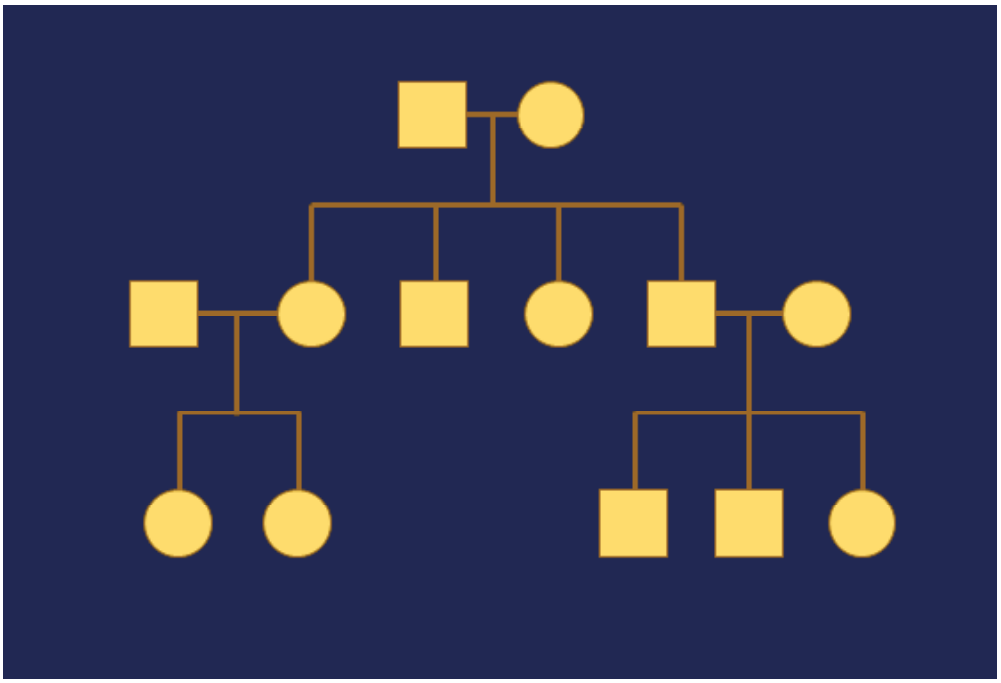
Sri Lanka Medical Association Oration 2008

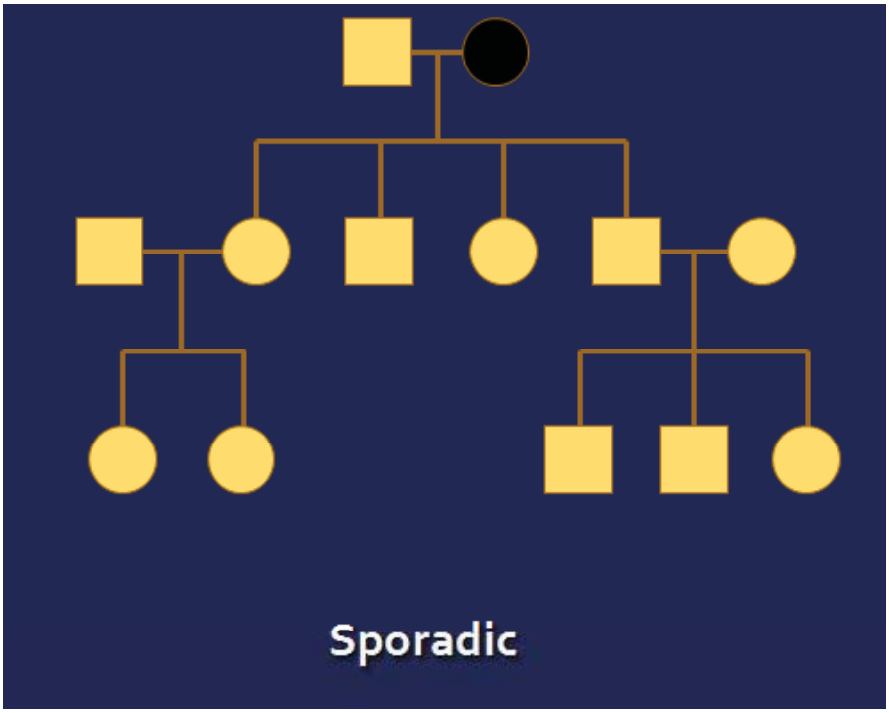
GENETICS OF PRE-ECLAMPSIA AND THE WEIGHT OF BABIES AT BIRTH : CLINICAL AND GENETIC STUDIES IN SRI LANKA

Dr. Vajira H.W. Dissanayake

In 1997, as a raw medical graduate, just out of medical school, I attended my first SLMA oration, in this very hall. Having been associated with the SLMA since then, I know the high expectations placed on the SLMA orator. I shall do my best to live up to those high expectations. Let me introduce you to the subject of my oration today using pedigrees - family trees. When we draw family tress we use squares to depict males, circles to depict females, and lines to link parents to children to grandchildren.

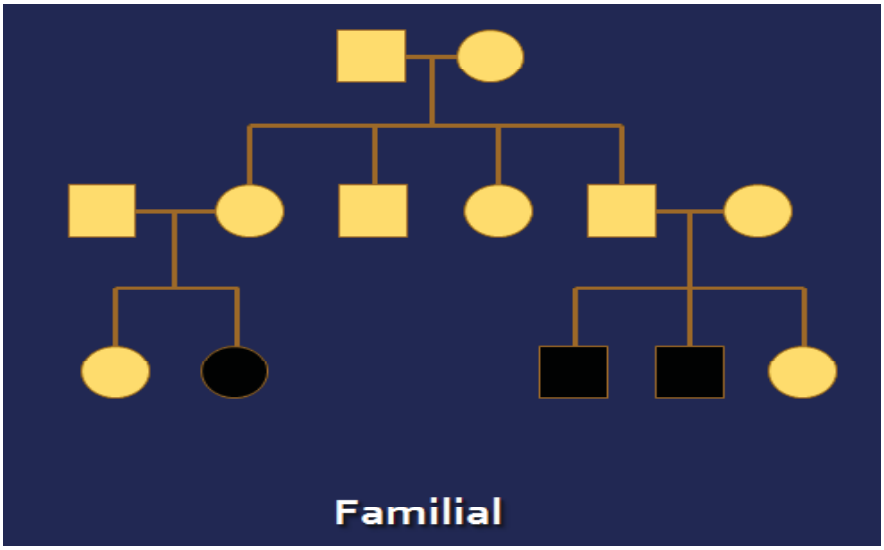


When we draw pedigrees like this, and mark on them individuals who are affected with any disorder, for example lets say diabetes, different patterns start immerging. The commonest pattern is one like this:



