The genetic cohorts: facing the new challenges in infectious diseases. The HIV model

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All physicians are confronted to the diversity of susceptibility to infectious diseases or response to medication among patients. Much work has been done to characterize the virulence factors of pathogens, while less is known about the host (human) factor. Two unrelated people share about 99.9% of their DNA sequences, while the remaining 0.1% contains the genetic variants that influence how they differ in their risk of disease or response to drugs. The completion of the Human Genome Project gives a new start to developing the field of host genetic susceptibility. This review highlights ethical and practical issues on developing genetic cohorts by using the model of the Swiss HIV Cohort Study.

Key words: **HIV. Cohort studies. Host genetics. Pharmacogenetics. Ethics in genetics. Toxicogenetics.**

Las cohortes genéticas: nuevos retos en enfermedades infecciosas. El modelo VIH

Todos los clínicos se enfrentan a la diversidad en la susceptibilidad que presentan los pacientes frente a las infecciones infecciosas o en su respuesta frente a la medicación. Se han efectuado avances importantes para caracterizar los factores de virulencia de los patógenos, aunque hay menos información acerca del factor huésped (ser humano). Dos personas genéticamente no relacionadas comparten aproximadamente el 99,9% de sus secuencias de ADN, mientras que el 0,1% restante presenta las variantes genéticas que influyen en sus diferentes riesgos de enfermedad o en sus distintas respuestas frente a los medicamentos. La finalización del Proyecto Genoma Humano hace que se abra una nueva era en el campo de la susceptibilidad genética del huésped. En esta revisión se subrayan los aspectos éticos y prácticos del desarrollo de cohortes genéticas mediante el modelo del Swis HIV Cohort Study.

Palabras clave: **VIH. Estudios de cohorte. Genética del huésped. Farmacogenética. Ética en genética. Toxicogenética.**

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Introduction

Since completion of the Human Genome Project, medicine is facing a new challenge – the identification of the determinants of genetic predisposition to infectious disease. The information needed to accomplish this role requires an understanding of human genetic variation. Considerable effort is placed in the identification and cataloguing of single nucleotide polymorphism SNPs, and other sequence variants in public (dbSNP, www.ncbi.nlm.nih.gov/SNP/) or private information resources, and in the completion of the HapMap (www.hapmap.org/). The goal of the International HapMap Project is to develop a haplotype map of the human genome, which will describe the common patterns of human DNA sequence variation. These efforts will accelerate understanding of the host genetic factors underlying infectious diseases susceptibility and interindividual differences in disease progression and outcome. Till date, only limited information exists (table 1). To illustrate one approach to building expertise in this field, I will present in this review the activities of the Genetics Project of the Swiss HIV Cohort Study (SHCS, www.shcs.ch).

The SHCS represents a model of integrated management and investigation on a disease. It has served to conduct research of sociological and cultural influences on the disease, on therapeutic strategies, and on basic science aspects: virology and immunology. Since 2001, the SHCS has undertaken a new challenge: bringing genetic research to the cohort structure. The specificity of this field has required that a number of steps be solved in order to create a "Genetics cohort" within the existing structure of the SHCS: (i) development of a information and consent form that reflects the constrains defined in the frame of the Swiss law (www.ofj.admin.ch/themen/genomanalyse/vn-ve-pdf), (ii) approval by the ethics commissions of the seven AIDS centers in Switzerland, (iii) the establishing of a central laboratory for storage of DNA, (iv) the creation of a protected data base for genetic data, and (v) the incorporation of know-how in interpretation of genetic variables in cohort analysis.

All these steps were essential as the Swiss Federal Constitution dictates that "The genetic patrimony of an individual can only be analysed […] with his/her consent, or as defined by the law" (art. 119 al. 2 let f). In addition, the current project on the genetic analysis dictates that "A genetic analysis for the purpose of research, using material obtained for other reasons, can be performed when the concerned person, or his/her legal representative:

a. Has been informed of his/her rights and has not refused,

b. The anonymity is guaranteed (art. 17 al. 2)".

