

## **PRENATAL GENETIC SCREENING AND TESTING**

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### **Preamble**

Genetic Screening is a highly contentious subject as it evokes different emotions and sensitivities. This broad arena includes Prenatal Screening and Testing for which there is currently no uniform approach in Singapore. This paper will attempt to objectively discuss the merits of establishing a standard protocol, bearing in mind the concerns of conscientious objectors and different interest groups even within the medical profession. It will be limited to the field of Prenatal Screening and Testing, as I cannot profess to be an expert in other areas.

### ***Rationale for Genetic Screening***

Genetic disorders like Haemophilia and Huntington's disease exist in every population. In our population the prevalence of Thalassaemia remains an issue. But over the past decade, an additional problem has arisen. The increasing maternal age of conception has led to an increased risk of having chromosomally abnormal conceptions, commonly Down syndrome. As such, universal screening should be practiced. Identified "at-risk" individuals can then be counselled and offered Prenatal Testing to determine if their progeny is affected. The prevalence of genetic disorders within the population can serve as a guide to decide what types of screening should be made available to the people.

### ***Goal of Pregnancy Screening***

In the case of Pregnancy Screening for Birth Defects, the goal is to ensure that parents are able to determine if they will have a normal, healthy baby. If test results indicate defects, such information early in the pregnancy allows parents the time to receive adequate counselling regarding the expected prognosis of the conditions. This will then facilitate the making of informed and deliberate choices, rather than hasty and emotive decisions. For the vast majority of women, however, screening will produce negative results. Negative results achieve the other objective, which is to give parents reassurance and peace of mind for the rest of the pregnancy.

### ***Rationale for Prenatal Testing and Diagnosis***

After undergoing universal screening and prenatal testing, at-risk individuals should be offered adequate counselling. For more specific problems (e.g. Haemophilia A or B), prenatal testing should be available to determine if the progeny is affected.

Prenatal diagnosis should be performed to give parents and physicians information about the health of the foetus. Use of prenatal diagnosis is not readily acceptable for paternity testing, except in cases of rape or incest, or for gender selection, apart from sex-linked disorders. The woman should be an important decision maker in all matters related to reproduction. There needs to be a clear understanding that testing is purely voluntary and there should be no coercion.

### **Pregnancy Screening**

Universal screening should be made available to all patients. All patients should be given adequate information to choose for themselves whether or not they want to receive screening during pregnancy. Screening should strictly occur on a voluntary basis.

### ***Pre-requisites***

**Public education** and **pre-test counselling** should be required before a screening test is offered. Every test should be offered in a manner in which individuals and families can freely refuse or accept according to their wishes and moral beliefs. Religious leaders and community leaders should be given regular updates on the most current pregnancy screening tests so that they can advise their wards about the acceptable mores. **Pre-test counselling** makes **post-test counselling** for patients with positive screen results (and eventually an affected foetus) much less difficult because prospective parents are better prepared. Pre-test counselling should include the general characteristics of the major disorders that the test may identify. The characteristics of the disorder(s) should also be described in terms of effects on the future child, on the parents and on family life.

Pre-test counselling should emphasise that most conditions diagnosed in the foetus cannot be treated before birth and that knowing about the condition may not help the foetus. The information also does not guarantee a healthy baby, as there are other conditions that may not be identified before birth.

### ***Synopsis of Available Methods***

Different modes of screening exist and may include history taking, using maternal age at delivery or maternal serum biochemistry with or without the use of ultrasound. With the advent of new diagnostic technologies, it becomes possible to look at screening service delivery in a different way, which may result in reduced family anxiety, more informed choice and a more efficient use of the healthcare professionals' time.

## Thalassaemia Screening

### *Understanding Thalassaemia*

There are two forms of Thalassaemia commonly found within our local population:  $\alpha$  and  $\beta$  Thalassaemia.

a **Thalassaemia** is mainly a gene deletion problem. The deletion of four alleles by the combination of two *cis* carriers would lead to a severely affected and lethal state of Bart's Hydrops. This condition also increases the risk of hypertensive disorder in the mother during pregnancy, putting her life in jeopardy. Hence prenatal diagnosis potentially prevents increased morbidity and possibility of mortality not only for the foetus but also for the mother.

