Béatrice Dambaya

Ms. Dambaya is currently a PhD fellow in Molecular Biology at the Chantal Biya International Reference Center (CBIRC) for Research on Prevention and Management of HIV/AIDS. Her main research interest is based on the identification of host genes impacting the evolution of HIV/AIDS infection in children.

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ISID Small Grant Report

Trim5α Gene Polymorphysm in Cameroonian infants: Restriction to HIV infection, implication in disease progression or protection

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Background

The general objective of this project is to understand and exploit the effects of various polymorphisms of Trim5α initially known as innate antiviral factors but now being associated to LTNP in order to provide essential insights into disease outcome and for the development of effective vaccine strategies.

Specific Objectives

- To perform DNA extraction from whole blood or from DBS;
- To perform CD4 and viral load testing;
- To perform TRIM5 alpha gene amplification and identification of mutation by RFLP;
- To perform sequencing for identification of mutations.

Patient Recruitment

Most of the activities that we have conducted since the beginning of the project have been emphasis in sample collection. We have already collected 359 samples respectively distributed in all the following groups:

1. Fast Progressor (FP)
Number of samples already collected = 64 (49 plasma+15 DBS of already dead children)

This group includes children born from HIV positive mother, presenting severe clinical manifestations or immunosuppression before 2 years from infection, with CD4 less than 50, presenting some opportunistic infections except tuberculosis (TB). This group include also children who are already dead before the age of two (retrospective study).

2. Slow Progressor (SP)
Number of samples already collected = 147

Slow Progressors include children born from HIV positive mother, diagnosed at age 5 (years of infection), on antiretroviral therapy (ARV) or not, without any critical events or with moderate clinical manifestation (CDC class A or B).

3. Long Term Non Progressor (LTNP)
Number of samples already collected = 68

This group include children born from HIV positive mother, diagnosed at age 10 or more (years of infection), never on antiretroviral therapy (ARV), and if on ARV the date of the beginning of ARV will be very important, without any critical events or with moderate clinical manifestation (CDC class A or B), and may be elite controller, but we have also included children more than 7 years but less than 10 years in this group. Only 10 of them have more than 10 years old.

4. HIV Exposed Children (HIV Negative)
Number of samples already collected = 30 (12 fresh blood+18 DBS)

This group includes HIV negative children born from HIV positive mothers distributed between the three different age groups: 0–2 years old (25 samples), 5–10 years old (6 samples), 10–15 years old (3 samples).

5. Negative Children
Number of samples already collected = 10

Healthy children (born from HIV negative mothers, aged 2–12 years old).

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Samples Analysis

Protocol Development

We started by testing some sample which are not included in our study in order to establish the protocol. So we have tested 25 samples with DNA extracted from whole blood and the same samples as DBS. Extraction, amplification by PCR with the Trim 5 primers and the Restriction Fragment Length Polymorphism (RFLP) were carried out. The results obtained were very satisfactory.

Major Findings During the First Year

A) Long Term Non Progressor Group

Over the years, the definition of long-term non progressors has varied from slow progressor to HIV controllers; with specification as virological controllers and immunological controllers. There is no internationally agreed definition of a long-term non-progressor (LTNP). As a result several different interpretations of what parameters can make someone a LTNP or HIV controller (HIC) exist. Either way these atypical patients are an important group to research as they have the potential to increase our understanding of HIV pathogenesis and develop prevention measure for HIV infection. Our question was: can we find pediatric LTNP in a setting where vertical transmission is still as high as 10%, where several endemic infectious diseases exist, where nutrition and hygiene conditions are still poor and where the frequency of protective gene mutations is low? If yes what can be their characteristics?

We performed a study, both prospective and retrospective on pediatric HIV patients to determine the prevalence of LTNPs or HICs in Cameroon, which is known to harbor all circulating HIV strains, where several tropical diseases exist. Inclusion criteria was: born to HIV positive mother.

CD4 count was determined for all infants enrolled and viral load was determined for those not yet on treatment at enrolment. Previous data from the patient medical booklet were recorded for analysis.

A total of 359 infants, aged 6 months to 17 years were enrolled in our study. According to our classification criteria, 61 were fast progressors (FP), 51 were slow progressors (SP) and 68 were classified as LTNPs. Some of the children enrolled could not be classified.

The 68 infants enrolled as LTNP, aged between 7 and 17 years, with the latest value of CD4 ranging from 239 to 1086 cells/m m3.

Of these LTNP, 47 were with no history of ART. Regarding their latest CD4 count value, 24 of them had a CD4 count <350 cells/mm3, 13 had CD4 value between >350and< 750, whilst 10 had a CD4 count of >750 cells/mm3, with the highest value of 4559 of a 13 year old infant. We could not have the CD4 count of all children enrolled. Twelve LTNP children had more than 2 values of CD4 counts that allow us to look at the dynamic of CD4 cells. We could not have the value of the CD4 count from all of our patients.

The two oldest children in the study population were 16 and 17 years old, with CD4 count of 321 and 189 respectively.

Because there were very few patients with their viral load count, this parameter was not used in the characterization of our patients. Nevertheless, from the 47 patients classified in the LTNP category, 4 infants had viral load below 5000 copies, aged from 9-14 years. These four children may be classified as real LTNP in our setting.

Also, we had 25 infants less than 3 years who were not on treatment as of WHO recommendation that states to systematically put under treatment all infants diagnosed as HIV positive from the national EID programme.

These preliminary data allow us to say confidently that among pediatric HIV patients in Cameroon there exist children capable of controlling their infection despite several unfavorable conditions like co-infections and poor nutrition.

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Also, these preliminary data allow us to say that at some point of the infection, the balance between the virus and the host is lost in favor of the virus.

A systematic enrollment and follow up of infants from three years old, HIV positive, not yet on ARV treatment will allow us to really define what LTNPs are in our settings.

B) Polymorphism Analysis

Although this first year was emphases on patient enrollment, we also started the polymorphism analysis with 30 samples included in the negative non exposed children, using Trim5 alpha allele. The different results obtain are shown in this table.

Alleles	n	Heterozygous	Homozygous	Wild type
Trim 5α	30	8	22	0

We have also started to analysis of some samples included in the Fast progressors group using Trim 5 primers. The results are in the following table.

Alleles	n	Heterozygous	Homozygous	Wild type
Trim 5α	39	37	2	0

There are most individual carrying the Trim5 alpha allele in homozygous form in Negative non exposed group (73,33%) than in the Fast progressors group (5, 12%). The percentage of the heterozygous allele among these children where respectively 26,66% and 94,87%.

C) Sequencing Polymorphism

We must sequence at least 25 DNA samples five in each group, DNA extraction have been already done for some groups, and we are still recruiting samples for this analysis. The sequencing analysis will be done with Trim 5 H 43 Y polymorphism and Trim 5-2GC primers pair:

Trim 5-F and Trim 5-H43-R (5'-GGCTGGTAACTGATCCGGCAC-3')

Trim 5-F and Trim 5-2GC-R (5'-GCAGGGATCTGTGAACAAGAGG-3')

Finally in other to have at least 50 children in each group of our study population we must emphasis our enrollment for the group who are still empty. And we are still continuing the analysis of others samples included in all the different groups.

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