



**UNIVERSITI PUTRA MALAYSIA**

**DOWN SYNDROME: DEVELOPMENT OF A NON-INVASIVE  
PRENATAL DNA SCREENING TEST USING SUPEROXIDE  
DISMUTASE 1 GENE IN MATERNAL BLOOD AND DETECTION OF  
CYSTATHIONINE  $\beta$ -SYNTHASE GENE MUTATIONS**

**THILAKAVATHY A/P KARUPPIAH**

**FPSK(P) 2004 7**



**DOWN SYNDROME: DEVELOPMENT OF A NON-INVASIVE PRENATAL  
DNA SCREENING TEST USING SUPEROXIDE DISMUTASE 1 GENE IN  
MATERNAL BLOOD AND DETECTION OF CYSTATHIONINE  $\beta$ -SYNTHASE  
GENE MUTATIONS**

**By**

**THILAKAVATHY A/P KARUPPIAH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**February 2004**



## **DEDICATION**

This thesis is dedicated to my spiritual master,  
*HIS DIVINE GRACE YOGA JNANA SITTAR OM SRI RAJA YOGA GURU*,  
without whom none of this would have been even possible.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**DOWN SYNDROME: DEVELOPMENT OF A NON-INVASIVE PRENATAL  
DNA SCREENING TEST USING SUPEROXIDE DISMUTASE 1 GENE IN  
MATERNAL BLOOD AND DETECTION OF CYSTATHIONINE  $\beta$ -SYNTHASE  
GENE MUTATIONS**

By

**THILAKAVATHY A/P KARUPPIAH**

**February 2004**

**Chairman: Associate Professor Rozita Rosli, Ph.D.**

**Faculty: Medicine and Health Sciences**

Down syndrome or Trisomy 21, is the most commonly occurring genetic disorder that stems from the failure of chromosome 21 to segregate normally during meiosis, resulting in an individual carrying an extra copy of chromosome 21. The main aims of this study were to develop a relatively non-invasive prenatal DNA screening method using maternal blood and to detect mutations on cystathionine  $\beta$ -synthase (CBS) gene, a folate pathway gene located on chromosome 21. As an initial step, the presence of foetal cells and DNA in the maternal blood was firstly determined by foetal haemoglobin (HbF) staining and polymerase chain reaction (PCR). It was found that the ratio of the nucleated foetal cell to maternal cell increased from 2 in  $10^6$  to 3 in  $10^6$  and 5 in  $10^6$  at the first, second and third trimester, respectively. By using Y chromosome specific primers, DNA from male foetuses could be detected as early as 6 weeks of gestation in 200  $\mu$ l maternal blood obtained from fingertip. This is in line with the current technology in non-invasive screening methods of foetal aneuploidies which is focused on detecting Y chromosomal sequences which is impossible to be used

for female foetus pregnancies. Therefore, the superoxide dismutase 1 (SOD1) gene sequence, which is located on the Down Syndrome Critical Region, was used to overcome this situation by using real-time quantitative PCR. The level of SOD1 sequences in maternal blood was found to be significantly elevated in the third trimester normal pregnancies (mean = 11728 copies/ $\mu$ l) when compared to the second trimester (mean = 5705.6 copies/ $\mu$ l),  $p < 0.005$  and non-pregnant normal women (mean = 3580.2 copies/ $\mu$ l),  $p < 0.0001$ . Down syndrome pregnancies have the greatest elevation compared to all the three trimesters of normal singleton pregnancies and twin pregnancies,  $p < 0.05$ . The traditional approach of prenatal chromosomal diagnosis using amniotic fluid was found to be cumbersome and time-consuming compared to the newly developed method. The mutation detection on CBS gene was carried out using DNA sequencer and denaturing high performance liquid chromatography (DHPLC). This study revealed that the Down syndrome patients have four mutations, which are in intron 1 (A9231C), exon 10 (C20628T) and exon 17 (T27796C and C27817T). The Down syndrome children were found to have the same genotype as their mothers. The number of mothers and children having the substitutions in the CBS gene was twice the number of mothers and children with normal genotype, suggesting that the mothers who have these substitutions are at higher risk of having a child with Down syndrome. In conclusion, non-invasive prenatal diagnosis at first trimester using Y chromosomal sequence is feasible for diagnosis of foetal-derived paternally-inherited polymorphism/mutations or genes. Quantitative analysis using gene associated with a disorder has a potentially significant advantage over the invasive techniques currently used widely for prenatal diagnosis. Finally, the discovery of the mutations in the CBS