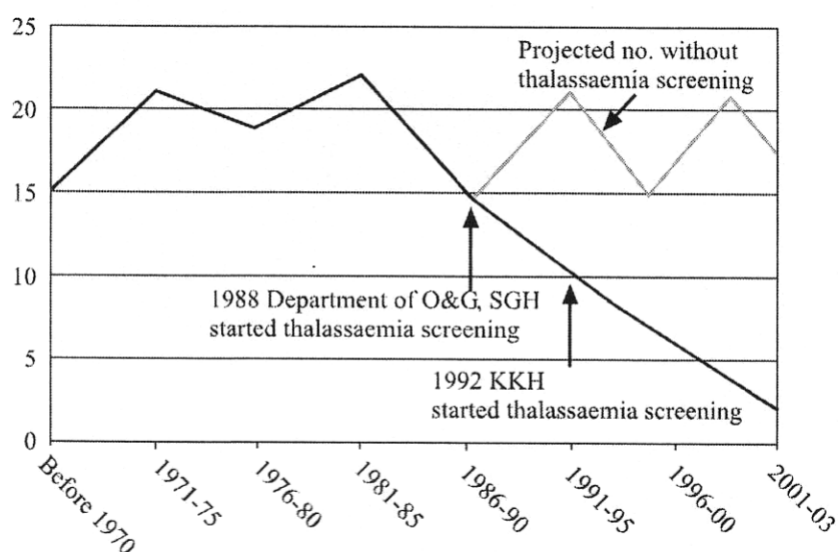
b **Thalassaemia** is a multifactorial problem and involves many different genetic permutations. Prenatal diagnosis is thus more difficult and should be performed when one can identify the specific marker for that couple. A  $\beta$  Thalassaemia major is a severely handicapped person requiring regular blood transfusions and the continuous use of iron chelating agents to maintain some semblance of a decent quality of life.

### *Testing for Thalassaemia*

The gene prevalence of Thalassaemia in our local population is approximately 3%. The carrier state is compatible with good quality life. It is impossible to distinguish a carrier from a normal person without performing targeted blood tests. A simple blood test should be performed on both the male and female partner. In the screening phase, testing involves only a Full Blood Count or more specifically, the Hb level and the MCV of the red corpuscle. Should the Hb be below 10g/dl and the MCV be below 80 fl, a Hb Electrophoresis should be sent and genetic testing can then be performed if needed. [GSH Yeo, KH Tan, TC Liu. The Role of Discriminant Functions in Screening for Beta-Thalassaemia Traits During Pregnancy. Singapore Med. J. 1995 Dec; 36(6):615-8.]

Since the initiation of routine Thalassaemia screening, the number of  $\beta$  Thalassaemia major births in Singapore has fallen dramatically from an average of 15 to 20 cases a year to that of one case per year. See graph of Number of Beta-Thalassaemia Major Births in Singapore 1997–2003.

### Number of $\beta$ Thalassaemia major births in Singapore, 1997-2003<sup>1</sup>



The **Thalassaemia Registry** is a rich resource of data because many family trees have been mapped and their specific genetic defects/deletion identified. Those with affected relatives can easily avail themselves of the resources available when the need arises.

## Down Screening

### *Understanding Down Syndrome*

Down syndrome is the most common chromosomal abnormality syndrome in humans. Humans were built with two sex chromosomes and two copies of each of the other 22 chromosomes. Trisomies occur when there are three copies of a chromosome. These children usually have decreased intelligence, but increased unselfconsciousness, openness and affection. Children with Down syndrome face many medical and development problems. Early intervention programmes can be very helpful in helping children with Down syndrome to develop to their full potential and thus be less of a social burden to their immediate families and society.

The extra chromosome may be derived from either the mother or father. Non disjunction is found in 95% of Down syndrome, the other 5% are caused by Translocation, Mosaicism or partial trisomy. The risk for many chromosomal defects increases with maternal age. Additionally, because foetuses with chromosomal defects are more likely to die in *utero* than normal foetuses, the risk decreases with gestation.

