Summary

Project no.012166

Project acronym Tuberculosis China

Project title

The diversity of *Mycobacterium tuberculosis* strains in China: tracing the origins of the worldwide dispersion of the multidrug-resistant Beijing genotype

Instrument Specific Support Action

Thematic Priority "Confronting the major communicable diseases linked to poverty": Tuberculosis

Title of report

Analysis of Mycobacterium tuberculosis antibiotic resistance and genetic diversity in 12 Chinese province.

Period covered: from December 1st2006 to May 30th 2008
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Start date of project: December 1st 2005 Duration: 30 months

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Summary

Section 1 – Project objectives and major achievements during the reporting period

The main goals of this project were 1) to organize the collect, storage and analysis of a large collection of *Mycobacterium tuberculosis* strains (6000) from the 31 Provinces of China, and of information on TB patients who provided samples, 2) to create a database and perform epidemiological studies to identify the origin of the Beijing lineage . The data were to be compared to the characteristics of strains collected in other countries in order to produce knowledge on the diversity of Chinese TB strains and show whether the dispersion of the Beijing type is of clonal origin.

The present SSA aimed at preparing the project in collaboration with Chinese scientists headed by Pr. Kanglin Wan, transfer the technical knowledge, follow the progress of the experiments, collect the data in a shared-database and produce population analyses.

During the past 18 months we have analysed data produced by genotyping and multidrug-resistance (MDR) assessment of a large collection of isolates from 12 Chinese provinces. These tasks were performed in close collaboration by the teams in Orsay (Christine Pourcel co-ordinator of the SSA and Gilles Vergnaud) and in Bilthoven (Dick van Soolingen, partner 2 and Kristin Kremer). Data on the BCG status, age and gender of the patients as well as other relevant clinical information have been collected. Some conclusion were obtained on the existence of ancestral strains in the South of China where the disease is likely to have emerged. This work was at the basis of Wan Kanglin PhD thesis which was defended on October 8th 2008 at University Paris-Sud Orsay. We are preparing a manuscript to be submitted for publication to an International Journal.

Section 2 – Workpackage progress of the period

WP1: Objectives

Defining the **patient cohort**, collect the samples to isolate *M. tuberculosis* strains, and characterize the strain phenotype (MDR).

Project leader: Wan Kanglin

Progress toward objectives

As discussed in the previous phase of the project, a repartition of patients according to age, gender and BCG status was made. We used Bionumerics, a multipurpose software for Image analyses and database management to store the data.

Pr Kanglin Wan together with Heads of health centres in all the Chinese provinces, have organised the collect of specimens from which *M. tuberculosis* were cultured. Different documents were gathered: patient inform consent and subject information sheet, questionnaire and Table of Examination of Patient and Samples Preservation. A precise identification tag was given to all the strains which were sent to

Beijing for checking the antibiotic resistance profile and for storing them alive in a secured -80°C freezer. The strain characteristics were entered in the database.

At the present time 3763 samples and patient information forms have been collected in the Beijing CDC and DNA was prepared for genotyping . The patient are distributed into four age groups: older than 60, between 60 and 30, between 30 and 18 years old and younger than 18 years, with an equal number of males and females. The major problem was in collecting strains from the youngest age group ie. less than 18 years old which will delay this phase of the project.

As planed in the present SSA the data for 103 patients of the Beijing province have been entered in the database in a first phase of the project. The remaining data is progressively stored in a computerised fashion after checking the quality of the information and when approval is obtained from ethical committees.

The antibiotic resistance of all the collected isolates was assessed and the data has been published in Chinese Journals and are also reported in Wan Kanglin PhD thesis. It was also discussed in a submitted work on the molecular characterization of rifampicin resistance gene *RpoB*.

WP2: Objectives

The objective was to select relevant genotyping techniques including spoligotyping, VNTR typing (MLVA16) and analysis of deletions, and to transfer the technology to laboratories in China. Training session were to be organised in Beijing for people responsible for health centres in the different provinces.

The different steps include: training of Chinese scientists in Orsay and Bilthoven, transfer of technical knowledge to Chinese laboratories and validation of techniques, follow-up of large-scale genotyping, analysis of images, centralisation of data and production of dendrograms.

Project leader: Christine Pourcel

Progress toward objectives

In the second part of our project it was important to verify that the data produced in China was reliable. During a 6 months stay in Orsay, Jinghua Liu collected and cross-checked all the data produced in Beijing and entered them in the Bionumerics database. She was able to identify some problems and make sure that all the necessary controls were efficiently performed. She also entered the patient information which were collected as paper forms, in excel tables. In total she collected and controlled data for more than 1000 isolates from nine provinces.

In addition she has genotyped new TB isolates using different techniques. She has spent three weeks in Bilthoven getting training on additional genotyping techniques.

These details can be found on the web site dedicated to this project.

The analysis of spoligotyping and MLVA genotyping data is reported in Wan Kanglin PhD thesis. It shows the distribution of the Beijing family strains in China and the existence of a potential ancestor to the Beijing family in the South of China. A manuscript is in preparation, which will be submitted to an International Journal.

WP3 Objectives:

This work package is intended to merge genotyping data present in the literature with our data to produce a **public database** on TB strains worldwide. This will allow phylogeography studies to try and understand the basis for the spreading of some particular lineages such as the Beijing family. Our own database containing also information on patients will be used to perform population studies.

Project leader: Christine Pourcel

Progress toward objectives

MIRU/VNTR data can be found in the literature together with other genotyping data such as spoligotyping or IS typing. We have started recovering this information and adding it to our own database. The genotyping data was recovered as images or Excell files. The person in charge, Ibtissem Grissa, was responsible for the quality of data included into the database. For this she has issued quality checking instructions and chose the format used to deliver the data. Bionumeric software has been used to analyse the images and as database manager.

In order to produce pertinent analyses on the emergence of new clones and evolution of older lineages it is necessary to know the mechanisms by which the genetic markers evolve. For this we analysed the frequency of VNTR alleles in different lineages using the data of the present project and other data from the IGM in Orsay, and we observed a tendency to a reduction in size during evolution (see figure). This work has been presented at a meeting in Zakopane (May 2008).