Project Name: Immunogenetics of Ageing

The aging process is very complex and longevity is a multifactorial trait, which is determined by genetic and environmental factors. The aim of the component "Immunogenetics of Ageing" is to identify new biomarkers for successful aging and an increased capacity to reach the extreme limits of life-span by analysis of immune response genes. The study will include unrelated elderly individuals (octogenarians and nonagenarians) and families with longevity members. HLA typing will be performed by NGS approaches. In addition to classical HLA loci (HLA-A,-B,-C,-DRB1/3/4/5, -DQB1,-DQA1, -DPA1, -DPB1), genes in the extended MHC region such as MICA will be included in the analysis. Polymorphisms in pro- and anti-inflammatory cytokine genes (IL-2, IL-6, IL-10, IL-12, IFNγ, TNFα, TGFβ) with possible correlation to the level of gene expression, KIR, MBL, Toll-like receptor genes could also be analyzed. Linkage and Association analyses will be performed.

Co-chairs:
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NGS HLA Component Survey Link:
https://docs.google.com/forms/d/10e-gn1ECdgQp4EsUjvXmM7Qq81g86MlWzsXLfwBJQjQ/viewform?c=0&w=1&usp=mail_form_link

A. Requirements Documentation:

Background:

Deterioration of the immune system with aging is associated with an increased susceptibility to infectious diseases, cancer and autoimmune disorders. Many studies have focused on age-associated changes in immune functions which might contribute to these pathologies. It has been demonstrated that aging is associated with chronic, low-grade inflammatory activity. The aging process is very complex and longevity is a multifactorial trait, which is determined by genetic and environmental factors, and the interaction of "disease" processes with "intrinsic" ageing processes. It is hypothesized that the level of immune response as well as possibly longevity could be associated with genes regulating immune functions. It is further hypothesized that the diversity of these genes might influence successful aging and longevity by modulating an individual´s response to life-threatening disorders. Several studies have focused on the role of immune gene polymorphisms for human longevity. However, available data do not allow at present to clarify the role of these genes due to major methodological problems, such as the typing approach and focusing on single loci, insufficient sample size, different inclusion criteria and age limits, inappropriate control matching and neglected considerations of sex-related effects and the different genetic makeup of studied populations. The 'Immunogenetics of Aging' program was a component introduced in the 14th International HLA and Immunogenetics Workshop (IHIWS) and developed further within the 15th and 16th. The aim of this component was to determine the contribution of immune genes to successful aging and an increased capacity to reach the extreme limits of lifespan. Results showed that longevity in the populations studied is positively associated with DRB1*11- and DRB1*16-associated haplotypes and with increased anti-inflammatory cytokine genotypes. Additionally, collaborative studies suggested that both activating and inhibitory KIR and functionally-relevant MBL2 haplotypes are important factors for control of CMV infection in the elderly and therefore for chronic low grade inflammation. Results showed that these genes might be predictive

biomarkers in ageing and longevity. Prevalence of MBL2 haplotypes determining absence of the protein (LYPB, LYQC, and HYPD) was observed in elderly people with a higher CMV antibody titer. The high CMV titer was also associated with a decreased frequency of the activatory KIR2DS5 and A1B10 haplotypes in elderly. However extended data on more different populations are required. A broad family-based analysis on the role of genes with immune functions on longevity in different racial and ethnic groups would be very informative and would allow clarification of the impact of these genetic markers in successful aging. However, such family-based studies are lacking so far in this important area of endeavour. Additionally HLA typing methods with a higher resolution, such as NGS, have to be applied in order to precisely identify immunogenetic determinants of successful ageing. Discovery of potential new markers that influence the longevity and susceptibility to age related diseases will contribute for development of strategies for rejuvenating the immune system and preventing replicative senescence.

Objective/Goals:

To determine new biomarkers, including extended HLA haplotypes, cytokine genes, other MHC-encoded loci and innate immunity genes, for successful aging and an increased capacity to reach the extreme limits of life-span.