<sup>1</sup> Recent Developments in Obstetric Care and Maternal Foetal Medicine in Singapore, Annals Academy of Medicine, November 2004, Vol.33 No.6

### *Testing for Down Syndrome*

Screening tests are used to look for potentially at risk pregnancies with the aim of performing a diagnostic test to confirm if the pregnancy is really affected. There are many modes of screening as the following table shows:

Screening Test	DR (%)	FPR (%)
MA	30 (or 50)	5 (or 15)
MA + foetal NT at 11-14 weeks	75 (or 70)	5 (or 2)
MA + foetal NT and serum $\beta$ -hCG and PAPP-A at 11 – 14 weeks	90 (or 80)	5 (or 2)
MA + foetal NT and NB and serum $\beta$ -hCG and PAPP-A at 11 – 14 weeks	97 (or 90%)	5 (or 2)
MA + serum biochemistry at 15-18 weeks	60-70	5
Ultrasound for markers at 16 – 23 weeks	75	10 – 15

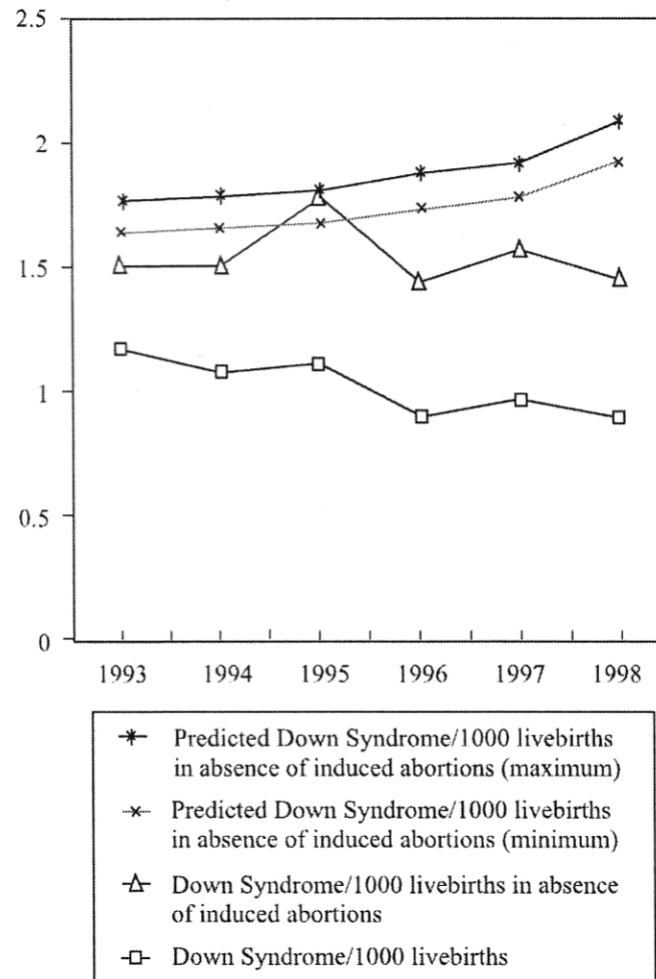
$\beta$ -hCG, beta human chorionic gonadotrophin; DR, detection rate; FPR, false positive rate; MA, maternal age, NB, nasal bone; NT, nuchal translucency; PAPP-A, pregnancy associated plasma protein A. (Ultrasound Obstet Gynecol 2003; 21:313-321)

In Singapore, First Trimester Screening using the MA, foetal NT and serum  $\beta$ -hCG and PAPP-A at 11 to 14 weeks was started in August 2003. Until Dec 31 2004, 1859 women had been screened and total of four anomalies had been detected, two cases of Trisomy 21, one of Trisomy 13 and one of Trisomy 18. Two of these cases were to mothers aged 28 and 29, respectively, who would have not been offered testing based on maternal age.

The test quickly gained recognition in the second half of 2004 when KK Women's and Children's Hospital and other centres started offering it to its patient population. With this test in place, the trend of increased public awareness of pregnancy screening and its benefits is set to improve.