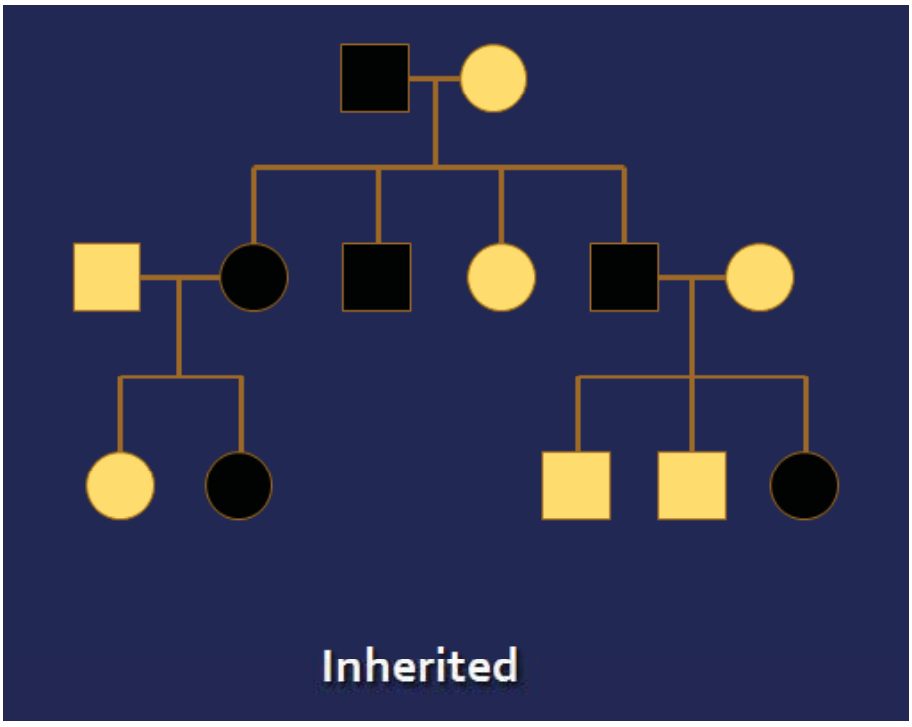
One family member affected with the condition, shown in black, everyone else is normal. We call this the sporadic occurrence of a condition in the family. There is no sign of a 'big' genetic contribution to the development of the condition.

The second pattern that we see is one of several members of the family, in the same generation affected with the condition, usually in old age.

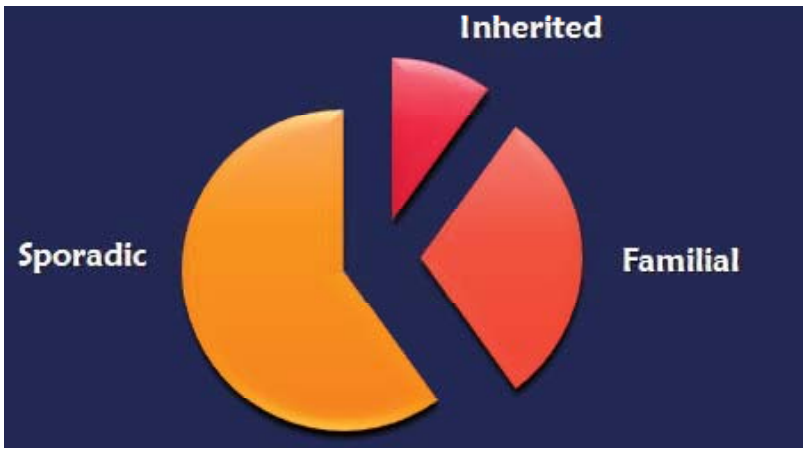


In this case the condition is familial. several relative developing the same condition as they age, a shared environmental factor, junk food, bad habits, such as alcohol and smoking, or even environmental pollution acting on an underlying genetic predisposition to bring about the development of the condition. But still there is no sign of a strong genetic contribution.

The third pattern is the one which excites us, the inherited pattern, showing many first degree relatives in several generations of the family affected with the condition. The condition running in the family according to a known pattern of inheritance, for example, in this case an autosomal dominant pattern.

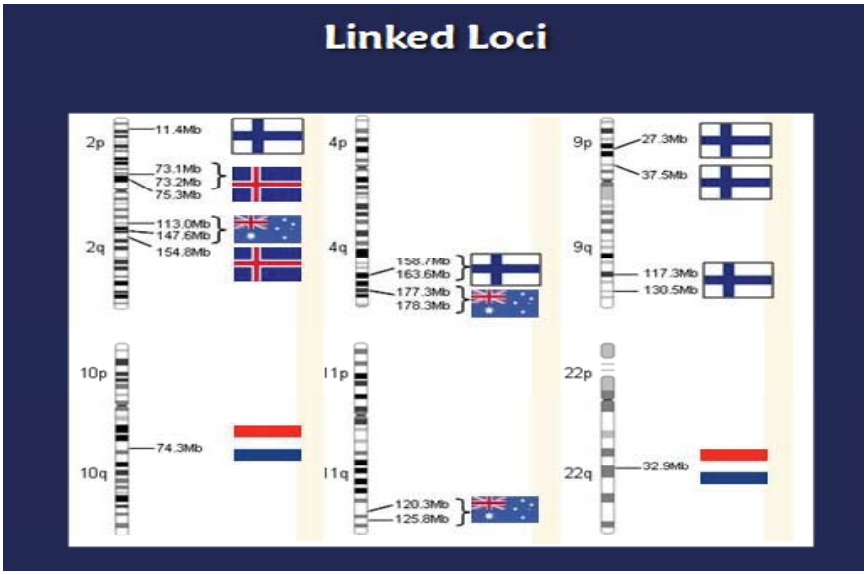


These three patterns, sporadic, familial, and inherited are found when we examine the family tress of families affected with any common complex disorder; diabetes, hypertension, ischaemic heart disease, or cancer to name a few.



In the case of pre-eclampsia it is difficult to demonstrate the inherited pattern in a straight forward manner because males do not get pregnant and therefore they do not develop the condition. The families that exhibit, the inherited pattern of any condition, usually have rare genetic disorders, cause by what we call high penetrant, low frequency, genetic variations, in one gene. In contrast the common complex disorders, found in families exhibiting the sporadic or familial occurrence of the condition, are cause by low penetrant, high frequency genetic variations, in several genes interacting with each other and the environment. As a result the pattern of inheritance of such conditions is not clear. They however leave behind genetic traces, which could be unmasked through epidemiological studies, twin studies, and pedigree analysis using special techniques.