gene of Down syndrome patients and mothers will help contribute to new knowledge and the future studies on the folate pathway genes mutation and occurrence of Down syndrome. It may also suggest an opportunity to improve public health strategies for the primary prevention of Down syndrome.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**SINDROM DOWN: PENGHASILAN UJIAN PENYARINGAN PRENATAL  
DNA YANG TIDAK MERBAHAYA MENGGUNAKAN GEN SUPEROXIDA  
DISMUTASE 1 DALAM DARAH IBU MENGANDUNG DAN PENGESANAN  
MUTASI GEN SISTATHIONIN  $\beta$ -SINTASE**

Oleh

**THILAKAVATHY A/P KARUPPIAH**

**Februari 2004**

**Pengerusi: Profesor Madya Rozita Rosli, Ph.D.**

**Fakulti: Perubatan dan Sains Kesihatan**

Sindrom Down atau trisomi 21, merupakan keabnormalan genetik yang paling biasa berlaku akibat kegagalan kromosom 21 untuk membahagi secara normal semasa meiosis, menyebabkan seseorang individu mempunyai tambahan satu kromosom 21. Objektif-objektif utama kajian ini adalah untuk menghasilkan sebuah teknik penyaringan prenatal DNA yang tidak merbahaya secara relatif dan juga mengesan mutasi-mutasi pada gene sistathionin  $\beta$ -sintase (CBS), satu gen yang terletak di tapak-jalan folat pada kromosom 21. Sebagai langkah pertama, kehadiran sel-sel dan DNA janin di dalam darah ibu hamil ditentukan dengan pewarnaan haemoglobin janin (HbF) dan tindakan rantai polimerase (PCR). Didapati bahawa, nisbah antara sel janin bernukleus dengan sel ibu hamil bertambah dari 2 dalam  $10^6$  kepada 3 dalam  $10^6$  dan 5 dalam  $10^6$  masing-masing pada trimester pertama, kedua dan ketiga. Primer-primer yang spesifik bagi kromosom Y dapat mengesan DNA dari janin-janin lelaki seawal enam minggu gestasi dengan menggunakan 200  $\mu$ l darah ibu hamil yang diambil dari hujung jari. Ini adalah sejajar dengan teknologi terkini dalam penyaringan janin

aneuploidi yang tidak berbahaya yang difokus pada pengesanan jujukan kromosom Y di mana ianya tidak mungkin dapat digunakan untuk kandungan janin perempuan. Oleh itu, jujukan gen superoksida dismutase 1 (SOD1), yang berlokasi pada Kawasan Kritikal Sindrom Down, digunakan untuk mengatasi situasi ini melalui aplikasi kuantitatif masa-benar PCR. Tahap jujukan SOD1 dalam darah ibu hamil didapati meningkat secara ketara pada kehamilan normal trimester ketiga (min = 11728 salinan/ $\mu$ l) apabila dibandingkan dengan kehamilan normal trimester kedua (min = 5705.6 salinan/ $\mu$ l),  $p < 0.005$  dan wanita normal yang tidak hamil (min = 3580.2 salinan/ $\mu$ l),  $p < 0.0001$ . Kehamilan sindrom Down mempunyai peningkatan yang paling tinggi berbanding dengan kehamilan tunggal kesemua trimester dan kehamilan kembar,  $p < 0.05$ . Diagnosis prenatal kromosom tradisional yang menggunakan cecair amnion didapati sukar dan mengambil lebih masa berbanding dengan teknik baru yang telah dihasilkan. Penentuan mutasi pada gen CBS dilakukan dengan menggunakan penjujuk DNA dan “denaturing high performance liquid chromatography” (DHPLC). Kajian ini telah menunjukkan bahawa pesakit sindrom Down mempunyai empat mutasi, iaitu di dalam intron 1 (A9231C), exon 10 (C20628T) dan exon 17 (T27796C dan C27817T). Kanak-kanak sindrom Down didapati mempunyai genotaip yang sama dengan ibu mereka. Bilangan ibu dan anak yang mempunyai substitusi ini adalah dua kali ganda daripada ibu dan anak dengan genotaip normal, mencadangkan bahawa ibu yang mempunyai substitusi ini berisiko tinggi untuk mendapat anak sindrom Down. Sebagai kesimpulan, diagnosis prenatal yang tidak berbahaya pada trimester pertama menggunakan jujukan kromosom Y boleh digunakan untuk mendiagnosis janin yang membawa polimorfisma/mutasi-mutasi atau gen-gen warisan-bapa. Analisis kuantitatif

