Genetics of Growth, Development and Human Migration

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This presentation will highlight some of the genetic research carried out at the Institute of Biochemistry, Molecular Biology and Biotechnology on growth and development as well as how genetic research was used to delineate maternal origin of Sri Lankans. Presentation is divided into three sections namely,

- Insulin like growth factor I & II genotypes and *H 19* genotypes in fetal growth
- *GHRH-R* codon 72 mutation in growth hormone deficiency
- Mitochondrial DNA analysis of Sri Lankan ethnic groups

Insulin like growth factor I & II genotypes and *H 19* genotypes in fetal growth

Nature dictates that the zygote (fertilized ovum) is genetically endowed from biological parents. However many environmental factors can affect the way that the fetal genome is expressed, thus nurture sometimes overrides the natural inheritance, for genes are not able to carry out the destined functions unless they are expressed and appropriate proteins are produced. Impaired fetal growth leading to low birth weight not only increases ones morbidity and mortality in the perinatal period but also predisposes an individual to diseases in later life. Growth may be impaired due to variations in the genes implicated in fetal growth or due to epigenetic changes (changes that can lead to gene expression without changing the nucleotide sequence) that result from insults from the perinatal environment. The resulting birth phenotype will influence the adult phenotype predisposing an individual to diseases later in life, especially to non communicable diseases.

Insulin like growth factors (IGFs) which are structurally related to insulin play a very important role in fetal growth. Many studies from elsewhere have reported a positive correlation of cord blood IGFI and IGF II levels and birth weight. IGFs are synthesized by adult liver and by feto-maternal tissues during pregnancy. IGF-I is involved in skeletal growth and cell differentiation whereas IGF-II is involved in cell migration and differentiation. Insulin like growth factor binding proteins regulate bioavailability and biological activities of IGFs. There are six binding proteins of which IGFBP-1 is the major binding protein for IGF-1 at the feto-maternal interface. IGF I mediates cellular action via IGF1 receptors and IGFII via either IGF1 receptors or insulin receptors. IGF2 receptors are mainly involved in clearing IGFII from the circulation.

Studies in humans from elsewhere had shown that serum levels of IGF-I and -II are positively correlated with birth indices while IGFBP-1 levels showed a negative correlation. We had previously carried out a longitudinal study on a limited number of healthy mother-newborn pairs in which we failed to confirm an association of IGF-I with birth indices but confirmed an association of IGFBP-1 with birth indices. Animal studies have demonstrated that complete deletion of *IGF-I* and *IGF-II* genes results in embryonic and neonatal mice becoming 40% smaller than their normal counterparts. Furthermore in transgenic mice, over expression of IGFBP-1 caused growth retardation

Several investigators have studied the association of genetic variations of *IGF I* and *IGF II* genes with birth weight, but results have been inconsistent. These investigations have included dinucleotide repeat polymorphisms, particularly a CA repeat in the promoter region, a CT repeat in intron 2 and a CA repeat in the 3' region of the *IGF I* gene. In Caucasians the promoter CA repeat was associated with IGF I levels and birth indices. CT repeat had been studied less, and one study reported it to modulate birthweight. With regard to the *IGFII* gene, Apa 1 polymorphism has been studied in relation to birth weight but results have been inconsistent. Similarly a few investigators have studied the genetic variations in the *H19* gene in relation to birth weight again resulting in inconsistent findings. *H19* is a gene which is maternally expressed and it codes for a long non coding RNA. *H19* is a reciprocal gene of *IGF-II* exclusively expressed by the maternal allele encoding a long non-coding RNA. Its function is not clear but is thought to regulate *IGF-II* expression.

There were no previous studies on the association of genetic variations in the *IGFI, IGF II* and *H19* genes with birth weight or other birth indices in Sri Lankans, thus we investigated the possible association of selected polymorphisms of these genes with birth indices in a cohort of healthy mother-new born pairs. *IGF-I* dinucleotide repeats were analysed using sequencing based fragment analysis. *IGF-II* Apa 1 polymorphism and selected *H19* single nucleotide polymorphisms (SNP) were analysed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) and confirmed by sequencing based SNP analysis.