TABLE 1. **Association of genetic polymorphisms with infectious diseases**[1,21-23]

Gene/Protein	Role	Polymorphism/Allele	Associated disease
Mannose binding lectine	Pathogen sensing	Codons 52, 54, 57	Meningococcemia, respiratory infections
Toll-like receptor 4	Pathogen sensing	D299G	Gram negative shock
Fc gamma receptor IIA	Pathogen sensing	H131R	Meningococcemia, pneumococcemia
CD14	Pathogen sensing	C160T	Septic shock
Tumor necrosis factor A	Inflammation	TNF2	Meningococcemia, septic shock, cerebral malaria
Tumor necrosis factor B	Inflammation	TNFB2	Severe sepsis
Interleukin-1B	Inflammation	IL-1B (511)	Meningococcal disease
Interleukin-1-ra	Inflammation	IL-1 RN2	Severe sepsis
C reactive protein	Inflammation	134 bp dinucleotide repeat polymorphism	Invasive pneumococcal disease
Interferon γ receptor 1	Inflammation	IFNGR1 non-functional alleles	Susceptibility to mycobacteria
Interleukin-10	Inflammation	Promoter polymorphism	Persistance of Hepatitis B and altered response to INF-α therapy in Hepatitis C treatment. Accelerated progression of HIV infection
Interleukin-12	Inflammation	Deficiency	Susceptibility to mycobacteria
Interleukin-12 receptor	Inflammation	Mutation	Susceptibility to mycobacteria
CC Chemokine receptor 5	Inflammation	CCR5-Δ32 →	Protection from HIV
		CCR5 p1/p1	Accelerated progression of HIV infection
CC Chemokine receptor 2	Inflammation	CCR2-I64	Accelerated progression of HIV infection
Macrophage inflammatory prot-1α	Inflammation	MIP-1α459T	Accelerated progression of HIV infection
Rantes	Inflammation	Rantes In1.1C	Accelerated progression of HIV infection
HLA class I	Immunity	B8	Susceptibility to tuberculosis
		B35	Susceptibility to AIDS
		B53	Protection from severe malaria
		B5701	Protection from AIDS
		Cw*04	Susceptibility to AIDS
HLA class II	Immunity	DRB1*1302	Clearance of Hepatitis B
		DRB1*1352	Protection from severe malaria
		DRB1*1101	Clearance of Hepatitis C
		DRB1*04	Protection from typhoid fever
		DR2	Susceptibility to tuberculosis and leprosy
		DR7	Susceptibility to Hepatitis B
Plasminogen activator inibitor-1	Coagulation	4G/4G	Meningococcemia, severe sepsis
Solute carrier family 11 (NRAMP1, SLC11A1)	Transporter	–	Susceptibility to tuberculosis
Vitamin D receptor	Metabolism	TaqI restriction polymorphism	Protection from tuberculosis and leprosy

TABLE 2. **Highlights of the information provided to the Swiss HIV Cohort Study participants wishing to join the Genetics Project**

Goals: study the influence of genetic (hereditary) traits on the evolution of HIV infection, on the response to treatment, and on the development of side (adverse) effects of medication

Participants: patients included in the Swiss HIV Cohort and that provide informed consent

Study procedure: analyze genetic (hereditary) factors by using blood samples stored in the Swiss HIV Cohort repository and relate them to the disease progression

Confidentiality and data protection: The blood sample sent to the laboratory is anonymous and identified by a Cohort number. The researchers don't know the identity of the person who donated the blood sample. If communication of a result was deemed necessary, this will be done by your physician, as he is the only person who is in the position to establish the link between the Cohort number and your name

Duration: undetermined

A summary of the information for the patient participating in the Genetic project is presented in table 2.

Below, (table 3) I describe the directions chosen for implementation of genetic data to answer the following questions in the field of HIV medicine: can we predict with more precision the evolution of HIV disease? *(When to treat)* and, can these new genetic data help identify the most effective drugs for the individual *(How to treat)*, with the least adverse events? *(How to prevent toxicity)*. The final goal will be assessing the possibilities for a more personalized medicine by using genetics[1].

