biopsy

After the subject has been included, the following procedures will be performed:

- Clinical assessments
  - Physical examination ;
  - Vital signs measurements ;
    - Blood pressure (2 supine measurements);
    - Heart rate;
    - Body temperature;
- Other assessments;
  - Lifestyle questionnaire including exercise habits ;
  - Concomitant treatments;
  - AE/SAE evaluation;
- Blood sample collection for (see appendix 18.5):
  - phenotypic assessment (baseline): 12mL
  - trial objectives: 75 mL
    - Genetic analysis (25mL);
    - TruCulture® - immune assays (50mL);
- Stools sample (collected at home within 24h before V1);
- Nasal swab;
- Skin punch biopsy (Appendix 18.2) for up to 500 subjects: for some subjects, the pose of strip or stitch will be made if clinically required. In case of stitches, they will be removed between 7 to 14 days after the biopsy and before V2.

Appropriate material and procedure for stool sampling will be given to the donor who will perform V2. Stool collection will be performed at home before or after returning at V1 (within 24hours before the visit and up to 2 days after).

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The subject will be asked if he/she has joined the study Nutrinet - Santé.

The subject will be instructed to phone the center in case of any issue in biopsy site healing.

#### **6.1.4. End of study visit (V2)**

This visit will only occur for the subjects who will have been biopsied at V1. The visit (V2) will take place 28 days after (V1). A time windows of +/- 14 days is allowed then visit (V2) can take place maximum 42 days after (V1) and minimum 14 days after (V1).

The subject will have to be on an empty stomach for at least 6 hours. In addition the subject must not have made any physical exercise within 8 hours before the visit V1.

The following investigations will be conducted at the end of study visit:

- Clinical assessments;
  - Physical examination ;
  - Vital signs measurements;
    - Blood pressure (2 measurements);
    - Heart rate;
    - Body temperature;
- Other assessments;
  - Concomitant treatments ;
  - AE/SAE evaluation;
- Skin biopsy site examination, if applicable;
- Urine pregnancy test for women of childbearing potential;
- Blood sample collection for (see Appendix 17.5):
  - standard analysis (8mL) (see Appendix 17.5):
    - Haematology and CRP;
  - trial objectives:75mL (see appendix 18.5)
    - Genetic analysis (25mL);
    - TruCulture® - immune assays (50mL);
- Stools sample (collected at home within 24 h before (V2));
- Nasal swab;
- End of study assessment.

The urine pregnancy test will be the first to be performed. In case of positive test, the subject is withdrawn from the study and no other tests will be performed.

The subject will be asked if he/she has completed the study Nutrinet.

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### **6.1.5. Collected data**

Collected data will be recorded in the subject's e-CRF.

#### **Physical examination data**

A complete physical examination will be carried out by a physician at enrolment visit (V0), inclusion (V1) and at last visit (V2), and will be evaluated overall as "normal" or "abnormal".

#### **Vital sign data and Electrocardiogram (ECG)**

Supine blood pressure (mean of two measurements), heart rate and intra-auricular temperature will be performed at visits (V0), (V1), (V2). Blood pressure and heart rates will be recorded after at least five minutes supine.

ECG, height, weight and waist circumference will be performed only at V0.

The normal ranges used by the Biotrial are the following:

- HR : [40-100]
- ECG : PR < 220 ms, QRS < 120 ms, QTc male < 450 ms, QTc female < 470 ms

#### **Medical histories and data**

- General information (demography, eye color, hair color, corrective lenses, age of graying, age at menarche, menstruation cycle, year of the last cervical smear, pregnancies and children ages, menopause, occupation, education)
- Past and current medical and surgical history, including childhood diseases, and perinatal history (premature birth, method of delivery, breast feeding including duration, ...)
- Food consumption and nutritional intakes
- Lifestyle (smoking, alcohol, drugs habits, physical activities, travel, etc)
- Family health history
- Vaccination history (polio, hepatitis B, influenza, measles, varicella, BCG) based on the vaccination certificate
- Medication intake
- Para-medication intake (essential oils, fish oils, herbal preparation, vitamin and mineral supplementation, probiotics ...)

#### **Standard laboratory data**

Standard laboratory data from blood sample and urine test will be assessed by the investigator.

Occurrence and intensity of abnormal biology values occurring throughout the study period will be reported into the e-CRF.

Any standards laboratory parameters that the investigator judges to be clinically significant will be recorded as an adverse event. As such, clinically significant laboratory abnormalities will be followed until clinical resolution, improvement or stabilization (only relevant for standard lab repeated at V2).

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## **7. STUDY COMPLETION AND SUBJECT REMOVAL FROM THE STUDY**

### **7.1. Study completion**

Subjects randomized to perform V2 (with biopsy or not at V1) will be considered to have completed the study after having attended visit V2.