We studied a cohort of predominantly Sinhalese healthy mother-newborn pairs in the first or second pregnancy. The commonest polymorphism of the *IGFI* intron 2 CT repeat was 189 bp (15 repeats) homozygosity and 34% of the mothers and 29% of the newborns carried this polymorphism. Second commonest polymorphism was 191 bp homozygosity and 23.5% of the mothers and 18.5% of the newborns carried this polymorphism. 189 bp/19 1bp heterozygosity was only 6% among mothers and 12% among the newborns. Birth weight, crown heel length and head circumference were significantly higher in newborns who were 189 bp homozygotes for the IGFI intron 2 CT repeat when compared to 191 bp homozygotes. Newborns of 189 bp homozygous mothers had a significantly higher birth weight and a crown heel length than the newborns of mothers with 191 bp homozygosity. When parity of mothers was taken into account these differences were limited to the first pregnancy. Furthermore, primiparous mothers with 191 bp homozygosity had lower maternal IGF-I levels than their counterparts with 189 bp homozygosity while the newborns showed the same pattern for cord blood IGF-I levels. Promoter CA repeat was associated with higher IGF-I levels but not with birth indices whereas the 3' region CA repeat did not show any consistent effect on IGF-I levels or birth indices.

With regard to the *IGF II Apa* polymorphism, the polymorphic allele (G) was more prevalent among Sri Lankans and the genotype had no effect on birth weight. *H19* genotype & birth indices were investigated in 196 mother baby pairs of which majority were from the original cohort of 200 and a few were new recruits. Three SNPs were studied of which only the TT homozygosity of maternal rs217727 SNP showed a positive association with birth weight.

We used multiple regression analysis to examine the effect of *IGF-I* intron 2 CT repeat and *H19* maternal rs217727 on birth weight in 173 Sinhalese mother-newborn pairs from the above cohorts and results confirmed that even in normal pregnancy birth weight is independently modulated by *IGF-I* intron 2 dinucleotide repeat (maternal and newborn) and *H19* rs217727 (maternal) polymorphisms. One need to examine the same variants in other ethnic groups before any interventions based on these genetic variants are introduced to improve birth weight.

GHRH-R codon 72 mutation in growth hormone deficiency

After birth, somatic growth is mediated by the growth hormone (GH) secreted from the anterior pituitary. Its synthesis and secretion is stimulated by growth hormone releasing hormone (GHRH) of hypothalamic origin. GHRH acts on G protein coupled GHRH receptors (GHRHR) on GH secreting cells to mediate its action. Both *GH* and *GHRHR* gene mutations are reported to cause growth hormone deficiency leading to short stature. There have not been any studies to identify mutations in *GH* and *GHRHR* genes in growth hormone deficient Sri Lankan children except a report on two migrant brothers from the Delft Island reported from France. These two migrant brothers carried the codon 72 mutation in the *GHRHR* gene which causes truncation of the GHRHR protein rendering it nonfunctional. The same mutation has been reported from Pakistan and India suggesting this to be the most prevalent genetic abnormality leading to growth hormone deficiency in the Indian subcontinent. We screened a cohort of 91 growth hormone deficient children for *GHRHR* codon 72 mutation. They were clinically and biochemically confirmed to have GH deficiency. DNA obtained from peripheral blood samples were amplified by PCR and sequenced to identify the mutation. Among the 91 patients screened only 8 carried the codon 72 mutation. As expected a higher number (6) of males were affected. Lower prevalence of the mutation compared to reports from India and Pakistan appeared to be due to the lower prevalence among Sinhalese who comprised of nearly 75% of the patient cohort. Further studies are needed on patients of Tamil and Muslim ethnicities to see whether these groups have a higher prevalence of the *GHRHR* codon 72 mutation than the Sinhalese.