menggunakan gen yang berkaitan dengan penyakit mempunyai potensi yang tinggi berbanding dengan teknik-teknik merbahaya yang digunakan kini secara meluas untuk diagnosis prenatal. Penemuan mutasi-mutasi pada gen CBS dalam pesakit sindrom Down dan ibu mereka akan membantu menyumbangkan pengetahuan baru dan kajian masa hadapan ke atas mutasi gen-gen di tapak-jalan folat dan kejadian sindrom Down. Ia juga mungkin memberi peluang untuk memperbaiki strategi kesihatan awam bagi pencegahan awal sindrom Down.

## AKNOWLEDGEMENTS

I owe my respectful gratitude to my spiritual master, **His Divine Grace Yoga Jnana Sittar Om Sri Rajayoga Guru**, whose blessings have accompanied me every step of the way in finishing this thesis work and made the impossible possible.

I am very grateful to the mothers and Down syndrome children who participated in this study for their time, effort, and flexibility.

My appreciation goes to Associate Professor Dr. Rozita Rosli, Associate Professor Dr. Jammal Ahmad Essa, Dr. Tan Boon Chong and Dr. TP Baskaran for their effort in helping to make this thesis a success. I would also like to thank Dr. S. Raman and Dr. Patrick for providing the positive samples, without which this prenatal diagnosis study would not have been possible.

Sincere thanks are due to Kak Norshariza and Syarilnizam for teaching me the techniques in molecular genetics and for the hospitality during my stays in Edinburgh and Oxford.

Loving thanks to my friends / learning partners especially Sharizah Alimat, who played such important roles along the journey, as we mutually engaged in making sense of the various challenges we faced and in providing encouragement to each other at those times when it seemed impossible to continue.

I would like to express my heartfelt thanks to my beloved mother, brother and sister for their endless encouragement, patience, care and sacrifices that had helped me in my undertakings and to complete this research study successfully.

This study was supported by a grant (06-02-04-0089) and fellowship from the Ministry of Science, Technology and Environment, Malaysia.



## TABLE OF CONTENTS

	<b>Page</b>
DEDICATION	2
ABSTRACT	3
ABSTRAK	6
ACKNOWLEDGEMENTS	9
APPROVAL	10
DECLARATION	12
LIST OF TABLES	16
LIST OF FIGURES	17
LIST OF ABBREVIATIONS	22
 <b>CHAPTER</b>	
<b>I</b> <b>INTRODUCTION</b>	24
Objectives of the Study	27
Specific Objective	27
Measurable Objectives	27
 <b>II</b> <b>LITERATURE REVIEW</b>	28
Down Syndrome	28
History of Down syndrome	28
Genetic Forms of Down Syndrome	30
Causes of Chromosomal Aberrations	38
Clinical Features in Down syndrome	39
Prenatal Testing for Down Syndrome	42
Prenatal Screening	42
Prenatal Diagnosis	45
Invasive Prenatal Diagnosis	46
Non-Invasive Prenatal Diagnosis	50
Ethical Aspects of Prenatal Diagnosis	53
Folate Metabolism and Down Syndrome	55
Biochemical Profile of Down Syndrome	59
Superoxide Dismutase (SOD)	60
Cystathionine $\beta$ -Synthase (CBS)	61
Polymerase Chain Reaction	63
Real-Time Quantitative Polymerase Chain Reaction	67
Denaturing High Performance Liquid Chromatography	71
 <b>III</b> <b>METHODOLOGY</b>	74
Foetal Cell and DNA Detection in Maternal Blood	74
Study Subjects	74
Foetal Hemoglobin Staining	75
Microscopy and Cell Scraping	75

