

CHAPTER 3

METHODOLOGY

3.1 Research design

The research design of this study is case control. Cases were defined as the mothers who have children with Down syndrome are from the lab of Rachanukul Institute and the King Chulalongkorn Memorial hospital. Additional, their children had karyotypically confirmed full trisomy 21. Control were defined as the mothers are from the King Chulalongkorn Memorial hospital and they do not have children with Down syndrome. From this design, previous exposure were interviews in both cases and controls.

3.2 Subject and Sample Collection

Samples of this study consisted of two groups, cases and controls. The study samples consisted of 108 women who have children with Down syndrome presenting to King Chulalongkorn Memorial hospital and Rachanukul Institute. Selection criteria of study population, their children with Down syndrome had to have karyotypically confirmed full trisomy 21. They domicile in Bangkok and used to participate in Sinthuwiwat (2004) study. In addition, they consent to participate in this study. The 101 woman who have children with Down syndrome were selected and referred to case groups.

For each case mother, one or two age-matched control mothers were recruited. The control populations were 187 women who have children without Down syndrome used to delivery at King Chulalongkorn Memorial hospital. Selection criteria of control population, they were Thai (They described themselves as “Thai”. They could speak Thai clearly and fluently). They consent participation in this study. In addition, they had to have experience no miscarriages, other abnormal pregnancies or had previous children with possible genetic factor related *MTHFR*, *MTRR* and *MTR*

SNRs, i.e. cleft lip with or without cleft palate (CL/P) and neural tube defect (NTD). The 177 women were used in this study and referred to control groups.

Data consists of two parts. The first parts are MTHFR, MTRR and MTR genotype of samples. This part is abstract from the record in the study of "Association Between Polymorphisms in Maternal Genes of Folate Metabolisms and Down Syndrome" (Thivaratana Sinthuwiwat, 2004). The second part, environmental factors of mothers, data are from interviews of a sample group of individuals in the first part.

3.3 **Ethical Consideration** (Appendix A)

The project was approved by the research advisory committee. After that, this project was proposed and permitted before the trial by Faculty of medicine, Thammasat University Ethical committee who would review both scientific and ethical research aspect. Moreover, this project was also proposed and permitted before the trial by King Chulalongkorn Memorial hospital ethical committee.

In addition, patient's information sheet and informed consent forms were also reviewed and approved by the ethical review committee.

3.4 **Data collection instrument** (Appendix B)

The researcher established data collection instrument. Review literatures and studies about environmental risk of Down syndrome are frames of the instrument. There were two categories of data collection instrument as follows:

3.4.1 General basic data was gathered from patients file. This category consisted of general information such as name, age, sex of target child, address and telephone number.

3.4.2 Questionnaire for interviewing the participants. It is composed of three parts as follows:

Part I Parental information of the participant's i.e. marital status, educational level, occupation, social-economic status and history of toxic agent exposure.

Part II Data concerning chronic diseased is composed of diagnosis, onset of disease and treatment

Part III Data concerning health behavior such as using contraception, smoking, drinking alcohol and eating habit.

For eating habit: the researcher asks about the frequencies of consumed foods. These foods have had quality sources of folate as good, very good or excellent. This part was consisted of 10 kinds of foods and was divided the eating frequencies in 3 levels:

<u>Answer</u>	<u>Scale</u>
Every day or 2-3 days per once	regularly
Once to twice a week or rarely	rarely
Never	never

3.5 Research instrument testing

3.5.1 Content validity was checked by 6 specialists. The specialists are obstetrician, nurse and genetic scientist who corrected and gave advice.

3.5.2 The researcher tried out the research instrument after correction with 30 mothers coming in pediatrics unit at Thammasat University hospital who were similar to the studied group according to their age.

3.6 Research process

3.6.1 Data collection

Permission letter for data collecting was sent to the Director of the Rachanukul Institute, and the director of the Faculty of Medicine, Chulalongkorn University.

Making form for data collection was done. The form included the following questions: parental data, chronic disease, and health behavior (contraceptive, smoking, drinking alcohol and

eating habit of mother before pregnancy). The form was tried out to test for content and consistency. Next step was collecting address and phone numbers of sample subjects.

After that, submit proposal to the Ethical Committee of the Faculty of Medicine of the Thammasat University and the Ethical Committee of Faculty Medicine of Chulalongkorn University.

Afterward; contact case and control via phone in order to ask for their participating and make an appointment for interviewing. Before interviews, the subjects were asked for permission from participants in collecting data and signing in informed consent form. Henceforth; use questionnaire to collect data by interviewing one by one.

3.6.2 Data analysis procedure

Descriptive statistics was used to describe general characteristics of study samples. This are age, gender, the number of children, educational level etc. Chi-square Test was used to test interaction between environmental factors and maternal genes polymorphisms of folate metabolism confidence level of 95 %.