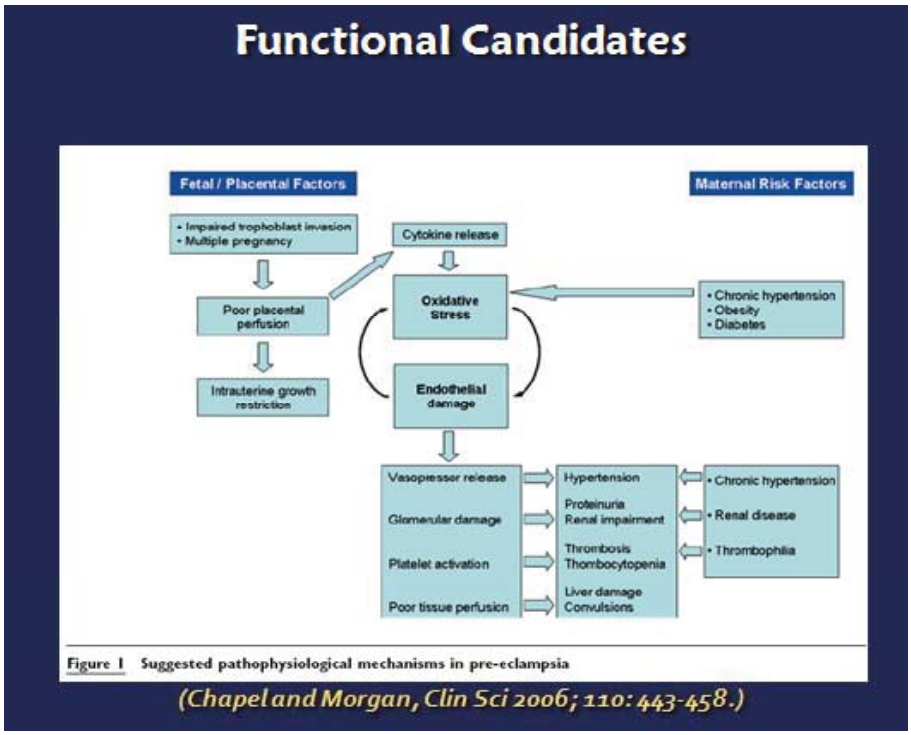
In the case of pre-eclampsia epidemiological studies show that both maternal and paternal genes acting through the fetus may be contribute to the development of the condition, twin studies confirm that and pedigree studies report that the pattern of inheritance of pre-eclampsia is consistent with an autosomal dominant pattern with reduced penetrance, It is in this background, ladies and gentleman that we talk of genetics of pre-eclampsia. Two strategies can be used to identify genes causing pre-eclampsia: Linkage studies and candidate gene disease association studies. Linkage studies involve recruiting families with multiple affected members such as the families affected with pre-eclampsia, shown here, recruited for a study in Iceland, and then identify regions of the genome that are co-inherited along with pre-eclampsia within the family using molecular genetic tests to analyse their DNA and statistical methods to analyse the genetic test results. There have been several genome wide linkage screens. The results of these genome wide linkage screens are summarized in this slide.



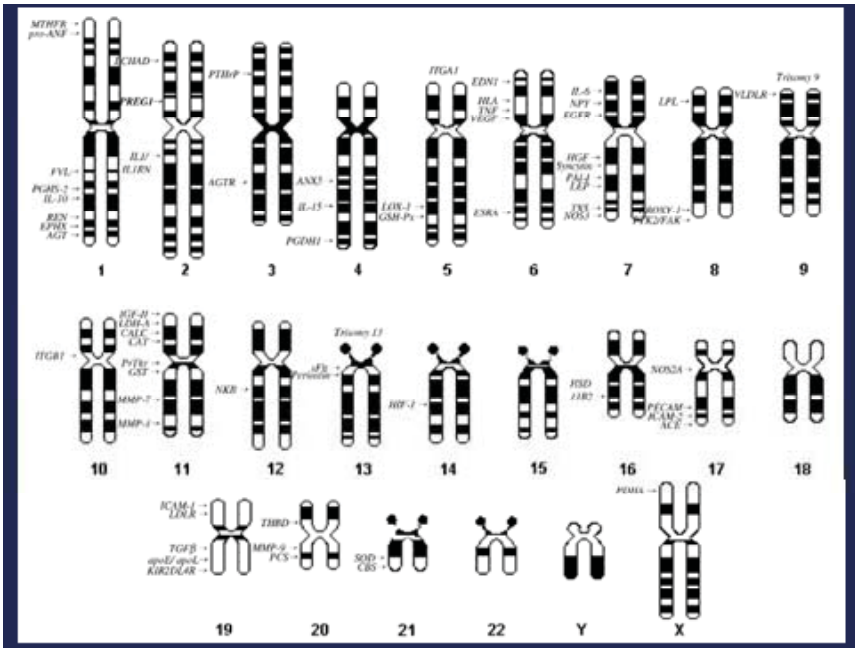
This slide shows the chromosomes and the chromosome locations in which a gene or genes causing pre-eclampsia may be located. You can see here that there is very little overlap between the loci identified in different populations, possibly due to their genetic differences, highlighting the importance of carrying out genetic studies in different parts of the world.

You would appreciate however that it would not be possible to carryout linkage studies in most parts of the world because medical and genological records that are necessary to ascertain such families are not widely available. Candidate gene disease association studies on the other hand can be performed any where. These studies involve prospectively recruiting cases and controls from a population. Then selecting a candidate gene or genes and the genetic markers in or near the candidate gene or genes and determining whether the marker is found more in cases than in controls using molecular genetic tests to analyse their DNA and statistical methods to analyse the genetic test results. This is the approach that we selected for our studies of genetics of pre-eclampsia in Sri Lanka.

You may wander what genetic markers are. Genetic markers are genetic variations. There are various types of genetic markers. The preferred markers for this type of study nowadays are single nucleotide polymorphisms or single base changes in the genome. Next you may wander how candidate genes are selected. There are two ways of doing that First, positional candidates. Positional candidates are genes located in genomic regions identified by genome wide linkage screens that I mentioned earlier. Second Functional candidates. Functional candidates are genes that code for any protein that is involved in the pathophysiological pathway of pre-eclampsia, shown on this slide.



Candidate gene disease association studies have been the popular approach to study genetics of pre-eclampsia. Many candidate genes across the genome have been studied using this approach. They are shown on this slide:



But the down side to these studies has been that the results of most of them have not been reproducible. You may ask why?