Subjects randomized to only perform V1 will be considered to have completed the study after having attended visit V1.

### **7.2. Criteria for withdrawal from the study and replacement policy**

If one or more of the following occurs, the subject will be discontinued from study:

- At any time, if the subject decides to withdraw his/her consent
- At any time, if the subject develops a non-inclusion criterion (e.g. development of an acute disease between V0 and V2) for example:
  - Clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory tests
  - Intra-auricular temperature  $\geq 38.4^{\circ}\text{C}$  between V0 and V2.
  - Pregnant woman: in case of pregnant woman, the data from the collected sample won't be use in the final statistical analysis.
- At any time, if the subject develops an AE or an SAE (see section 11)
- At any time, for safety reasons at the investigator's discretion.

If a subject is withdrawn from the study (i.e for subjects performing V2: ceases participation in the study prior to completion of the V2 assessments planned in the protocol), the primary reason should be recorded in the end of study form in the Case Report Form (CRF).

The investigator will provide or arrange for appropriate follow-up (if required) for subjects withdrawing from the study. If the subject has withdrawn due to an AE or SAE the Investigator should follow the procedures documented in Section 11.

In the event of a premature withdrawal, the subject will be replaced by a subject of the same gender and age decade.

If blood, urinary or ECG assessments lead to exclude the subject from the study after V0, he/she will be compensated for his participating in the first visit.

## **8. CONCOMITANT MEDICATIONS**

It is recommended that the subject does not modify his/her treatment during the study. Nevertheless he may receive any treatment required in case of a concomitant health problem.

For all concomitant treatments taken during the study, the following information must be collected in the relevant section of the e-CRF:

- The name of the treatment and its form,
  - The reason for prescription,
  - The route of administration,
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- The daily dose,
  - The duration.

### **8.1. Authorized treatment**

Hormonal contraception and HRT for menopause are authorized during the study, provided these treatments were started at least 3 months prior to inclusion (V1) and are kept stable until the end of the study.

Inhaled and topical steroids are allowed with the exception of medium or high dose inhaled glucocorticosteroids for asthma (see non-inclusion criteria).

Treatment with low dose of aspirin (maximum 250 mg/daily) is allowed but should be kept stable until end of study.

Long term prophylactic tamoxifen (or related molecules) for breast cancer in women who are disease free 3 years or more after the breast cancer treatment is allowed and should be kept stable until end of study.

### **8.2. Prohibited treatments**

The following therapies are not allowed during the study:

- Anti-depressant
- Immunosuppressant or other immune-modifying drugs
- Chemotherapies and treatments against cancer except long term prophylactic tamoxifen (or related molecule) for women who are disease free 3 years or more after the treatment for breast cancer
- Anticoagulants (e.g. warfarin derivatives or heparin or antiplatelet medications with the exception of low dose of aspirin)
- NSAIDs including aspirin [exception for low dose aspirin, see 8.1]
- Any anti-diabetic treatment
- Oral corticosteroids at a dose equivalent to 20 mg/day of prednisone or equivalent for persons for > 2 weeks
- Antibiotics
- Antiseptics (intestinal, nasal or respiratory)
- Vaccination
- Blood products or immunoglobulins
- Investigational product
- Bisphosphatate

Any subject taking one of these prohibited treatments will be excluded from the study analysis.

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## **9. BIOLOGICAL SAMPLE**

### **9.1. Biological samples**

Biological material will be collected for:

(i) the standard analysis for determining inclusion / exclusion criteria:

- Blood sample testing
- Urinalysis and pregnancy test (if applicable).

(ii) the phenotypic assessment of the donors (baseline)

- Blood sample for serology and immunology assessments

(ii) the evaluation of the trial objectives:

- Blood, stools, nasal swabs and skin biopsies

After collecting samples, these will be labeled with the subject's identification number.

### **9.2. Standard laboratory analysis**

#### **9.2.1. Blood sample analysis**

20mL of blood sample will be collected at enrolment visit (V0) for determining inclusion/exclusion criterion and 8mL at the last study visit (V2) and analyzed at local center.

#### **Hematology/Hemostasis**

Hematology assessments will include red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), white blood cell count (WBC) including differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and platelet count.

#### **Biochemistry**

Chemistry assessments will include uric acid, albumin, total protein, phosphate, calcium, sodium, potassium, chloride, bicarbonates, blood urea nitrogen (BUN), creatinine, fasting glucose, gamma GT, alkaline phosphatase (AP), aminotransferase AST and ALT, total bilirubin, total Cholesterol, HDL, LDL, triglycerides, CRP.