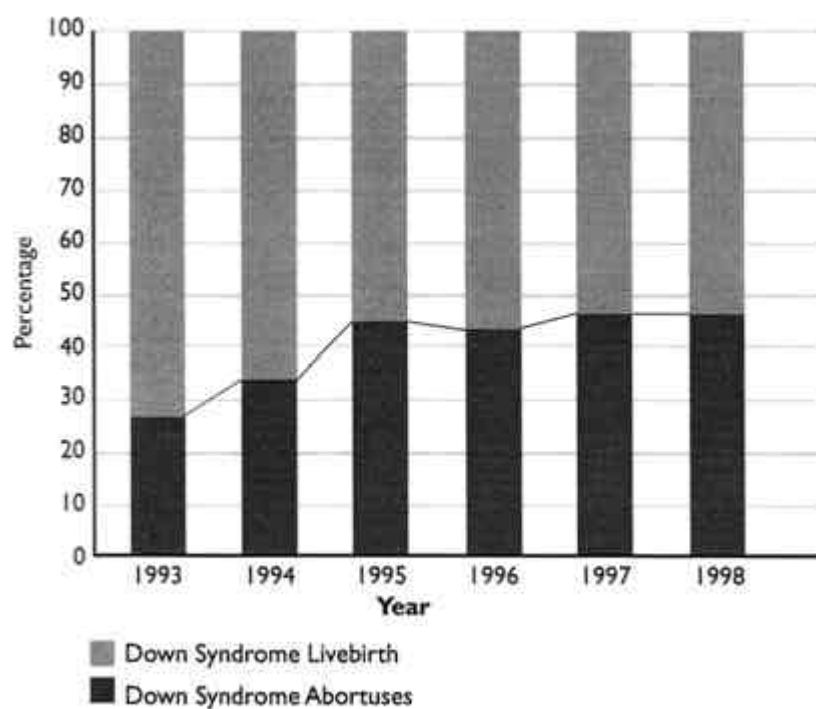
**Down screening has contributed definitively to the reduction of Down syndrome live births over the years**

**Down syndrome per 1000 livebirths in Singapore from 1993 to 1998<sup>2</sup>**



<sup>2</sup> Recent Developments in Obstetric Care and Maternal Foetal Medicine in Singapore, Annals Academy of Medicine, November 2004, Vol.33 No.6

### Trend of Down Syndrome Livebirths vs Abortions 1993-1998<sup>3</sup>



### Down syndrome livebirths, stillbirths and abortions, and actual and expected Down syndrome livebirth rates, Singapore 1993 to 1998<sup>4</sup>

	1993	1994	1995	Year 1996	1997	1998	Total (93-98)
Down Syndrome Livebirth	59	53	54	44	46	39	295
Down Syndrome Stillbirth	2	1	0	0	0	1	4
Down Syndrome Abortuses	22	28	43	34	38	32	197
Total	83	82	97	78	84	72	496
Total Livebirths I	50225	49554	48635	48577	47333	43664	287988
Down Syndrome rate /1000 livebirths	1.17	1.07	1.11	0.91	0.97	0.89	1.02
Down Syndrome to livebirths ratio	1:851	1:935	1:901	1:1104	1:1029	1:1120	1:976
Expected Down Syndrome Livebirths in the absence of induced abortions	76	75	87	70	75	64	447
Expected Down Syndrome rate/1000 livebirths in absence of induced abortions	1.51	1.50	1.79	1.44	1.59	1.46	1.55
Expected Down Syndrome to livebirths ratio in absence of induced abortions	1:661	1:665	1:558	1:692	1:629	1:686	1:645

I Report on Registration of Births and Deaths 1998, Singapore Immigration and Registration

<sup>3</sup> Birth Prevalence of Down Syndrome in Singapore from 1993 to 1998, Singapore Med J, 2002 Vol 43(2): 070-076

<sup>4</sup> *Ibid.*

## Screening for Other Chromosomal Disorders

Lethal chromosomal abnormalities (e.g. Trisomy 13, Trisomy 18 and Triploidy) can be detected through screening methods similar to those of Down syndrome. Less lethal forms of XO, XXY, XXX, XYY etc can only be predetermined through karyotype as these do not present any physical abnormalities in general. The significance of detecting these prior to birth are again for the parents to be fully counselled as to the long term prognosis of their children, as early intervention in childhood may be required for some anomalies and lethal anomalies should not pose a threat of operative deliveries to the mother.