The reasons are as follows: First, there are issues relating to the definition of pre-eclampsia, stemming from the fact that blood pressure and urinary protein are variable measurements, and establishing the diagnosis involve passing a pre-defined threshold or cut off on which there has been some controversy in the past. Second, researchers fail to rigorously phenotype cases. Some medical conditions and obstetrics states are known to increase the risk of development of pre-eclampsia. This means that one has to identify the trees from the woods, as it were, by rigorous phenotyping when recruiting cases and controls for these studies. Then there is the possibility of population admixture, i.e. the presence of different in-breading subpopulations within cases and controls. This could happen when careful attention is not paid to determine the ethnicity of recruits and when cases and controls are not matched for ethnicity. Finally, most studies do not have sufficient numbers of cases and controls. This makes such studies under powered to detect any genetic effect. We were mindful of these problems when we began our studies into genetics of pre-eclampsia in Sri Lanka - The Inherited Factors in Pre-eclampsia (IFPE) in Sri Lanka Study.

The diagnosis of pre-eclampsia is defined by the International Society for the Study of Hypertension in pregnancy as de novo development of hypertension, i.e. blood pressure, of 140 over 90mmHg or more on two occasions six hours a part, and proteinuria of 300mg or more on a 24 hour urine collection or in the absence of a 24 hour urine collection 2+ or more on dipstick testing. For research settings a stricter definition of proteinuria of 500mg or more on a 24 hour urine collection has being recommended and used by researchers.

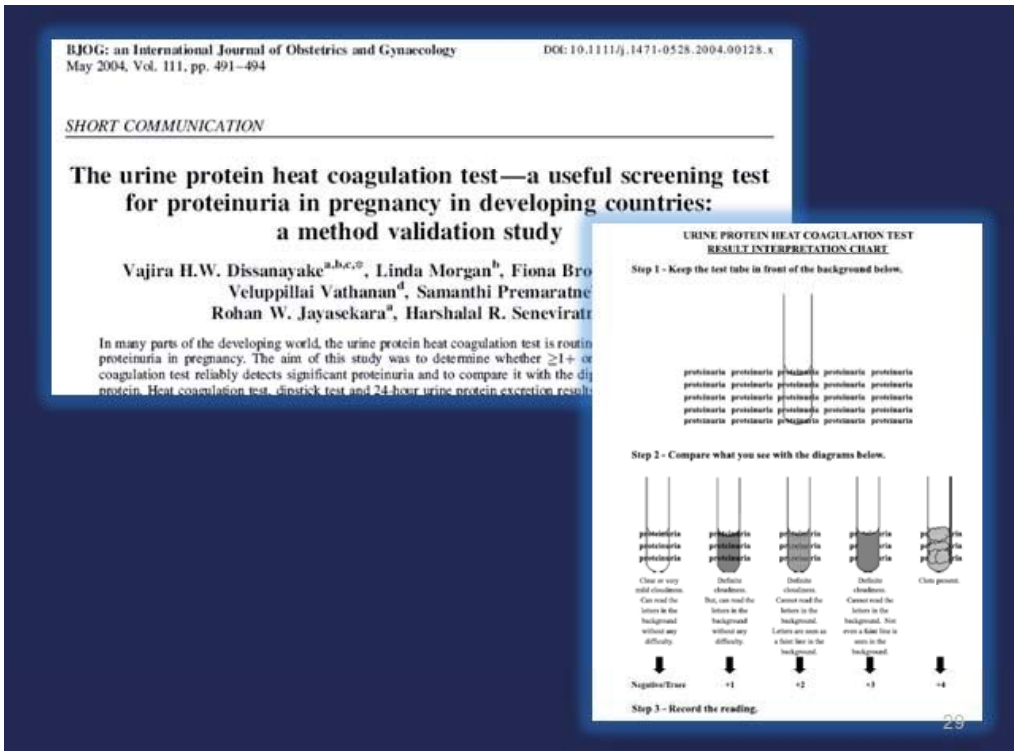
The problem in establishing this diagnosis in Sri Lanka is that we do not perform either of these tests for proteinuria routinely in our wards. What is performed in our wards is the urine protein heat coagulation test. This test, surprisingly, in spite of its widespread use, had not been validated against quantitative methods. Therefore the first step in our investigations was to carry out a study to validate the urine protein heat coagulation test performed according to a standardized protocol as a reliable screening test to screen for proteinuria in pregnancy.

The results of this validation study are summarised in this slide.

	Dipstick		HCT
	≥1+	≥2+	≥1+
Total No Tested	71	71	71
Proteinuria ≥500 mg/day	31	31	31
True Positives	26	20	26
False Negatives	5	11	5
Sensitivity	84%	65%	84%
Proteinuria < 500 mg/day	40	40	40
True Negatives	28	37	35
False Positives	12	3	5
Specificity	70%	93%	88%
Positive Predictive Value	68%	87%	84%
Negative Predictive Value	85%	77%	88%

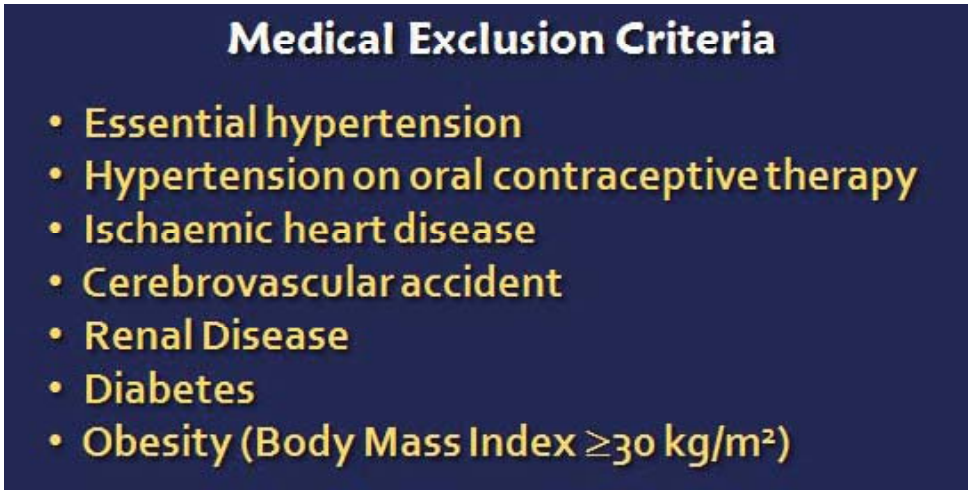
≥1+ HCT ≈ ≥2+ on dipstick ≈ ≥500 mg/day

The conclusion was that a reading of 1+ or more on the heat coagulation test was almost as good as 2+ or more on the dipstick test in predicting proteinuria of 500mg or more on a 24 hour urine protein estimation. These results were presented at the British Meeting of the International Society for the Study of Hypertension in Pregnancy in July 2003 where the paper was placed third in the best oral presentations category and later published in the British Journal of Obstetrics and Gynaecology, now called the BJOG.



