CHAPTER I

INTRODUCTION

Background and rationale

Down syndrome (DS), a genetic disorder that affects 1 in 700 live births across all ethnic groups, is the leading genetic cause of mental retardation (*1*). Other clinical abnormalities associated with DS include congenital heart disease, hypotonia, facial dismorphology, immune system defects, gastrointestinal anomalies, and the early development of the pathological and neurochemical changes of Alzheimer's disease (*1, 2*). The extensive studies on DS patients with partial trisomy 21 have allowed the researchers to narrow the search for genes associated with the phenotypic features of DS to a segment of chromosome 21, the DS region (*2*). Among the genes located in the DS region is the DS critical region 1 (*DSCR1*) gene, nearly 45 kb. *DSCR1* is normally expressed in the central nervous system and the heart, but overexpressed in fetal DS brain (*3,4*). *DSCR1* belongs to a highly conserved calcineurin inhibitor family called calcipressin. Biochemical studies have shown that *DSCR1* and other proteins in the calcipressin family can bind to and inhibit calcineurin, a serine/threonine protein phosphatase important for learning and memory (*4,5,6,7,8,9,10,11*).

RNA interference (RNAi) is a naturally occurring cellular mechanism that induces post-transcriptional gene silencing in diverse cell types and has attracted much attention as a potential therapeutic strategy (*12*). Small interfering RNAs(siRNA) are short duplexes of RNA (21–23nt) that are the key intermediaries in this process (*12*). The technology of siRNA was borne out of work to elucidate the process of RNAi and post-transcriptional gene silencing initially pioneered in plants (*13*). In the early 1990s it was known that the introduction of transgenes into plants led to inhibition of the endogenous gene and the transgene itself-a phenomenon termed as "co-supression". It was also known that gene silencing occurred post-transcriptionally as transcripts from both genes were produced but were then degraded rapidly in the cytosol; hence, it terms "post-

transcriptional gene silencing". The subsequent identification of small (21–23nt) fragments of double stranded (ds) RNA in plants strongly implied the involvement of dsRNA in this process (13,14). This led to the pioneering finding in the nematode, *Caenorhabditis elegans*, that long term gene silencing could indeed be achieved through the injection of dsRNA (15). Soon after, similar findings were described in other organisms: fruit flies (16), frogs (17), mice (18) and now human cells (19) implying that the natural RNAi process is conserved amongst a variety of invertebrate and vertebrate species. RNAi operates by cleaving dsRNA with Dicer, an RNAse III nuclease, to form siRNA (12); the siRNA then causes significant down-regulation of homologous messenger (m) RNA. The mechanism of action is thought to differ between organisms. The introduction of long dsRNA (greater than 30nts in length) into mammalian cells leads to a global inhibition of gene expression via a protein kinasedependent- mediated interferon response. However, siRNAs (less than 30nts) do not elicit this toxic immune response and cellular administration of these molecules can yield potent gene silencing. Thus, administration of siRNAs to cells offers a convenient way of activating the intracellular RNAi machinery against a specific gene of interest. The power of this application was realized that exogenously applied siRNAs can be used in cultured mammalian cells for targeted inhibition of gene expression (19,20). Since its discovery in the late 1990s (15), RNAi/siRNA technology has made a tremendous impact in molecular biology and has the potential to revolutionise future healthcare. The siRNA/RNAi technology has broad applications in target validation, probing gene function (transgenomics), and may serve as a potential therapeutic strategy for the inhibition of disease-associated genes (21,22,23,24,25). Indeed, many now consider RNAi/siRNA technology as the simplest, most effective gene silencing tool that has largely superceded its predecessors such as antisense oligonucleotides, ribozymes and DNAzymes (26,27,28).

Sine *DSCR1* is overexpress in DS fetal brain (3,4), it's possible that normalizing *DSCR1* expression may restore normal brain function in DS individual. In this study, the RNAi/siRNA technology was used for *DSCR1* gene silencing in fibroblast cell. The

siRNA induced post-transcriptional gene silencing. The results from this study may serve as a potential therapeutic strategy for Down syndrome in future.