## Screening for Structural Birth Defects

Structural defects are more likely to be associated with genetic disorders (e.g. 50% of Down syndrome affected foetuses have a cardiac anomaly). In the National Birth Defect Registry, structural anomalies account for a large proportion of the registered anomalies. Cardiac anomalies are the most common at 7 to 8 per 1000 live births, whilst chromosomal abnormalities including Down syndrome account for only 3 per 1000 live births.

Structural anomalies usually necessitate early neonatal and paediatric surgical care to correct or decrease harm to the baby's development. Prenatal diagnosis helps give prospective parents the opportunity to prepare for their child's special needs and to cope with the "well-wishers" around them.

The use of ultrasound equipment provides a clear view of the state of the developing foetus. Anomalies can be detected as early as the 11th week of pregnancy. Certain structural defects are incompatible with life (e.g. anencephaly, Barts' hydrops and hypoplastic left heart syndrome). Early detection again allows the relevant paediatric surgeons to pre-empt the birth and decide on the mode of operations that may be required. Conjoined twins can be detected from the first trimester by the presence of a single yolk sac with two foetal poles.

Ultrasound for genetic markers can be performed as part of a genetic sonogram. The chance of a chromosomally affected child is detected on the presence or absence of these markers. Likelihood ratios have been derived for the presence of many different individual markers. However, these are rarely used in the practice by regular obstetricians. A reduction of the age related risk of Down syndrome is reduced by 50% should the genetic sonogram prove negative for any marker.

Ultrasound screening for foetal anomalies is currently performed by varying practitioners (e.g. sonographers, general practitioners and the majority of obstetricians and gynaecologists). A genetic sonogram is only as good as the person who performs the scan and the equipments used.



## Screening for Genetic Disorders

If there is a known family history of a genetic disorder (e.g. Huntington's disease, Haemophilia) then screening the mother for the risk of having an affected progeny should be discussed with her and the partner. In certain types of diseases in which there is no treatment (e.g. Huntington's disease), some people have questioned the need to screen for it. Sex-linked diseases (e.g. Haemophilia, Red/Green colour blindness) are potentially preventable if the mother refuses to pass these onto her children. If the mother is determined to be a carrier, prenatal diagnosis for each pregnancy should be offered to her or the possibility of pre-implantation genetic screening can be discussed.

## Genetic Testing

### *What is Genetic Testing?*

**Genetic Test** (as defined by the National Institutes of Health, Department of Energy Working Group on Ethical, Legal and Social Implications of Human Genome Research) is the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease related genotypes, mutations, phenotypes or karyotypes for clinical purposes. Such purposes include predicting risk of disease, identifying carriers and establishing prenatal and clinical diagnosis. Prenatal, newborn and carrier screening, as well as testing high-risk probability thaw and excess or deficiency of the metabolite indicates the presence of heritable mutations in single genes.

It must be emphasised that little can be done to change a pre-existing genetic defect or even to improve the resulting situation. Negative test results may not rule out future occurrence of disease whilst positive tests do not mean that the disease will inevitably develop.

In the realm of prenatal diagnosis, a genetic test involves the harvesting of foetal tissue either through an **amniocentesis, chorionic villous sampling or cord blood sampling** for a full karyotype. Each of these procedures carries a risk of pregnancy loss. As such, one should not assume that all persons of high-risk status or those with positive screen results will immediately undergo such an invasive diagnostic test.

## Diagnostic Procedures

### *Amniocentesis*

**Amniocentesis** is currently the most frequently performed diagnostic procedure in Singapore, as it follows the most common prenatal screening test – the second trimester serum screening test. The second trimester screen is performed at 15 to 20 weeks and gives a 5% positive screen rate to determine 65% of Down syndrome. By the time a

diagnostic test is performed and a full karyotype is obtained, the pregnancy is therefore usually far advanced at 20 weeks gestation. This inevitably leads to great psychological and physical distress, as a decision not to continue with the pregnancy is not only ethically and emotionally difficult but also physically stressful and not without morbidity.

The use of **FISH** (Fluorescent In Situ Hybridisation) or **PCR** (Polymerase Chain Reaction) has led to improvements in this area, as it can determine the presence or absence of a Trisomy within 24 to 48 hours. It gives information only about the chromosome tested. Other karyotypic anomalies cannot be identified without the full karyotype. Thus, these procedures remain as a rapid first step to reduce anxiety prior to receiving full information.