#### **Serology**

Serology assessments will include:

- Hepatitis B (HBs Ag),
  - Hepatitis C (anti-HCV IgG, viral load only if positive antibody result),
  - HIV (anti-HIV IgG, anti-HIV IgM),
  - CMV (anti-CMV IgG\* only even in case of positive result),
  - HTLV-1 (anti-HTLV-1 IgG)
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\* In case of positive anti-CMV IgG result, no other dosages will be performed for confirming or not the infection. CMV infection will be assessed on investigator's judgment.

### **9.2.2. Urine analysis**

Urinary test will be carried out at (V0) to screen for cannabinoid use.

Proteinuria, glycosuria using urine test strip will be tested at (V0).

A urinary pregnancy test dipstick will be used at (V0) and (V2) in female subjects.

### **9.3. Phenotypic assessment of the donors (baseline)**

12mL of blood sample will be collected at inclusion visit (V1) and analyzed at local center for the assessment of the phenotypic of the donors to have a baseline for each donor:

#### **Serology**

4mL will be collected for serology assessments will include: Influenza.

#### **Immunology assessments**

8mL will be collected for immunology assessments that will include serum immunoglobulins (IgM, IgG, IgA, IgE).

Any laboratory parameters that the investigator judges to be clinically significant will be recorded as an adverse event. As such, clinically significant laboratory abnormalities will be followed until clinical resolution, improvement or stabilization (only relevant for standard lab repeated at V2).

### **9.4. Biological sample for evaluation of trial objectives**

#### **9.4.1. Blood samples**

- Blood sample for genetic analysis (25mL)

Whole blood samples will be collected at V1 and V2 on heparin (20 mL) and EDTA (5 ml) and immediately shipped at temperature between 18°C and 25°C and within 6 hours to Institut Pasteur to the Center for Human Immunology (CIH) for storage before performing study analysis.

The following procedures will be performed from the sample (see appendix 18.1):

- 1) immunophenotyping of fresh whole blood samples by flow cytometry
- 2) Determination of the genetic architecture of the population-based study achieved using whole genome sequencing and SNPs haplotyping. (using DNA isolated from PBMCs/PMN cells)
- 3) EBV induced cell lines

EBV lymphoblastic cell lines will be generated by EBV transformation of B-Lymphocytes from the peripheral blood lymphocytes populations.

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- Blood sample for TruCulture® - immune assays (50mL)

Blood samples will be used to measure cytokine/chemokine stimulated by 40 pattern-recognition receptors agonists (PRR agonists) or immune stimulators.

In vitro/ex vivo analyses on whole blood assays will be performed at V1 and V2 and are described in details in appendix 18.1.

All immune stimulations will be performed using whole blood TruCulture® medical devices. TruCulture® tubes, filled with the different stimulants will be provided in prepacked sets.

About 50mL of blood will be collected from the antecubital fossa (elbow pit) using regular pre-heparinized syringes. Syringes will be transferred to the laboratory where the blood filling into the TruCulture® tubes will be performed.

Specific work instructions for collection, incubation and handling will be provided within a TruCulture® handling manual.

The material is cryopreserved in dry ice (-80°C) at local center and will be shipped about once a week to Institut Pasteur to the Center for Human Immunology for storage before performing study analysis.

#### **9.4.2. Stools and nasal swab samples**

The stools sample and nasal swabs will be collected at V1 and V2 for characterizing the **evaluation of the metagenomic architecture of the population based on sequence analysis of bacterial, fungal and viral populations in fecal and nasal samples** (human Microbiome Phenotype).

##### ➤ Stools sample

A collection device and a plastic bag for the transport labeled with the subject's identification number will be supplied to the subjects at V0 and V1. Subjects will produce and collect the faecal specimen at their home within 24 hours before their visits (V1, V2) and up to 2 days after the visits, using the collection device. The collection device containing the sample should remain in the subject's fridge until subject's visit to the unit.

Upon reception at the clinical site, the specimen container is cryopreserved in a freezer (-80°C) and will be shipped about once a week to Institut Pasteur to the Center for Human Immunology for storage before performing study analysis.

##### ➤ Nasal swab

Specimens will be obtained with sterile, dry swabs, which are rotated five times around the inside of each nostril while applying constant pressure. Right and left nostril will be sampled separately. All swabs will be frozen (-80°C) upon collection from healthy individuals (Frank et al., 2010 PLOS One 5, e10598)<sup>(2)</sup> at local site.

The material is frozen at -80°C at local center following and will be shipped about once a week to Institut Pasteur to the Center for Human Immunology for storage before performing study analysis.