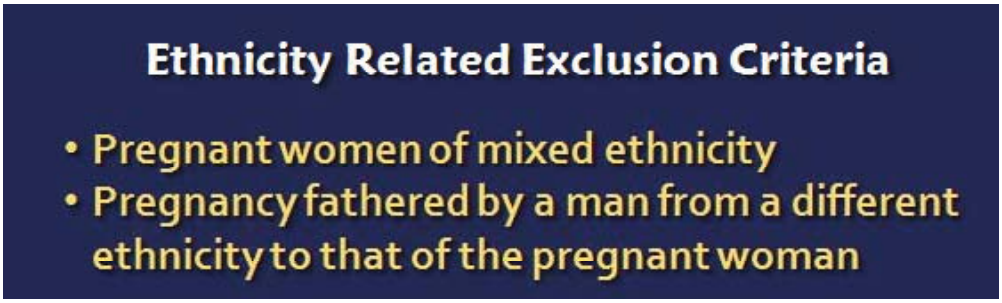
I am pleased to inform you, ladies and gentleman, that this validated standardized urine protein heat coagulation test is now being used in pre-eclampsia studies underway in Africa and in Pacific Islands funded by the Medical Research Council of Canada. Madam President, the SLMA can share the credit for this study, because it was funded by the SLMA-Glaxo Welcome Research Award awarded to me in 2002.

Having found a solution to the main problem that we faced in diagnosing the condition, we turned our attention to phenotyping our recruits rigorously. To do so; we had to ensure that none of our recruits had any of the conditions known to place them at increased risk of developing pre-eclampsia. At the same time we had to minimize the possibility of population admixture among our recruits. The list of general medical exclusion criteria that we applied when recruiting subjects, both cases and controls, are shown on this slide:



This general list was further extended to a list of pregnancy related exclusion criteria that ensured that only women with true pre-eclampsia were recruited.

To reduce the possibility of population admixture we had to ensure that recruits were not of mixed ethnic origin. These ethnicity related exclusion criteria were applied for that purpose



A woman or her husband was considered as belonging to a particular ethnic group when both their parents as well as their maternal and paternal grand parents belonged to the same ethnic group. Resorting to such strict recruitment criteria made recruitment a laboriously slow process. In fact it took nearly one and a half years to recruit the required number of women from the two recruiting hospitals, De Soysa Hospital for Women, and the Castle Street Hospital for Women. At this point, I would like to acknowledge the support I received from the entire obstetrics and gynaecology community in Colombo, for these studies. These studies would not have been possible if not for their extraordinary support. I salute them.

Acknowledgements

For giving access to wards/patients and support:

Dr. R. Almeida
Dr. R. Colonne
Dr. A. Kaluarachchi
Dr. K.D.S. Ranasinghe
Dr. G.A. Ranatunga
Dr. C. Randeniya
Dr. H.M. Senanayake
Dr. L. Senanayake
Prof. Harsha Senevirathne
Dr. L.A.W. Sirisena
Dr. A. Sivasuriya
Dr. R. Sriskanthan
Dr. D. De S. Suriyawansa
Dr. B.G.D. Vidyathilaka
Dr. S. Warnakulasuriya
Dr. A. Wijesundara
Dr. K.K.S. Karandagoda
Dr. V.S. Pannila

Junior Doctors & Nursing and Midwifery Staff of DSHW & CSHW

Clinical research assistants with subject recruitment:
Dr. Dilan Samarasinghe
Dr. Samanthi Premaratne
Dr. Velupillai Vathanan

The summary of recruitment is on this slide.

Summary of Recruitment

Total referred (G1P0, G2P0)		360
Consent not given		8 (2%)
Excluded / Withdrawn	Failed proteinuria criteria	64 (18%)
	Chronic hypertension	19 (5%)
	Twin pregnancy	12 (3%)
	Gestational diabetes	11 (3%)
	Failed blood pressure criteria	10 (2%)
	Body Mass Index ≥ 30	8 (2%)
	Mixed race marriage	7 (2%)
	G1 IUD at POG>12	3 (1%)
	Blood sampling could not be done	1
	LAMA before contacting for consent	4 (1%)
	Tamils & Moors excluded	30 (10%)
Total recruited		180 (50%)

(Sri Lanka Journal of Obstetrics and Gynaecology 2004,26(Supple1): 28-29)

This summary highlights the fact that many women with hypertension and proteinuria in pregnancy do not meet the research criteria for pre-eclampsia. To match the 180 primiparous Sinhalese women with pre-eclampsia that formed the cases for our candidate gene disease association studies, we recruited a control group of 180 primiparous Sinhalese women who had completed an uncomplicated first pregnancy and delivered a normal child. The cases and controls were matched for age and body mass index.

We reported the clinical phenotype of these women at the Annual Scientific Sessions of the Sri Lanka College of Obstetrician and Gynaecologists in 2004 and at the World Congress of the International Society for the Study of Hypertension in Pregnancy in 2004; and there after reported it in a paper published in the Journal of Obstetrics and Gynaecology Research.

doi:10.1111/j.1447-0756.2007.00475.x J. Obstet. Gynaecol. Res. Vol. 33, No. 1: 56–62, February 2007

Morbidity and mortality associated with pre-eclampsia at two tertiary care hospitals in Sri Lanka

Vajira H. W. Dissanayake[1,2,3], Harshana D. Samarasinghe[1], Linda Morgan[2], Rohan W. Jayasekara[1], Harshalal R. Seneviratne[4] and Fiona Broughton Pipkin[3]

[1]Human Genetics Unit, Faculty of Medicine, University of Colombo, Sri Lanka, [2]Division of Clinical Chemistry, Institute of Genetics, School of Molecular Medical Sciences, University of Nottingham, [3]Division of Obstetrics, School of Human Development, University of Nottingham, Nottingham, UK and [4]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Colombo, Sri Lanka

Abstract

Aim: To report the occurrence of morbidity and mortality associated with carefully phenotyped pre-eclampsia in a sample of nulliparous Sinhalese women with strictly defined disease.

I am sure, ladies and gentleman, that you would agree with me, that by applying a strict definition for pre-eclampsia, and strict exclusion criteria, we have recruited a group of women who have been rigorously phenotyped for pre-eclampsia and that the issue of population admixture has been addressed as far as possible. There was however, one remaining question to answer. That was one of statistical power. Do 180 cases and 180 controls have adequate statistical power to detect a genetic effect in a case control analysis?