	Genomic DNA Extraction	75
	Polymerase Chain Reaction (PCR)	76
	Prenatal Chromosome Analysis	77
	Study Subjects	77
	Amniotic Fluid Cell Culture	77
	Changing of Medium	78
	Harvesting	78
	Slide Making	79
	Staining and Banding	80
	Karyotyping	80
	Non-Invasive Prenatal DNA Screening Test of Down Syndrome	80
	Study Subjects	80
	Preparation of Samples	81
	DNA Extraction	82
	TaqMan Real-Time Quantitative PCR	82
	Cystathionine $\beta$ -Synthase Gene Mutation Screening	86
	Study Subjects	86
	DNA Extraction	86
	PCR Primer Design	86
	Annealing Temperature Optimisation	87
	Touchdown PCR	87
	Sequence Analysis	89
	Denaturing High Performance Liquid Chromatography (DHPLC) Analysis	89
IV	RESULTS	91
	Foetal Cell and DNA Detection in Maternal Blood	91
	Foetal Haemoglobin Staining and PCR of Foetal Cell	91
	PCR of Foetal DNA	92
	Prenatal Chromosome Analysis	95
	Non-Invasive Prenatal DNA Screening Test of Down Syndrome	103
	Real-Time Relative Quantitative Polymerase Chain Reaction	103
	Real-Time Absolute Quantitative Polymerase Chain Reaction	106
	Cystathionine $\beta$ -Synthase Gene Mutation Screening	110
V	DISCUSSION	117
	Foetal Cell and DNA Detection in Maternal Blood	119
	Prenatal Chromosome Analysis	124
	Non-Invasive Prenatal DNA Screening Test of Down Syndrome	130
	Cystathionine $\beta$ -Synthase Gene Mutation Screening	136
VI	CONCLUSION	147
	Suggestions for Future Studies	149
	BIBLIOGRAPHY	151

<b>APPENDICES</b>	<b>172</b>
<b>A</b> <b>Ethical Approval Letter</b>	<b>173</b>
<b>B</b> <b>Consent Form for Prenatal Diagnosis Study</b>	<b>174</b>
<b>C</b> <b>Questionnaire for Prenatal Diagnosis Study</b>	<b>177</b>
<b>D</b> <b>Consent Form for CBS Mutation Screening Study</b>	<b>178</b>
<b>E</b> <b>QIAamp Blood and Body Fluid Spin Protocol</b>	<b>181</b>
<b>F</b> <b>Foetal Haemoglobin Staining Kit Protocol</b>	<b>182</b>
<b>G</b> <b>QIAquick PCR Purification Kit Protocol</b>	<b>183</b>
<b>H</b> <b>STR Gene Sequence</b>	<b>184</b>
<b>I</b> <b>SOD1 Gene Sequence</b>	<b>185</b>
<b>J</b> <b>Purine-Purine &amp; Pyrimidine-Pyrimidine Pairing</b>	<b>186</b>
 <b>BIODATA OF THE AUTHOR</b>	 <b>187</b>

**LIST OF TABLES**

<b>Table</b>		<b>Page</b>
1	Frequency of Down syndrome per maternal age (From Chromosomal Abnormality Rates at Amniocentesis and in Live-Born Infants. <i>JAMA</i> 249:2034-2038, 1983)	43
2	Dilutions for standard curve	85
3	Primer sequences and conditions	88
4	Foetal cell quantitation by HbF staining	91
5	Average C <sub>T</sub> value for SOD1 and GAPDH at different input amounts	104
6	Quantitative analysis of SOD1 sequence in blood using TaqMan assay	109