##### ➤ Stools and nasal swab analysis

The analysis are described in Appendix 18.1

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### 9.4.3. Skin punch biopsy

The skin punch biopsy will be performed at V1 to **establish cell bank of genetically annotated fibroblasts cell lines and iPS cell lines.**

➤ Skin punch biopsies at V1

Skin punch biopsies will be performed at V1 under local anesthesia with appropriate disinfection of the skin with antiseptics, by a qualified physician. The biopsy will be taken using a sterile single use biopsy punch. The standard depth of the punch blade is 7mm. The biopsy is a standard 3mm round dermal punch, taken from the inner site of the upper arm. The skin biopsy is usually performed in less than 5 minutes.

Hemostasis will be achieved by pressure with a gauze pad.

Specific work and handling instructions for collection of the punch biopsy of the skin tissue will be provided within a biopsy handling manual. More details are given in appendix 18.2.

The subject will be recommended to clean the skin at biopsy site each day for reducing the infection risk.

The material collected will be shipped the same day of the collection at 4°C to the subcontractor Genethon (Evry, Ile de France, France) and before 3:00 PM.

The skin biopsies will be used to create an annotated collection of human fibroblast cell lines and potentially generate induced pluripotent stem cells (iPS cell lines) from fibroblasts, in turn permitting establishment of a wide variety of cellular populations for experimental study.

The iPS bank will be performed by Ectycell then it will be sent to Institut Pasteur to the Center for Human Immunology for performing study genetic analysis. The remaining samples will be stored at Institut Pasteur in the same department.

### 9.5. Sample storage

After the study, if the subject agrees their biological samples may be stored until their complete use for further research related to immune responsiveness.

If the subject agrees, the stored biological may be used for additional genetic analysis.

The collection will be stored at Institut Pasteur at the Center for Human Immunology (CIH).

## 10. RESULTS COMMUNICATION TO SUBJECTS

Upon completion of the study, the global results of the research will be communicated to the investigators. According to the French regulation, the subject may ask the investigator for the results or can consult the dedicated website.

Concerning the individual data, clinical examination findings including ECG, standard laboratory tests (biochemistry, haematology, hemostasis, serologies, immunology and urinary analysis including dipstick) will be communicated to the subjects. If these results are clinically significant or relevant the subject will be sent to a physician or to an appropriate unit for medical care. After the visits are completed, if clinically significant abnormal laboratory results are found, which could indicate a health hazard, the subject will be contacted in order to inform him/her so that he/she will be taken care of in appropriate manners.

The global results of the research will be available for volunteers through a website dedicated to the research.

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## **11. SECURITY AND SAFETY EVALUATION**

### **11.1. Definitions**

#### **Adverse Events (AE)**

Any untoward medical occurrence in a clinical research subject and which does not necessarily have a causal relationship with this research.

#### **Adverse Reaction (AR)**

Any untoward medical occurrence in a clinical research subject and which has a causal relationship with this research.

#### **Serious Adverse Event (SAE)**

A serious adverse event is any untoward medical occurrence that:

- Results in death,
- Is life threatening (subject at risk of death at the time of the event),
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistence or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious (ICH E2A).

### **11.2. Expected Adverse events**

The only unwanted effects expected during the participation of the subjects are:

- Those related to the blood collection (discomfort at puncture site, transient hematoma, more rarely faintness or syncope)
- Those related to the skin biopsy (Allergic reaction due to the local anesthesia, local infection, bleeding, scar risk, vagal faintness)
- Those related to the nasal swab (low bleeding, irritation)

### **11.3. Reporting of the Adverse Events**

Any Adverse events occurring during the study will be reported in the CRF appropriate sections. For each adverse event, relevant information should be reported in the CRF including the nature, the timing of event occurrence and resolution, the severity, the outcome, the relationship to the research, the measures taken for the subject.

The severity of the event will be evaluated by the following scale:

- Mild: does not interfere with the usual subject activities
  - Moderate: partially prevents the subject to perform his/her usual activities
  - Severe: prevents totally the subject to perform his/her usual activities
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The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the subject is lost to follow up, or the subject withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

## **11.4. Notification of Serious Adverse Events to the sponsor**

### **11.4.1. Investigator responsibilities**

Any Serious adverse events (SAE) related to the research will be immediately (within 24h) reported to the selected CRO Clinical Service' Pharmacovigilance department by telephone or fax.

- Phone: 02 99 59 91 91
- Fax: 02 99 59 91 99

The relationship to the research will be first evaluated by the investigator then by the sponsor.

A written confirmation and a complementary detailed report will be sent to the select CRO.

### **11.4.2. Sponsor responsibilities**

Once the sponsor is informed of the SAE it must assess the relationship between the serious adverse event and the research.

The sponsor assesses if the SAE is expected or unexpected and the CRO Clinical Service' Pharmacovigilance department proceeds to the regulatory reporting, in particular to the Afsaps and to the CPP, and informs the investigators.