The sample size required to detect a genetic effect at different risk levels based on the frequency of a genetic marker is given in this table:

Sample Size

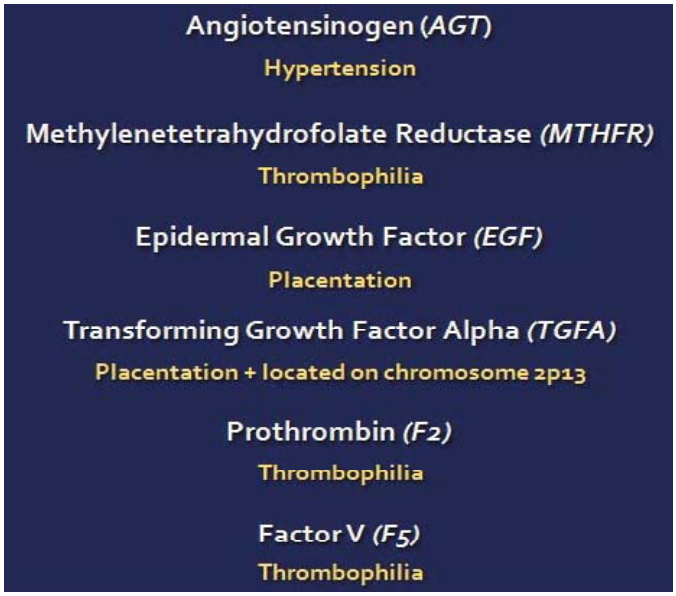
conventional power of 80% to detect association with α=0.05 (dominant model; 1 control per case)

	Frequency of the susceptibility allele of a genetic marker			
Genotype Risk Ratio	0.1	0.3	0.5	0.7
1.5	557	389	566	1371
2.0	137	179	211	529
5.0	29	30	54	147

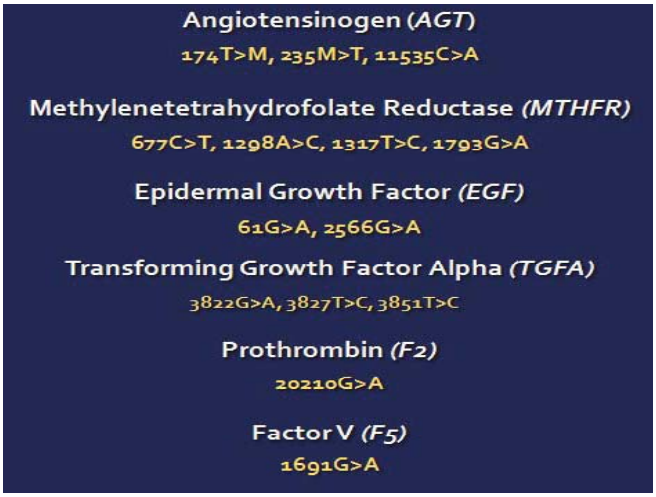
The risk level that we were aiming for was a doubling of risk when the frequency of the susceptibility allele of the genetic marker that increased pre-eclampsia risk, ranged from 0.1 to 0.5. Our case control sample size was then, within the range that we expected it to be.

Therefore, ladies and gentleman, in our study we had addressed all the issues that seemed to have plagued previous candidate gene disease association studies. We were then ready to focus our attention on genetic laboratory work.

We selected the candidate genes listed in this slide after a review of scientific writing in 2001.



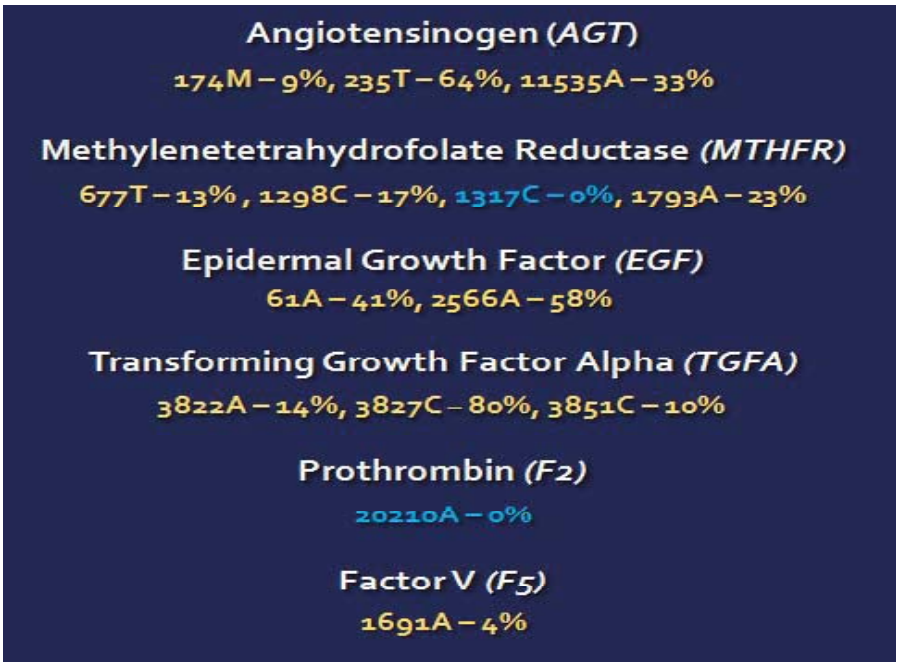
We selected both positional and functional candidates. We selected genes that have been studied before, and genes that have not been studied before. I would like to draw you attention to the epidermal growth factor gene which was a novel candidate gene selected by us. After selecting the candidate genes we selected the genetic markers. They are shown on this slide. They were all single nucleotide polymorphisms.



There was however, one final hurdle that we had to overcome before moving on to candidate gene disease association studies, that was to find an answer to the question, are these genetic markers polymorphic in our population? We needed an answer to this question, because, if a genetic marker is not polymorphic then it can not be used in case control studies. To answer that question we had to resort to another round of recruitment to establish a population based DNA resource. We established this resource by recruiting 240 random population based volunteers consisting of equal numbers of Sinhalese, Sri Lankan Tamils and Moors. Ladies and gentleman, I do not wish to burden you with the mundane details of how molecular genetic analysis was done in the laboratory. I shall move directly to

the results of our genetic studies. But before I do that, I would like to acknowledge the other members of our research teams, both in Colombo and in Nottingham. Their names are listed in this slide.