## LIST OF FIGURES

Figure		Page
1	Trisomy 21 male karyotype (From <a href="http://worms.zoology.wisc.edu/zooweb/Phelps/ZWK99024k.jpeg">http://worms.zoology.wisc.edu/zooweb/Phelps/ZWK99024k.jpeg</a> )	31
2	The origin of trisomy 21 is non-disjunction of chromosomes 21 either at meiosis I or meiosis II (From Molecular Genetics for the Clinician, Brock, DJH, 1993)	32
3	Possible types of foetus resulting from the fertilisation of a gamete with 14/21 translocation chromosome by a normal gamete (From Heredity and Disease, Porter, IH., 1968)	35
4	The production of chromosomal mosaicism by mitotic non-disjunction during cleavage of a normal zygote (From Heredity and Disease, Porter, IH., 1968)	35
5	A schematic representation of chromosome 21 showing the duplicated region leading to the classic Down syndrome phenotype and an additional region, which may be responsible for more variable features (From Molecular Genetics for the Clinicians, Brock, DJH, 1993)	36
6	Down syndrome phenotype. (a) Facies (b) Single palmar crease in affected foetus (From Essential Medical Genetics, Michael, C and Ferguson-Smith, M, 1997)	40
7	Methylenetetrahydrofolate reductase activity in the folate pathway (From Homocysteine, Folate Enzymes and Neural Tube Defects, <i>Haematologica</i> 84: 53-56, 1999)	59
8	Metabolic functions of folate pathways. Two genes (CBS and SOD) on chromosome 21 that are overexpressed in individuals with Down syndrome are shown in circles. Arrows indicate direct and indirect alterations in metabolites, induced by CBS overexpression in individuals with Down syndrome (From Homocysteine Metabolism in Children with Down Syndrome: <i>In Vitro</i> Modulation, <i>Am. J. Hum. Genet</i> 69: 89-95, 2001)	63
9	Polymerase Chain Reaction(PCR). Primers are in green colour (From <a href="http://www.web-books.com/MoBio/Free/Ch9E.htm">http://www.web-books.com/MoBio/Free/Ch9E.htm</a> )	65

- 10 Fluorogenic 5' nuclease chemistry. (1) Forward and reverse primers are extended with *Taq* polymerase as in a traditional PCR reaction. A probe with two fluorescent dyes attached anneals to the gene sequence between the two primers. (2) As the polymerase extends the primer, the probe is displaced. (3) An inherent nuclease activity in the polymerase cleaves the reporter dye from the probe. (4) After release of the reporter dye from the quencher, a fluorescent signal is generated (From <http://www.abrf.org/JBT/1999/March99/mar99grove.html>) 69
- 11 Heteroduplex formation and identifying heterozygosity. (A) Wild Type and mutant PCR products are heated to denature each strand, then allowed to cool slowly. The result is a mixed population of the original homoduplexes, plus heteroduplexes containing the missed match bases. (B) PCR product from a heterozygote naturally forms heteroduplexes because of the sequence variation of each allele. This heterozygosity is easily identified by DHPLC when analysed under partially temperatures (From <http://gatc.arl.arizona.edu/services/fragment%20analysis/whatisdhplc.html>) 72
- 12 Foetal haemoglobin staining of maternal blood smear. Arrow indicates the stained foetal cell while maternal cells appear as 'ghost' cells; (oil immersion, 1000X) 92
- 13 Polymerase chain reaction amplification of Y specific region of foetal cells scraped from foetal haemoglobin stained slides. M = 50 bp marker; 1-3 = foetal cells from women with male foetus at 38, 38, 39 weeks gestation, respectively; 4 = male positive control 92
- 14 Annealing temperature optimization of polymerase chain reaction amplification of *SRY* gene from cord blood, maternal blood and amniotic fluid of a male pregnancy. M = 50 bp DNA marker; 1-7 = DNA from cord blood (lane 1: 54°C, 2: 54.8°C, 3: 56.3°C, 4: 57.1°C, 5: 58.0°C, 6: 59.3°C, 7: 59.9°C); 8-14 = DNA from maternal blood (lane 8: 54°C, 9: 54.8°C, 10: 56.3°C, 11: 57.1°C, 12: 58.0°C, 13: 59.3°C, 14: 59.9°C); 15-21 = DNA from amniotic fluid (lane 15: 54°C, 16: 54.8°C, 17: 56.3°C, 18: 57.1°C, 19: 58.0°C, 20: 59.3°C, 21: 59.9°C). Black arrow shows the amplification of *SRY* gene in maternal blood at 58°C 93
- 15 Polymerase chain reaction amplification of *SRY* gene from maternal blood taken from the forearm. 1 = male positive control; 2 = female positive control; 3-9 = DNA from pregnant women with male foetus at 11, 20, 20, 38, 38, 26, 39 weeks gestation, respectively; 10-12 = DNA from pregnant women with female foetus at 12, 18, 26 weeks of gestation, respectively;