## **12. DATA MANAGEMENT AND STATISTICAL METHODS**

### **12.1. Data Management**

A database located at Institut Pasteur (CIH) will be created under the responsibility of Matthew Albert to record all the study data (data base from Biotrial 's site and data base from Nutrinet-santé).

An electronic CRF (e-CRF) will be used to capture the study data on each investigational site. Any queries generated during the data management process will be tracked by the contracted data management CRO. It will be the study monitor's responsibility to ensure that all queries are resolved by the relevant parties. At the end of the study, the data base will be sent to Institut Pasteur in a way totally securised.

The nutrition data collected from the Nutrinet-Sante Study will be sent directly to Institut Pasteur at CIH. The data will be sent under pseudo-anonymised code (enrolment number) in a way totally secured. The Nutrinet's data that will be transferred will respect the MR001 (see 14.6.2).

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## **12.2. Sample size and power**

It is considered that the sample planned for this study (a total of 1000 healthy donors with 100 in each gender x age strata combination) will be large enough to ensure sufficient statistical power to the planned analyses, based on the considerations described below:

- The power of association testing depends on several criteria: sample size, marker density and the QTL genetic effect on expression levels (Zondervan)<sup>(12)</sup>. Our sample size, particularly when it comes to eQTL analyses, are significantly larger than those recently used to successfully detect steady state eQTLs, based on 60 and 69 individuals of European and African-descent, respectively (Montgomery) (Pickrell)<sup>(7)(9)</sup>.
- In terms of precision of the estimations, the total sample size of 1000 donors will provide a precision (half the 95% CI coverage) of 0.062 standard deviations to estimations of means, and a precision of at least 0.03 to estimations of percentages. Focusing on each gender x age strata combination, the sample size of 100 in each cell will provide precisions of 0.20 standard deviations to estimates of means and 0.10 to estimates of percentages.

## **12.3. Statistical analysis**

The statistical analysis will be performed at CIH (Institut Pasteur) under the responsibility of Matthew Albert.

# **13. QUALITY CONTROL AND QUALITY ASSURANCE**

## **13.1. Monitoring**

### **13.1.1. Source document**

All trial-related documents must be kept by the Investigator in appropriate file folders. Records of subjects, original informed consent forms, source documents, electronic case report forms (e-CRFs), Ethics Committee and Sponsor's correspondence pertaining to the study must be kept on file.

The Investigator authorizes direct access to source documents for monitoring for any representative of the sponsor who will be in charge of the monitoring.

The Investigator will retain a list identifying the names (with address and/or number of medical file) of the subjects, their respective code number and the dates of entry into and completion of the trial period, in order to allow checking of data reported on e-CRFs against those in source documents.

This link will be maintained for at least 15 years after the end of the study.

Data should be available for at least 15 years after the end of the study.

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### **13.1.2. Monitoring visits**

In accordance with all applicable regulations, GCP and procedures defined within the protocol, monitors acting on behalf of the sponsor will contact the participating centers before recruitment is started in order to review with center staff the protocol and data collection procedures.

The study will be monitored by regular visits and telephone contacts to the investigator.

All the monitoring visits will be performed by a study monitor who will be assigned by the sponsor. Additional contact related to the visits, by telephone, fax or in person, will be performed as necessary.

### **13.2. Audits and inspections**

The sponsor is responsible for making sure that the study is conducted as specified by the GCP guidelines, the French regulations and the protocol. Audits may be done at the investigational sites where the CRFs are matched against source documents. It is compulsory to provide direct access to all study documentation. If an audit is performed, the Institut Pasteur will ensure the independence of the auditor.

The Health Care Authorities may audit any investigational site or the sponsor during the course of the study or following its completion, to verify the conduct of the study and quality of the data. The investigator will provide direct access to source documents.

## **14. REGULATORY ASPECTS AND ETHICS**

### **14.1. General**

This is an interventional non-pharmacological study. The sponsor is Institut Pasteur

This experimental protocol has been designed and will be conducted in accordance with:

- The ethical principles that have their origin in the Declaration of Helsinki <Version – October 2008 >
- Good Clinical Practices as outlined in the ICH Guideline for Good Clinical Practices (<ICH E6 R1 – Guideline for Good Clinical Practice – Current Step 4 version, 10 June 2010, Adopted by CPMP, July 1996, issued as CPMP/ICH/135/95/Step5, / Published in the Federal Register, 9 May 1997, Vol. 62, No. 90, p. 25691-25709>)
- All the French legislation and regulation, especially as stated by articles L 1121-1 and following of the French Public Health code pertaining to the protection of participants in biomedical research

### **14.2. Ethical committee**

It is the responsibility of the sponsor to obtain approval of the study protocol/amendment from the Comité de Protection des Personnes (CPP, Ethics Committee). Prior to the initiation of the study, the sponsor will submit:

- The study protocol
  - The IC and any other written documents to be provided to the subject
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- Details of any compensation to subjects
  - Details of modes of recruitment
  - Current curriculum vitae of the Principal Investigators
  - Any other requested document (s)

to the CPP of Brest (CPP Ouest VI) for approval.