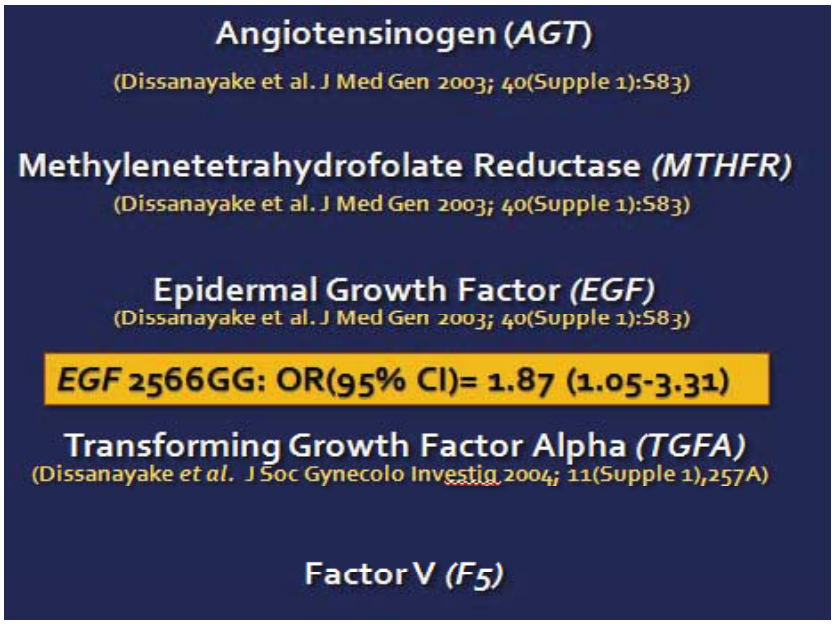
The results of genotyping the population based DNA collection for the genetic markers mentioned before are summarised on this slide. It lists the susceptibility alleles of the genetic markers and their frequencies in Sinhalese. The markers that are highlighted were not polymorphic.



The susceptibility allele frequencies of the Tamils and Moors, although not shown here, were similar to that of the Sinhalese. This observation was important to us because that gave us further confidence that any unsuspected population admixture would not affect the results of our candidate gene disease association studies.

Let me now move on to the results of the candidate gene disease association studies. In the case control analysis, to our delight, we found that the G allele of the 2566G>A polymorphisms in the Epidermal Growth Factor (EGF) gene increase the risk of pre-eclampsia. With women who were

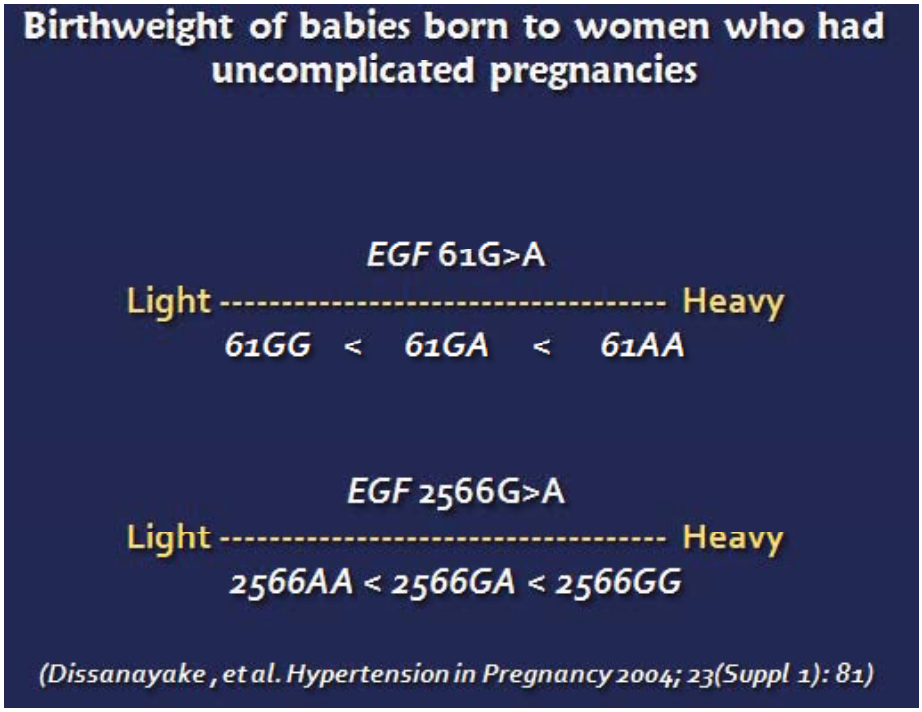
homozygous for the allele almost having a doubling of risk.



The collection of large amounts of clinical and genetic data such as we have done in these investigations opens up opportunities for innovative analysis. We decided to explore the contribution of these genetic variants to determining the weight of babies at birth.

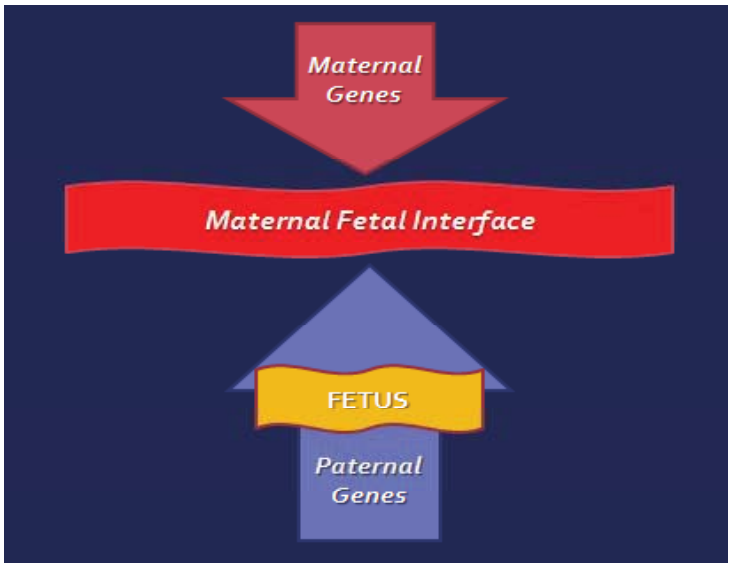
We know that the birthweight of babies born to women with pre-eclampsia is usually low. We also know that birth weight is a mutifactorial trait, which has a genetic component. We decided therefore to carryout further analysis of our data to find out whether any of the susceptibility alleles that we studied were associated with the weight of babies at birth either in women with-preeclampsia or in women who had completed uncomplicated pregnancies and delivered a normal baby.

To our delight, once again, we found that the birthweight of babies born to women who had uncomplicated pregnancies was also associated with the mother's epidermal growth factor genotype. These results are summarised in this slide.