Research questions

1. Can siRNA, designed by this study, suppress *DSCR1* expression in normal fibroblast?

2. What is the optimal amount of siRNA that can silence *DSCR1* expression in Down syndrome fibroblast?

Objectives

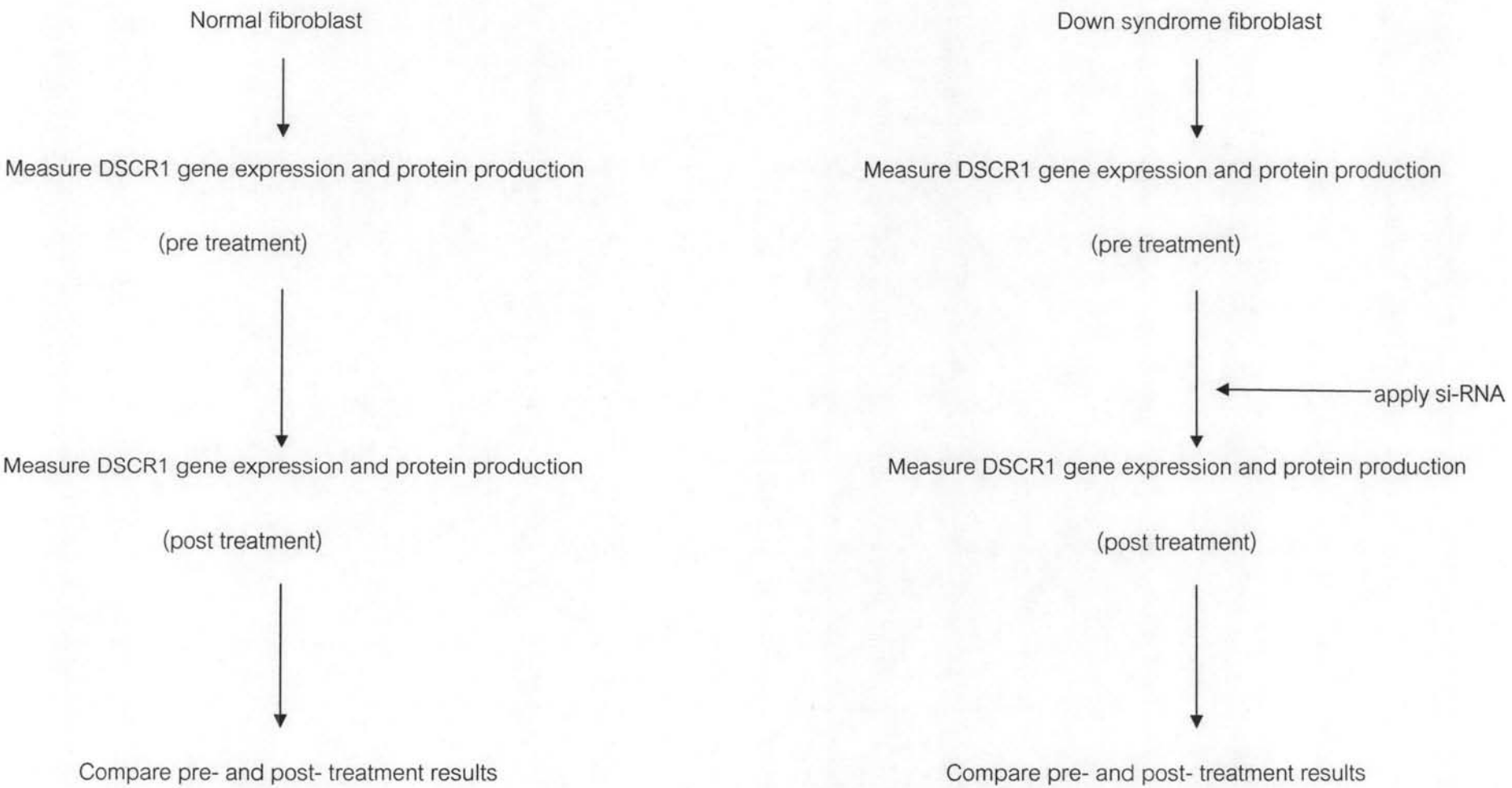
1. To study the *DSCR1* gene silencing by siRNA in fibroblast cells.