The trial will only start once a written favorable opinion has been received from the CPP and competent authorities.

Any modifications made to the protocol after receipt of the CPP approval must also be submitted by the sponsor (or designee), to the CPP in accordance with local procedures and regulatory requirements.

### **14.3. Competent Authorities**

According to the French law/regulation, the study will not be initiated before it has been approved by the relevant Competent Authorities (Agence nationale de sécurité du médicament et des produits de santé, ANSM).

### **14.4. Subject information and written informed consent procedure**

Subjects considered eligible for inclusion by the investigator will be informed of the characteristics and the consequences of the study both verbally and in writing with the participant's information sheet and consent form. If they accept to participate in the study, they will sign the informed consent and will keep a copy of it.

The subjects will also be informed that their participation is voluntary and that they may withdraw from the study at any time.

### **14.5. French database of participants involved in biomedical research « Fichier des Volontaires se prêtant à la Recherche Biomédicale (VRB) »**

Subjects participating in this study will be registered in the French database "Fichier des Volontaires se prêtant à la Recherche Biomédicale (VRB)" of people taking part in biomedical researches. This register includes for each subject:

- the identification of the research
- his/her family name
- his/her first name
- his/her birth date and location
- his/her sex
- the start and stop date of his/her participation in the study

if applicable:

- the interdiction to simultaneously participate in another study
  - the expiry date of the exclusion period
  - the amount of the past indemnity(/ies) and current indemnity for the study
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This register is completed, consulted and updated by the investigators. The registry can be accessed through the use of a confidential access code provided to the investigators by the Ministry of Health. Subjects have the right to access information stored within the “fichier national” through the investigator or the Ministry of Health, check the accuracy or request personal details to be removed. Access and destruction to the national registry are bound by timelines specified within the national regulation.

The subjects will receive a maximum compensation of 300 euros (V0: 50 euros, V1: 150 euros, V2: 100 euros) for their participation.

## **14.6. Data protection**

### **14.6.1. Anonymisation**

The data will be collected under pseudo-anonymised conditions: the identity of the subject is coded in a way that does not allow third-party persons to detect the identity of the person. However, the investigator will retain the key with which the person can be identified.

The investigator must ensure that the subjects’ pseudo-anonymity will be maintained. On the CRF or other documents submitted to the sponsor, subjects will NOT be identified by their names, but by the assigned subject code.

Cell lines generated from the subjects biological material will be irreversibly anonymized.

### **14.6.2. Data collection and computerized treatment**

The current research is covered by article L1121-1 of the French Public Health Code (Code de Santé Publique) and included in the scope of the MR-001 “Reference Methodology” for automated management of personal data (Méthodologie de Reference pour le traitement automatisé des données personnelles)<sup>(1)</sup>.

## **14.7. Insurance**

In accordance with the provisions of the law and the GCP, the Institut Pasteur has an insurance policy intended to guarantee against possible damage resulting from the research. The policy has been taken out with “Zurich Insurance PLC, sis 112 avenue de Wagram – 75808 Paris cedex 17” (n° 07 401 372 U) (see appendix 18.7).

The studies and / or experiments performed on behalf of Institut Pasteur are specifically and expressly guaranteed.

It is advisable to underline that non compliance with the Research Legal Conditions is a cause for guarantee exclusion.

## **15. ARCHIVAL STORAGE**

In order to respect the duration of legal regulatory, the sponsor and the investigator will archive the entire study documents for at least 15 years after the end of the study.

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## **16. PUBLICATIONS**

Results of this study could be published only after agreement of scientific coordinators of the two scientific coordinators involved in this project. Position of collaborating authors will be determined according participation of each.