The trend from lightest to heaviest birth weights was as shown here. We were able to replicate these findings in a similar group of white western European women in the UK. We presented these original findings at the World Congress of the International Society for the Study of Hypertension in Pregnancy in November 2004. Subsequently we were able to replicate these finding in another similar group of white Western European women, in the UK. Therefore, Ladies and gentleman, we have replicated our original finding, not once, but twice indicating that this is very likely to be a true association.

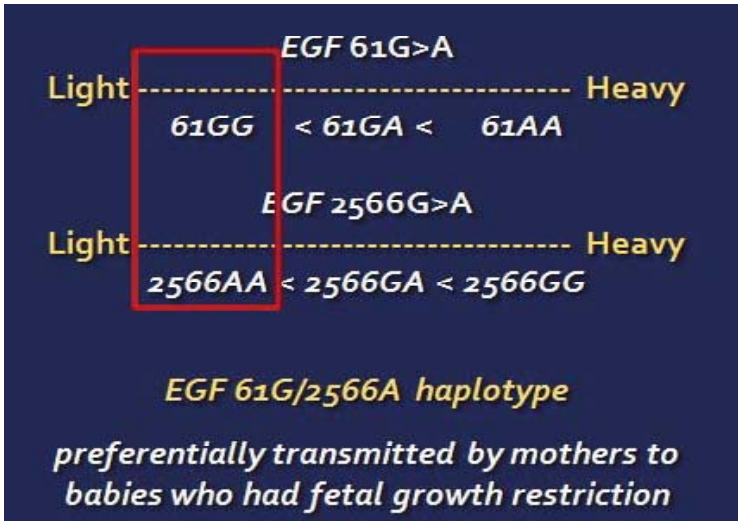
You would appreciate that the maternal-fetal interface in pregnancy is a unique one. It is the only site in an organism where genes from the mother and genes from the father acting through the fetus interact.



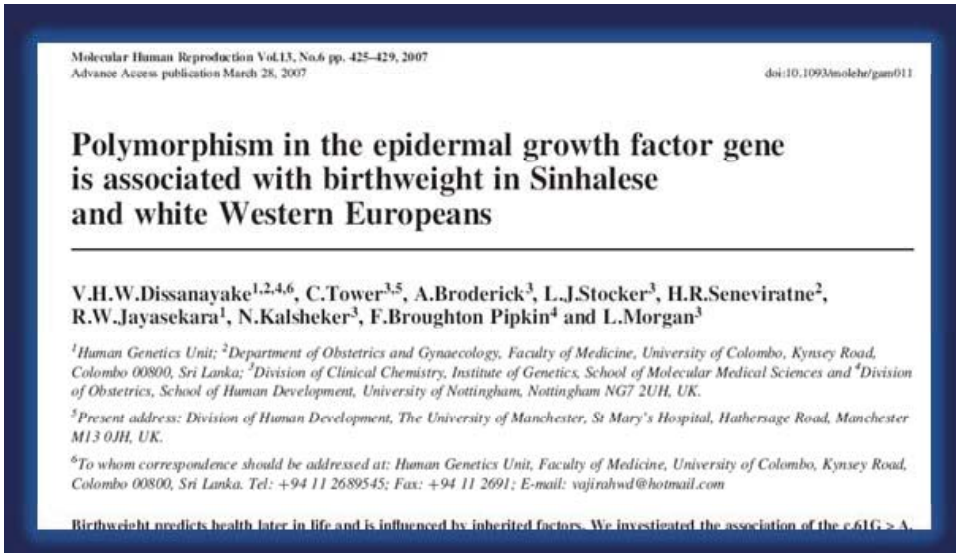
To study this interaction we have to study the genetic make up of the father, the mother, and the fetus or the baby - the so called parent-child trios.

We had access to such a collection of trios in Nottingham, UK. This collection contained DNA samples from women who had delivered growth restricted babies, DNA samples from their partners, and DNA samples of the babies themselves; in addition it also contained control trios consisting samples of women who delivered normal babies following uncomplicated pregnancies, samples from their partners, and samples from their babies.

When we genotyped these trios and analysed that data we found that the chromosomes that contained the 61G and the 2566A alleles, of the EGF gene, or in other words the 61G/2566A haplotype, which was associated with the lower birthweight, was preferentially transmitted mothers to their growth restricted babies; another original finding.



These three findings were the main breakthroughs in our investigations resulting in a notable publication in the Journal, Molecular Human Reproduction published by the European Society of Human Reproduction and Embryology.



Molecular Human Reproduction Vol.13, No.6 pp. 425–429, 2007
Advance Access publication March 28, 2007
doi:10.1093/molehr/gam011

Polymorphism in the epidermal growth factor gene is associated with birthweight in Sinhalese and white Western Europeans

V.H.W.Dissanayake[1,2,4,6], C.Tower[3,5], A.Broderick[3], L.J.Stocker[3], H.R.Seneviratne[2], R.W.Jayasekara[1], N.Kalsheker[3], F.Broughton Pipkin[4] and L.Morgan[3]

[1]*Human Genetics Unit;* [2]*Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Colombo, Kynsey Road, Colombo 00800, Sri Lanka;* [3]*Division of Clinical Chemistry, Institute of Genetics, School of Molecular Medical Sciences and* [4]*Division of Obstetrics, School of Human Development, University of Nottingham, Nottingham NG7 2UH, UK.*

[5]*Present address: Division of Human Development, The University of Manchester, St Mary's Hospital, Hathersage Road, Manchester M13 0JH, UK.*

[6]*To whom correspondence should be addressed at: Human Genetics Unit, Faculty of Medicine, University of Colombo, Kynsey Road, Colombo 00800, Sri Lanka. Tel: +94 11 2689545; Fax: +94 11 2691; E-mail: vajirahwd@hotmail.com*

Birthweight predicts health later in life and is influenced by inherited factors. We investigated the association of the c.61G > A

Ladies and gentleman, these discoveries have the potential to have huge implications, when taken in the context of the barker hypothesis, a hypothesis that has stood the test of time, the premise of the barker hypothesis is that adult onset disorders have origins in fetal life. Birthweight, which is the surrogate marker for development in fetal life, is an important predictor of health. Low birthweight is related to an increased risk of diseases in adult life, including cardiovascular disease, hypertension and diabetes.

Therefore ladies and gentleman, I leave you with the question, Is the epidermal growth factor gene the link between low birthweight and adult onset disorders? As I pose this question to you the research proposal to answer this question is on its way for ethics review.

Ladies and gentleman, this is not the end of this research journey, it is not even the beginning of the end, it is still the beginning. This work could not have been undertaken if we did not receive funding and other forms of support from many sources. They are listed here. I wish to thank them all.