When to treat

Current treatment guidelines are based on the evolution of CD4 T lymphocyte numbers. The loss of CD4 T cells is to a large extent determined by the level of HIV replica-

TABLE 3. **Inherited differences in disease progression and in the metabolism, transport and disposition, and toxicity of anti-retroviral drugs**

Gene/protein	Consequence of genetic variant
Chemokine/Chemokine receptors	
CCR5	CCR5 delta 32 variant associated with protection from infection and slow disease progression. CCR5 promoter variants are associated with rapid progression
CCR2	CCR2 64I variant associated with slow disease progression
SDF1	SDF1 variants reported associated with slow disease progression
CX3CR1	CX3CR1 variants reported associated with accelerated disease progression
RANTES	Various variants (RANTES –403A, in1.1, –28G), grouped in haplotyes, associated with slow or rapid disease progression
Cytokines	
Interleukine 4	IL-4 589T variant reported (controversial) associated with modified disease progression
Interleukine 10	IL-10 promoter variants reported associated with accelerated progression
MHC	
HLA class I	HLA-A B and C heterozygosity (slow), HLA-B*35 (rapid), HLA-Cw*04 (rapid), HLA-B57 (slow)
Phase I Enzymes	
CYP3A	Role of polymorphisms in CYP3A4 unclear. CYP3A5*1 express high amounts of CYP3A5. Inter-individual variation in 20% of bioavailability of substrates. HIV protease inhibitors are substrates.
CYP2C19	CYP2C19 poor metabolizer (PM), heterozygous/extensive metabolizer (hetEM), (EM). CYP2C19 PM better cure rates. PM implicated in adverse drug reactions. HIV protease inhibitors are substrates.
CYP2B6	Enzyme has variant alleles associated with PM. Associated with modification of plasma levels of Efavirenz and adverse events.
Other: CYP2D6, CYP2C9, CYP2E1, CYP1A	All enzymes have variant alleles associated with PM. Protease inhibitor and non-nucleoside reverse transcriptase inhibitors are substrates, inducers and /or inhibitors of various isoenzymes
Transporters	
P-glycoprotein (MDR1)	Protease inhibitors are substrates. Variants implicated in differences in plasma and intracellular drug concentration, and in immune recovery in HIV-infected individuals
MRP2 (ABCC2)	Dubin-Johnson syndrom-related nonsynonymous mutations with reduced bilirubin efflux. Protease inhibitors are substrates of this transporter
MRP4 (ABCC4)	Multiple variants. Unknown consequences for, azidothymidine, lamivudine, stavudine
MRP5 (ABCC5)	Multiple variants. Unknown consequences for, azidothymidine, lamivudine, stavudine
OAT1 (SLC22A6)	Adefovir, Cidofovir are substrates, insufficient information on genetic variants
OAT2 (SLC22A7)	Azidothymidine is substrate, insufficient information on genetic variants
OCT1 (SLC22A1)	Variants associated with reduced in vitro uptake, possible reduced hepatic clearance/intestinal absorption. Known substrates are: Azidothymidine. Indinavir,Saquinavir,Ritonavir, and Nefinavir are inhibitors
Other	
HLA haplotypes	Haplotype HLA-B*5701, HLA-DR7, HLA-DQ3. Hypersensitivity reaction to abacavir
TNFα	Variants associated with earlier onset lipoatrophy
APOCIII, APOE	Variants are associated with increased risk of hypertriglyeridemia and triglyceride-rich dyslipoproteinemia associated with PI therapy
SPINK-1, CFTR	Susceptibility to pancreatitis
Mitochondrial DNA	Host genetic factors unknown; MtDNA depletion implicated in 'mitochondrial toxicity', especially NRTI-associated lipoatrophy

Adapted from Nolan et al[24].

tion. The latter, expressed as viral load, is highly variable among individuals, and so is the rate of immune damage. These are dependent of a number of factors, including the type of viral strain(s) and the influence of host genetics. While the relative virulence or fitness of the virus may evolve over time, the genetic patrimony is a constant of the individual.

An increasing number of genes present variations (alleles) that have been associated with differences in the natural evolution of HIV disease. These can be classified as immunogenetic factors (differences in HLA), variant chemokine, chemokine receptor and cytokines modulating the response to HIV, and more recently, variants in genes participating to the life cycle of HIV in the cell[1-3]. The effect of these genetic variants can be assessed through influences in the rate of loss of CD4 T cells, or in the time to AIDS or death in comparison to individuals carrying the common copies of those genes. The progression of HIV disease will reflect the cumulative effects (favorable or deleterious) of many different genetic variants in multiple genes. Thus, HIV is best described as a "complex genetic trait" where the effect of any single genetic variant is in general very small, explaining only 1 to 8% of the disease progression[4]. Work in the SHCS Genetics Project is assessing multiple genetic influences simultaneously, modeling them into predictive scores. It is expected that the identification of a larger number of validated gene variants may eventually lead to an accurate and possibly clin-

ically applicable tool in predicting individual patterns of disease progression and need for treatment.