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## 17. REFERENCES

1. CNIL: Méthodologie de référence MR-001 pour les traitements de données personnelles opérés dans le cadre des recherches biomédicales October 2010 - [http://www.cnil.fr/fileadmin/documents/declarer/mode\\_d-emploi/sante/MR-001.pdf](http://www.cnil.fr/fileadmin/documents/declarer/mode_d-emploi/sante/MR-001.pdf)
  2. Frank et al., 2010 PLOS One 5, e10598
  3. Global Initiative for Asthma (GINA) - Global Strategy for Asthma Management and Prevention, 2010. <http://www.ginasthma.org/>
  4. Ghannoum, M.A., R.J. Jurevic, P.K. Mukherjee, F. Cui, M. Sikaroodi, A. Naqvi, and P.M. Gillevet. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6:e1000713.
  5. Howie, B.N., P. Donnelly, and J. Marchini. 2009. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5:e1000529
  6. Mahowald, M.A., F.E. Rey, H. Seedorf, P.J. Turnbaugh, R.S. Fulton, A. Wollam, N. Shah, C. Wang, V. Magrini, R.K. Wilson, B.L. Cantarel, P.M. Coutinho, B. Henrissat, L.W. Crock, A. Russell, N.C. Verberkmoes, R.L. Hettich, and J.I. Gordon. 2009. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A 106:5859-5864.
  7. Montgomery, S.B., M. Sammeth, M. Gutierrez-Arcelus, R.P. Lach, C. Ingle, J. Nisbett, R. Guigo, and E.T. Dermitzakis. 2010. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature 464:773-777.
  8. National Kidney Foundation (2002). "K/DOQI clinical practice guidelines for chronic kidney disease". [http://www.kidney.org/professionals/KDOQI/guidelines\\_ckd/toc.htm](http://www.kidney.org/professionals/KDOQI/guidelines_ckd/toc.htm)
  9. Pickrell, J.K., J.C. Marioni, A.A. Pai, J.F. Degner, B.E. Engelhardt, E. Nkadori, J.B. Veyrieras, M. Stephens, Y. Gilad, and J.K. Pritchard. 2010. Understanding mechanisms underlying human gene expression variation with RNA sequencing. Nature 464:768-772.
  10. Reyes, A., M. Haynes, N. Hanson, F.E. Angly, A.C. Heath, F. Rohwer, and J.I. Gordon. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466:334-338.
  11. Soules et al. - Executive summary : Stages of Reproductive Aging Workshop (STRAW)-Menopause, Vol. 8, No. 6, 2001
  12. Zondervan, K.T., and L.R. Cardon. 2004. The complex interplay among factors that influence allelic association. Nat Rev Genet 5:89-100.
  13. Hercberg et al. – The Nutrinet-Santé Study: a web-based prospective study on the relationship between nutrition and health and determinants of dietary patterns and nutritional status – BMC Public Health 2010, 10:242.
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## 18. APPENDICES

### 18.1. procedures for analysis on whole blood, stools and nasal swab

- In vitro/ex vivo analyses on whole blood

**Genetic analysis.** The genetic characterization of the study individuals will be performed using a two-step approach. We will first investigate in a subset of 100 donors, the levels of genome-wide variation using the Illumina HiSeq 2000 technology. We plan to achieve 30-32x coverage.

For the entire sample of 1000 individuals, we will use the Illumina Omni2.5 BeadChip, which contains 2.5 million SNPs. Then, we will use IMPUTE v.2 to estimate (“impute”) the genotypes at all SNPs identified using whole genome sequencing (Howie)<sup>(5)</sup>.

**Whole blood based immune assays.** We will focus our immune assessment on simple-to-perform whole blood assays and, where possible, employ clinically validated assays utilizing GMP-grade reagents, thus providing the highest quality data and reliable parameters for a healthy immune response.

All immune stimulations will be performed using whole blood. We will employ TruCulture® medical devices, further limiting the heterogeneity in sample processing associated with traditional culture-based ex vivo analyses (e.g. transportation of samples to the lab, Ficoll separation).

**Transcriptional Phenotypes.** Transcriptional responses, using both the mRNA and miRNA fractions, will serve as an important component of both the baseline and induced Immune Phenotypes. mRNA and miRNA will be extracted, purified and assessed for quality prior to use. Only samples with no evidence for RNA degradation (RNA integrity number > 8.5) will be retained for further experiments.

In the initial phase of the project (Phase I), we will have characterised genome-wide expression levels for 10 donors across the stimuli using Illumina sequencing for both mRNA-Seq (paired-reads of 2x100bp) and miRNA-Seq (single-reads of 1x50 bp). We will use the expression estimates based on read depth to identify those transcriptional units (i.e., exons and miRNAs) whose expression levels have changed following activation of the cells with the different stimuli (induced fold-changes).

In the current study (Phase II of the project), we will focus the analysis of the 1000 persons in the population based study on the 100 - 200 genes (exons and miRNAs) displaying the strongest fold-induction differences in gene expression following the different immune stimulations (based on the RNA-Seq results). The selected genes will be tested on the entire samples set using the Fluidigm BioMark™ 96.96 Dynamic Array for gene expression. The BioMark arrays enable up to 96 real-time qPCRs assays to be run on up to 96 samples, simultaneously (9 216 qPCR assays). This self-customised array will allow us to

1. interrogate transcriptional units of interest, thus preventing the known limitations of commercial pre-designed arrays that can only interrogate loci for which probes are included,
  2. utilize precise quantitative assays to establish gene expression variance on the larger sample across the chosen immune stimuli, and
  3. explore differences in exon usage and alternative splicing among samples.
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**Proteome Phenotypes.** One of the important read-outs used for establishing our Immune Phenotypes will be the quantitative measurement of secreted proteins.