	13 = non template control; M = 50 bp DNA marker	94
16	<p>Polymerase chain reaction amplification of SRY gene from maternal blood taken from the fingertip.</p> <p>1 = male positive control; 2-6 = DNA from pregnant women with male foetus at 6, 7, 8, 9, 10 weeks gestation, respectively; M = 50 bp DNA marker; 8-12 = DNA from pregnant women with female foetus at 6-10 weeks gestation, respectively; 13 = non template control</p>	95
17	A culture amniotic fluid: Arrow indicates a cell attached to the flask; (400X)	96
18	Cell contact and migration: (A) Cells extend protrusions ( ← ); (400X) (B) Adhesion of a cell to matrix contacts; (400X)	96
19	Cells differentiating after attachment; (200X)	97
20	Amniotic fluid cells (A) Epithelial cells: White arrow indicates a columnar epithelial cell, while the black arrow indicates a cuboidal epithelial cell; (200X) (B) Amniocytes; (400X)	97
21	A colony of cells in the amniotic fluid culture; (400X)	98
22	A confluent primary colony which is ready to be harvested; (200X)	99
23	Mitotic cells floating after colcemid treatment; (200X)	99
24	Cell detachment after trypsin treatment; (200X)	100
25	Metaphase spread prepared using potassium chloride hypotonic solution; (200X)	101
26	Metaphase spread prepared using sodium citrate hypotonic solution: (A) 400X magnification (B) Oil immersion, 1000X magnification	101
27	Metaphase resulted from yeast contaminated culture; (oil immersion, 1000X)	102
28	Karyotype of a male foetus metaphase	103
29	The standard curves for the amplification of the SOD1 and GAPDH targets detected using FAM and JOE labelled probe, respectively	104
30	Validation curve for $\Delta C_T$ of SOD1 and GAPDH versus the log of total input DNA	105

- 31 Amplification plot fluorescence intensity ( $R_N$ ) versus time (cycle number) based on real-time measurements of fluorescence intensity ( $R_N$ ) due to free fluorescence reporter molecules 107
- 32 The samples shown in Figure 31 are graphed here as a logarithmic plot  $\Delta R_N$  versus cycle number. The horizontal red line represents the target fluorescence threshold. This graph clearly illustrates the exponential growth of the amplicon during geometric phase of the PCR 107
- 33 Standard curve of SOD1 gene. Blue dots indicate the standards, red dots are samples. The dilution series was made from  $10^4 - 10^1$  107
- 34 Levels of SOD1 sequences as measured by real-time quantitative PCR in pregnant and non-pregnant individuals. *Horizontal bars, Means* 109
- 35 Sequence profile of exon 1-intron 1, CBS gene. (A) Normal CBS gene sequence (B) Down syndrome patient having a mutation at the 9231 nucleotide (C) Magnification of nucleotide at position 9231 of CBS gene in normal gene sequence individual (D) Magnification of the nucleotide at position 9231 of CBS gene in Down syndrome patient. Rectangular box indicates nucleotide at position 9231 112
- 36 Identification of a novel mutation, 9231 A>C, on the sense strand of intron 1 of the CBS gene. Denaturing high performance liquid chromatography profile of the PCR product using denaturing conditions of 64.5 °C from the (A) Normal CBS gene sequence individuals and (B) Down syndrome patients and their mothers with the polymorphism in the intron 1 112
- 37 Sequence profile of Exon 10, CBS gene. (A) A mother with a Down syndrome child having 2 different nucleotide (C and T) at position 20628 of CBS gene (B) Transition of C to T in a Down syndrome patient at the same position (C) Magnification of (A), the nucleotide at position 20628 of CBS gene (D) Magnification of (B), the nucleotide at position 20628 of CBS gene. Rectangular box indicates nucleotide at position 20628 114
- 38 DHPLC chromatogram of wild type, heterozygous and mutant genotypes of exon 10 114
- 39 Sequence profile of Exon 17, CBS gene. (A) A mother with a Down syndrome child having 2 different nucleotides (C and T) at position 27796 and 27817 of CBS gene (B) Transition of T27796C and C27817T of CBS gene in a Down syndrome patient (C)