### ***Chorionic Villous Sampling***

**Chorionic villous sampling** is performed at 11 to 14 weeks and readily obtains a large amount of foetal DNA. This procedure is favoured for genetic testing of Thalassaemia and Haemophilia. Technically, it is a more demanding test for the clinician. This test can be offered immediately following a positive screen from the First Trimester serum screen and Nuchal Scan.

As these procedures are invasive and pose a risk of miscarriage, informed consent is imperative. Currently, these procedures can be performed not only for those with a positive screen but can also be performed as an initial investigation. Rigorous training programmes are set out to ensure there are fairly uniform levels of expertise available amongst the practitioners.

### ***Role of Laboratories in Genetic Testing***

Not only are the persons performing these tests should be accredited, the **laboratories** responsible for chromosome culture should also be of the highest quality. Genetic testing should be governed by guidelines and standards set for the laboratories by the Ministry of Health and other international accreditation bodies.

### **Information Available with regards to Prenatal Screening / Testing**

Prenatal Screening is acceptable to the general public as evidenced by its use of currently available methods (e.g. ultrasound and second trimester screening for Down syndrome). Obstetricians, clinics and some family physicians offer prenatal screening.

There are no predetermined schedules for offering these screening tests and they are offered at the discretion of the doctor concerned. The quality of the available pre-test and post-test counselling has been improving by continuing medical education of medical staff in general.

Foetal Maternal Medicine specialists in Singapore have been the driving force in improving public knowledge with regards to prenatal screening and diagnosis.

In Singapore, a **National Birth Defect Registry** was set up for the purpose of maintaining a registry of all the various birth defects so that epidemiological data can be easily obtained and studies can be performed to determine local patterns to facilitate prenatal screening. The data is collated from abortions performed for abnormalities, from miscarriages/stillbirths registry and from perinatal units.

### **Accuracy of Screening Tests**

The traditional method of prenatally screening based on maternal age at delivery yields the lowest detection rate of approximately 30%. The current widely available method of second trimester serum testing allows 65% of Down syndrome for a 5% screen positive rate. These are either based on double or triple analyte testing involving serum beta HCG, alpha foetoprotein with or without unconjugated estriols. The ultrasound examination of the nuchal translucency alone detects 75% of Down syndrome cases.

Presently, the most promising method combines maternal age, nuchal translucency and first trimester serum testing to yield a detection rate of 89% for Down screening and 90% of other chromosomal anomalies using alternative algorithms. The analytes include serum free  $\beta$ -hCG and PAPP-A. The addition of the nasal bone imaging has improved the accuracy further with a reduction in false positives.

Screening tests using cervical swabs or foetal blood to isolate foetal cells are still not optimised. One is unable to determine a full karyotype from the few isolated cells. These tests could prove, however, to be THE way to screen each pregnancy because they are non-invasive to the foetus.

### **Medical and Legal Considerations**

Again, a screening test merely identifies a woman at an increased risk for an anomaly, but does not permit a diagnosis. This has to be emphasised to her and her partner, if required, and consent must be taken prior to the test.

No screening test is compulsory and all benefits and potential problems arising after screening should be discussed prior to signing the consent form. Options following a screen test positive must be advised.

### **Ethical Considerations**

More importantly, ethical tenets should be followed in all things pertaining to this realm. These include:

1. Respect for autonomy of choice: respecting the self-determination of individuals and protecting those with diminished autonomy.
2. Beneficence: giving the highest priority to the welfare of persons and maximising their health.
3. Non maleficence: avoiding and preventing harm to persons or, at least, minimising harm.
4. Justice: treating persons with fairness and equity, and distributing the benefits and burdens of health care as fairly as possible in society.

## **Conclusion**

Prenatal screening should be offered universally to all women who desire to know the health status of the child they bear. All women must thus be aware of the available screening tests and the purpose of each test. There must also be clear understanding of the difference between a screening test and a diagnostic test.

Education about genetics for the public and health care professionals is now of paramount importance because genetics is playing an increasing role in medical practice and many people are concerned about the possible abuse of this new knowledge. Geneticists and health care professionals must also learn from the support and advocacy groups representing those with genetic disorders.

Accreditation and self-evaluation should also be performed for those who wish to provide prenatal screening services.