Specific Aims/Objectives

The objectives of the project will include:

1. To analyze extended HLA haplotype using NGS approaches. These approaches will include screening for other relevant genes in the extended HLA region. Samples collected during 14th, 15th and 16th IHIWS, including: 1344 healthy randomly selected elderly individuals, 1163 young controls, and 12 families with long-lived members from 10 populations will be analyzed. Additionally, new samples will be collected.
2. To analyze polymorphisms in regulatory and/or coding regions, with a possible impact on the level of gene expression of pro- and anti-inflammatory cytokines in elderly and young/middle age individuals.
3. To analyze innate immunity gene polymorphisms in elderly and young/middle age individuals.
4. To perform linkage and association analyses in order to identify extended immunogenetic profiles that could be relevant to understand better the mechanisms of longevity and could be applied as new molecular targets for prevention, immunopharmacology and immunotherapy.

Samples type:

The study focus on unrelated elderly individuals (octogenarians and nonagenarians) and families with longevity members.

Families and case-control pairs will be studied through the collaborative participation of laboratories throughout the world. The participation of those laboratories previously involved in the field of immunogenetics and aging is strongly encouraged, as well as the participation of new laboratories. Investigators previously involved in studies in the international histocompatibility workshops have access to a wealth of untapped data and material that could already provide valuable contributions to answering the questions posed above. Additionally, more individuals could be recruited during the course of the study.

The following selection criteria will be used to identify families for the study:

- Extended families with a family history of at least two generations with longevity members (octogenarians and nonagenarians) including: elderly individuals, their children and grandchildren
- Sufficient demographic data should be available
- Data on family history of diseases should be available

Elderly individuals (in family-based analyses and unrelated case-control analyses) selected should ideally be characterized according to the SENIEUR protocol.
Ethnically matched unrelated young controls should ideally be characterized according to JUNIER protocol.

NOTE: Investigators may submit any of the following:

- Samples
- NGS data
- Both samples and data

If samples are being submitted without NGS data, contact the Component Chair to identify a collaborating laboratory who will perform the NGS testing. NGS could also be performed by a core laboratory if your group does not have a collaborating laboratory identified.

DNA requirements (if submitting samples only)

- 2µg DNA is required. For example, DNA at a concentration adjusted to 100 nanograms per microliter, in a volume of 20 microliters per tube.
- DNA must have > 10 kb fragments visible as a strong band when checked for quality.

Test Requirements:
HLA typing will be performed by NGS approaches. In addition to classical HLA loci (HLA-A,-B,-C,-DRB1/3/4/5, -DQB1,-DQA1, -DPA1, -DPB1), genes in the extended MHC region such as MICA will be included in the analysis.
There are no specific requirements for NGS platform/reagent combinations.

Polymorphisms in pro- and anti-inflammatory cytokine (IL-2, IL-6, IL-10, IL-12, IFNγ, TNFα, TGFβ) genes with possible correlation to the level of gene expression could be analyzed.

KIR, MBL, Toll-like receptors gene polymorphisms could be analyzed.

Proficiency testing:
Labs performing NGS HLA testing are required to perform proficiency testing (NOTE: labs who successfully participated in the Pilot project are not required to perform proficiency testing)

Instructions to request panels:

1. Choose the number of DNA reference panels desired (each is composed of 24 DNAs). Each participant may select as many as four reference DNA panels in addition to one Proficiency DNA panel.
2. Complete the **IHWG Nonprofit Order Form** attached here. Be sure to complete all the information requested.

Nonprofit Order Form-IHIW-Referenc

3. Indicate the total number of panels being ordered (additional panels may be ordered at a later time by completing another form). Enter the total number of panels in the yellow box as shown below

SPD40304	**HLA IHIWS Reference Panel**	$ 120.00		$ -

4. Once the form is completed, email to rcb@fhcrc.org or by fax to 206-667-5255 (please include tamara.vayntrub@stanford.edu)
5. Please contact the Fred Hutchinson Cancer Research Center IHWG Cell and DNA Bank for PO or other ordering information (please note: you must use the form attached here)
6. Make sure to indicate in the body of the email to rcb@fhcrc.org and tamara.vayntrub@stanford.edu if you are requesting a Proficiency DNA panel and how many additional reference DNA panels you would like (total max = 1 PT + 4 reference)

IRB Requirements
Samples and data submitted must de-identified, it should not contain personally identifiable patient information and should not include "Protected Health Information" as defined under the United States Health Insurance Portability and Accountability Act (HIPAA), http://www.hhs.gov/hipaa/index.html.
An IRB submitted by Stanford University will cover de-identified samples and data from participating centers that contain no PHI or clinical data and samples were not collected specifically for this project.