How to treat

Currently, treatment choice is dictated by the physician using (triple) combinations of greater than 14 antiretroviral agents currently in the market. There is little room for an a priori personalized choice, as tolerability and efficacy is only determined once treatment has started. Overall 15-30% of patients will fail the initial treatment, or change it because of poor tolerance or other intervening problems[5]. It is in this context that the field of pharmacogenetics attempts at assisting in treatment choice by predicting individual pharmacokinetics and pharmacodynamic profiles. These is a field of great "hope" but also "hype". Since 1965 there have been more than 800 pharmacogenetics/genomics reviews - most suggesting that we are on the verge of offering individualized drug therapy to everyone[6].

Given the relevance of the CYP450 detoxification system in the metabolizing of protease inhibitors and non-nucleoside reverse transcriptase inhibitors, this enzymes have been targeted in first place by the SHCS Genetics cohort. Extensive analysis of CYP 3A4, 3A5, 2C19, and 2D6 lead to modest success[7-9]. Analysis of CYP2B6 variants have been described[10] that influence plasma levels of efavirenz[11], a frequently used non-nucleoside analog. High plasma levels are associated with adverse neurocognitive events[12].

The other group of genes of interest are those coding for multidrug transport proteins. Protease inhibitors are substrates (and/or inhibitors) of a number of transporters, and this is also the case for nucleoside reverse transcriptase inhibitors. Most data are available on variants of *MDR1,* coding for the P-glycoprotein that are associated with differences in biodisponibility (due to its presence in the intestinal barrier), and probably access of the medication to pharmacological privileged compartments, such as brain, placenta, and subsets of cells considered to be target of HIV[7]. There is however significant controversy about the specific role of certain *MDR1* variants, underscoring once more the challenges of the new field of pharmacogenetics[13].

How to prevent toxicity

Toxicity is a central issue in HIV medicine because of the need for life-long use of suppressive therapy. Up to 47% of patients describe clinical adverse events, and 15% laboratory abnormalities probably or definitely associated to antiretroviral therapy[14]. Metabolic disorders in the form of treatment-induced hyperlipidemia, redistribution of the subcutaneous fat (lipoatrophy), and diabetes are prominent, and contributing to increasing cardiovascular risk, and alteration of self-image. The Genetics Project is addressing these issues by investigating genetic markers, such as *ApoE* and *ApoCIII* variants, in models that evaluate the influence of specific medications on the development of hyperlipidemia. The central interest is the identification of those at highest risk, in particular through gene-drug potentialization[15]. This will complement the assessment of genetic susceptibility to lipoatrophy. Only one variant, in the human tumor necrosis factor 1 alpha (*TNF1*α-238A) has been proposed to date as accelerating the development of this complication[16,17].

Additional areas of interest are the identification of genetic markers of allergic reactions, pancreatitis and diarrhea. Hypersensitivity reactions are a frequent complication after initiation of Abacavir, Efavirenz, and Nevirapine. A breakthrough has been the identification of a allele in an ancestral HLA haplotype (HLA-B*5701, HLA-DR7, and HLA-DQ3) highly predictive of hypersensitivity reaction to Abacavir[18,19]. Pancreatitis may also represent a complication of therapy. At any given time, 4.4% of patients receiving antiretroviral therapy present hyperamylasemia[14], and mutations in *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) and *SPINK-1* (Serine Protease Inhibitor Kazal-1) which encodes a trypsin inhibitor in the cytoplasm of pancreatic acinar cells, may help identify those at highest risk of developing clinical pancreatitis[20]. Finally, diarrhea is one of the most common adverse drug events, mostly among those using protease inhibitors. The mechanism is not well defined, and any breakthrough about the genes involved, would lead to potential interventions.

Conclusions

There is broad agreement that the era of genomic medicine has begun. In addition to analysis of increasing number of individual genes, there is major development in the areas of whole genome analysis. This demands that clinical and translational research structures, build the conditions for assessment, and later implementation of new knowledge. Cohorts studies provide large amounts of prospective information on the natural history of infectious diseases and on treatment response. Thus, they are appropriate settings for the establishment of Genetics projects where the main difficulties and challenges: validation of new markers and modeling of complex traits, can be approached. And genetics is not only about prediction, it also offers insights in pathogenesis of the disease.

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