2. To determine the appropriate amount of siRNA that can silence *DSCR1* gene expression in Down syndrome fibroblast.

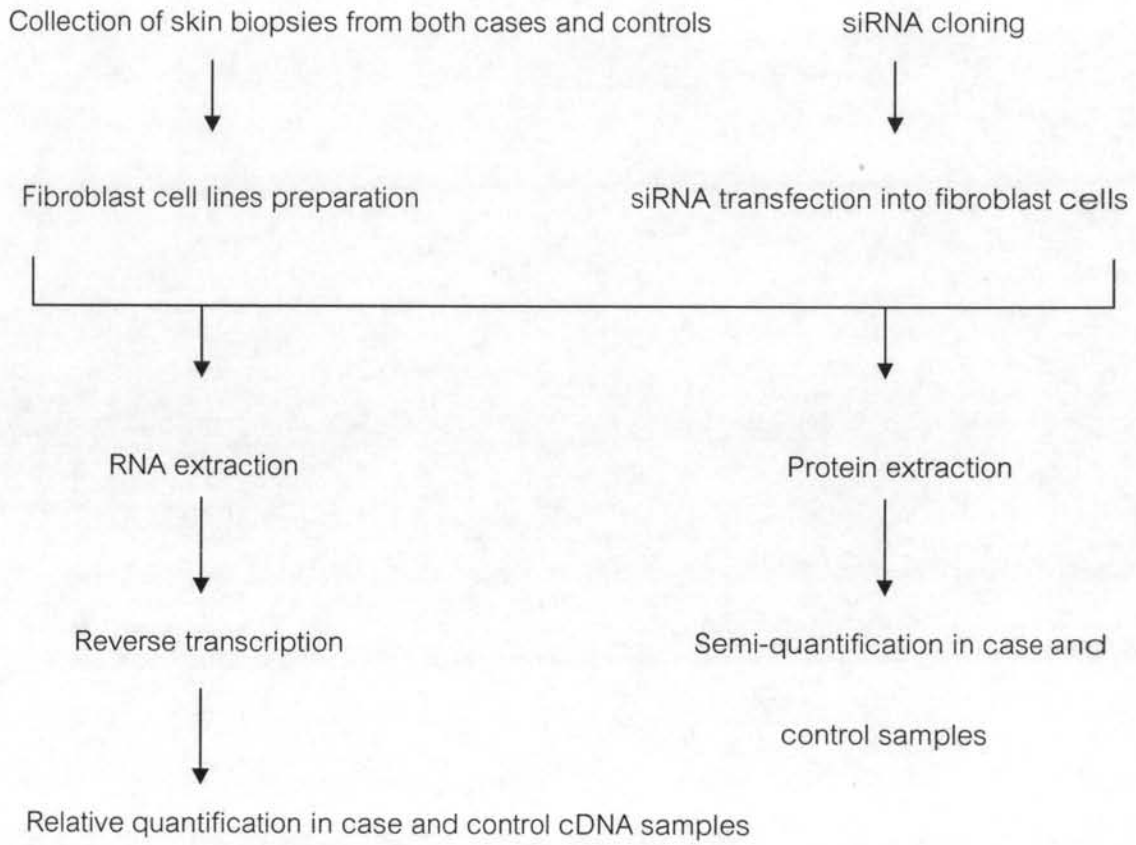
Hypothesis

1. *DSCR1* expression level is suppressed by siRNA.

Conceptual framework



Methodologies



Limitation

In this study, the limitation is slowly growth of fibroblast cells.

Expected Benefit

The result from this study may serve as a potential therapeutic strategy for Down syndrome in future.

Research Methodology

1. Collection of skin biopsies from DS patients and controls
2. Preparation of fibroblast cell lines
3. siRNA design and cloning
4. siRNA transfection into fibroblast cell lines
5. Selection of transfected cell lines
6. RNA extraction
7. Two-step RT-PCR
8. Agarose gel electrophoresis
9. Relative realtime PCR
10. Protein extraction
11. Calcineurin activity
12. Western blotting
13. Data analysis