Required NGS HLA loci: HLA-A, B, C, DRB1, DRB3/4/5, DQB
Optional NGS HLA Loci: HLA-DQA, DPA, DPB, MICA, MICB

Cost: participants of this project will be expected to perform NGS typing of a proficiency panel to be provided by the Workshop organizers. Panels consist of 24 DNAs. These will be shipped directly from the Fred Hutchinson Cancer Research Center IHWG Cell and Gene Bank. The cost of the panel is $150.31 plus shipping (shipping cost are about $60 within USA and $100 - $200 international) Participants may also provide an overnight courier shipping account number at the time the form with the number of panels requested is submitted.
Data analysis and data entry:
Database requires entry of HLA types in GL string format. Depending on the analysis software, a macro may be used to convert the output to the desired format. Example of Output file if not in GL string format should be provided.

Required for all projects:

- LabCode: six character alphabetic code provided by the 17WS organizers
- SampleID: As labeled
- GL Genotyping: a locus-level HLA genotype recorded using GL String format, as defined by Milius et al. 2013 (doi: 10.1111/tan.12150)


GL String.pdf

- HLAtyping: Allele in the GL String

Project Specific data:

- HLA typing of subjects elderly individuals, young controls and family members
- Age and health status of individuals
- Race, ethnicity and country of origin of the subject
- Data on family history of diseases
- Families with longevity members: a family history for longevity members should be available and the following members should be submitted: elderly individuals, their children and grandchildren
- Data on other immune response genes such as: IL-2, IL-6, IL-10, IL-12, IFNγ, TNFα, TGFβ,KIR, MBL, Toll-like receptors gene polymorphisms could also be submitted

Other data entry:

- Does the procedure involve mixing Amplicons (if yes, at what step(s) of the testing protocol)
  – *This information will be captured in the accessory methodological information either provided as a text/pdf file or referenced via a DOI or a published citation when providing the method documentation as part of the typing submission*

- Software_related
  Software_Manufacturer: the manufacturer of the software
  Software_Name: the name of the software applied

- Hardware related
  Instrument_Firmware: the version number, or other identifier, defining the software used on the instrument for data-analysis
  Instrument_Model_Number: the model number, or other identifier, defining the type of instrument used for the typing
  Instrument_name

- Alignment_Reference_DB: the IMGT/HLA Database release version (e.g., IMGT/HLA Database 3.18.0) or Genome Reference Consortium release version (GRCh37) used for aligning reads for consensus generation

- Reagent_Protocol

- BaseCalling_Reference_DB: the IMGT/HLA Database release version (e.g., IMGT/HLA Database 3.18.0) used to identify the genotype from the consensus sequence.

- Consensus_Sequence: A nucleotide sequence representing a contiguous phased region of DNA. This can correspond to a single feature, or to multiple contiguous features. If a locus is absent, this is not reported.

- Locus_name: (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, MICA, MICB) the locus for which the sequence data and metadata in a given Locus element are reported

- *NovelPolymorphism: describe any novel sequence polymorphisms resulting in a sequence that does not correspond to an allele in the reference database using a notation that identifies the reference database version, reference allele acccession number, feature in which the novel polymorphism is found, and difference from the referenced feature. For example, IMGT/HLA|3.18.0|HLA00001|1.4|G56C identifies a G>C transversion at position 56 of exon 2 in a sequence that is otherwise identical to the exon 2 sequence of HLA-A*01:01:01:01.*

B. Other Information that may be requested:
Raw data