Based on assays performed on a small group of donors, we will generate customized multiplex arrays, which will be employed for the analysis of the 1000 donors collected in the current study. All assays proposed have received CLIA certification (CV < 10%). The cut-off values for the assays are derived by determining the average of >200 media controls and adding 3 standard deviations to the mean. Values above the least detectable dose (LDD) possess excellent precision with coefficients of variation (CV) < 10%.

Analyte measurements reported below the LDD and above the lower assay limit (LAL) may be real values whose precision will be examined closely. For data mining, values below the LAL will be replaced with a value that is 50% of the lowest value in the data set.

**Cellular Phenotypes.** Cytometric analysis will be performed to determine cellular phenotypes, by employing clinically validated reagents from BD Biosciences. This includes truCOUNT™ tubes and the multitest 6-colours T/B/NK antibody cocktail (CD3-FITC /CD56-16-PE /CD45-Percp-Cy5.5 /CD4-PE-Cy7 /CD19-APC /CD8-APC-Cy7). Additional panels will be created and validated for analysis of additional cell populations of interest (e.g., T, B, NK, NKT, MAIT, Monocytes, conventional dendritic cells and plasmacytoid dendritic cells). In addition, we will include one panel with activation markers for T cells (e.g. CD69, CD38) and monocytes (e.g. CD86 and HLA-DR) to confirm that donors are not actively fighting infection or experiencing ongoing immune stimulation (n.b., donors with abnormally elevated levels of cell activation markers will be excluded from further analysis).

Thus, cytometric data will be utilized in several ways:

1. in its own right, absolute cell counts will serve as an Immune Phenotype as there is expected to be a genetic association with homeostatic set points for different cell populations;
2. the cell counts will help normalize data obtained from proteomic and gene expression studies; and
3. detection of cells with an activated phenotype will serve as an exclusion criteria, helping to ensure that the donors under investigation are healthy according to both history and laboratory analysis.

In addition to conventional cytometry, we will also be developing novel whole blood based assays using a multispectral imaging cytometer.

- **Analysis of stools and nasal swab**

The majority of symbiotic microbiota remains non-cultivable. Furthermore, the microbial diversity is estimated at approximately  $10^4$  unique strains of bacteria and unknown numbers of fungal and viral strains. These two constraints thus demand high-throughput sequencing technologies. Alternative approaches, will be considered as the technology and knowledge about enterotype profiling evolves.

Bacterial diversity will be analyzed by single molecule sequencing of 16S rDNA isolated from the stools sample and nasal swab (Mahowald)<sup>(6)</sup>.

Fungal diversity will be analyzed by single molecule sequencing of internal transcribed spacer (ITS) regions (Ghannoum)<sup>(4)</sup>.

Viral like particles (VLPs) will be purified by serial filtration and centrifugation, followed by DNA or RNA extraction and single molecule sequencing (Reyes)<sup>(10)</sup>.

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## **18.2. Skin punch biopsy: surgical procedures**

The biopsy will be performed by a physician trained on this procedure:

- 1- Clean the site of the biopsy with antiseptics. One injection of 1% lidocaine is performed. Wait 1 ½ to 2 minutes to achieve maximum local anesthesia.
  - 2- The biopsy will be taken in less than 5 minutes using a disposable punch. The standard depth of the punch blade is 7 mm. The biopsy is a standard 3 mm round dermal punch, taken from inner side of the upper arm.
  - 3- Immobilize the skin site of the biopsy with the fingers of one hand, applying pressure perpendicular to the skin wrinkle line with the skin punch.
  - 4- Core out a cylinder of skin by twirling the punch between the fingers of the other hand.
  - 5- As the punch enters into the subcutaneous fat, resistance will lessen. At this point, the punch should not be removed.
  - 6- The core of tissue often pops up slightly and can be cut at the level of the subcutaneous fat with curved iris scissors without using forceps.
  - 7- If tissue core does not pop up, it may be elevated by use of a hypodermic needle of fine tooth forceps. Include a portion of the subcutaneous fat in the specimen.
  - 8- Transfer the sample immediately to a Transport Medium collection tube (cryovial).
  - 9- Hemostasis can be achieved by pressure with the gauze pad.
  - 10- Bandage put on the area
  - 11- Recommendations on asepsis and care provided to the subject
  - 12- If necessary, stitches will be used to suture the wound. The stitches will be removed between 7 to 14 days after the biopsy. During this period, violent and water sports, sun and artificial ultra-violet exposures are contraindicated.
-