- Magnification of (A), nucleotides at position 27796 and 27817 in the mother with a Down syndrome child (D) Magnification of (B), the nucleotides at position 27796 and 27817 in Down syndrome patient. Rectangular box indicates nucleotide at position 27796 and 27817 116
- 40 The SRY gene sequence amplified to determine the presence of the foetal cell and DNA in the maternal blood 184
- 41 The SOD1 gene sequence used for the non-invasive prenatal DNA screening test 185
- 42 Purine-purine and pyrimidine-pyrimidine base pairing with a water molecule (From <http://www.bi.umist.ac/users/mjfasjw/2MMB/mutants/diagrams.asp>) 186

## LIST OF ABBREVIATIONS

AF	Amniotic fluid cell
AFP	Alphafetoprotein
bp	Base pair
CBS	Cystathionine beta-synthase
C <sub>T</sub>	Threshold cycle
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanine triphosphate
DHPLC	Denaturing high performance liquid chromatography
DNA	Deoxyribonucleic acid
dUTP	Deoxyuridine triphosphate
E-type	Epitheloid cell
F-type	Fibroblast like cell
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
HbF	Foetal haemoglobin
hCG	Human chorionic gonadotropin
ME-THF	5-methyltetrahydrofolate
MgCl <sub>2</sub>	Magnesium chloride
MTHFR	5,10-methylenetetrahydrofolate reductase
MTRR	Methionine synthase reductase

<b>NaCl</b>	<b>Sodium chloride</b>
<b>NF</b>	<b>Nuclear factor</b>
<b>PCR</b>	<b>Polymerase chain reaction</b>
<b>R<sub>N</sub></b>	<b>Fluorescence intensity</b>
<b>SAM</b>	<b>S-adenosylmethionine</b>
<b>SNPs</b>	<b>Single nucleotide polymorphisms</b>
<b>SOD1</b>	<b>Superoxide dismutase 1</b>
<b>SRY</b>	<b>Specific Region of Y</b>
<b>UE<sub>3</sub></b>	<b>Unconjugated estriol</b>

## **CHAPTER I**

### **INTRODUCTION**

Chromosomal abnormalities are the most frequent genetic disorders seen in both live born babies and miscarriages. Down syndrome is a chromosomal abnormality, which manifests itself in a set of common physical and mental characteristics. This abnormality is due to the presence of an extra chromosome (chromosome 21). Hence, the scientific name, trisomy 21.

Most people are aware that the chance of having a baby with Down syndrome is greater in older women. But Down syndrome can occur at any maternal age. In fact, 75-80% of babies with Down syndrome are born in younger women simply because that age group has more babies (Benke et al., 1995). Studies have proven that inadequate folate status at the time of conception increases the risk of Down syndrome. Women with genetic mutations which interfere with the body's ability to absorb folic acid, are at higher risk for having children with Down syndrome (James et al., 1999).

Down syndrome is a major reason for prenatal diagnosis. Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn foetus. Such diagnosis is usually performed by means of karyotyping and depends on analysis at 11-18 weeks of gestation. Karyotyping is not always possible, especially when the number of cells obtained is limited, where cell culture fails (in 1-2% of patients), or when the