Mitochondrial DNA analysis of Sri Lankan ethnic groups

Genetic tools for the study of human migration include mitochondrial DNA which is passed from the mother to both sons and daughters with only daughters being able to pass it onto the next generation, Y chromosome markers which are passed from fathers to sons and autosomal markers which are inherited from both parents. Each cell contains many mitochondria which are the sites of energy production. Each mitochondrion has circular DNA molecules of 16569 bp known as mitochondrial DNA. A non-coding region in the mitochondrial (mt) genome known as D loop contains 3 hypervariable regions. This region acquires most of the mutations while some mutations are also seen in the coding region.

A set of DNA variations, or polymorphisms that tend to be inherited together is referred to as a haplotype. A group of similar haplotypes sharing a common ancestor having the same mutation in all haplotypes is referred to as a haplogroup. Contemporary view is that we all are descendents of a group of few women (mitochondrial Eve – haplogroup L0) who lived in Africa 130,000 to 200,000 years ago. Due to certain mutations 6 haplogroups named L1 to L6 have arisen in Africa and of this L3 has migrated out. During migration out of Africa and subsequent movement to different parts of the world further mutations has led to emergence of other haplogroups such as macrohaplogroup M and N. Now a large number of haplogroups that arose from M and N can be found in the rest of the world and some of these are continent specific. Migration out of Africa towards the Indian subcontinent is thought to have taken either a Northern route towards west Eurasia and then to the Indian subcontinent through land mass, or crossing the red sea moving along the coastline of Arabian peninsula and then crossing the Gulf and moving along the Indian coastline (beachcomber route).

As the mitochondrial DNA can give us information on our maternal inheritance we studied contemporary Sri Lankan ethnic groups and Vedda population to identify their maternal relatedness through mitochondrial DNA. Individuals who reported absence of mixed marriages between their parents, grandparents and great grandparents were studied. Sinhalese, Muslims and Malays were from the Western province, Sri Lankan Tamils were from the Northern Province (or those who had recently migrated to the Western province from the Northern province), Indian Tamils were from the Central province and Vedda individuals were from the Uva and Eastern provinces. DNA

isolated from peripheral blood samples or buccal swabs were PCR amplified and sequenced to identify nucleotide variations in the hypervariable regions I and II. Furthermore, coding region of the mt genome was analysed using PCR/RFLP to identify mutations characteristic of different haplogroups. PCR/RFLP results were confirmed by sequencing.

We identified 157 haplotypes among a total of 202 individuals (Sinhalese=60, Sri Lankan Tamils, Muslims, Malays and Vedda =30 each, Indian Tamils=22), of which 135 were unique (not shared with any others). Of the 22 shared haplotypes 10 were shared between ethnic groups and 12 within an ethnic group. Bioinformatic analyses of the HVI and HVII sequences as well as coding region analyses confirmed that all individuals studied belonged to the M and N major haplogroups. Further analyses demonstrated that the majority of the haplogroups belonged to the South Asian (Indian) haplogroups. Interestingly among Sinhalese, Sri Lankan Tamils and Vedda people a significant presence (20 to 25%) of West Eurasian haplogroups was evident. West Eurasian haplogroups were absent among Indian Tamils and a very low prevalence was seen in Muslims and Malays. Vedda separated as a distinct group whereas Sinhalese and Sri Lankan Tamils were closer to each other. Malays and Muslims were more closer to each other while Indian Tamils were further from these groups.

South Asian (Indian) mt DNA haplogroups being the commonest among Sri Lankans, supports historical evidence of migrations from India or more or less simultaneous colonization of both Sri Lanka and India through the beach comber (coastal) route. West Eurasian haplogroups among the Sinhalese, Sri Lankan Tamils and Vedda, population groups with a longer history in the island suggest early migration of women carrying these haplogroups into the country. Our data led us to conclude that contemporary Sri Lankans share very close maternal ancestors and that ethnicity is created by linguistic, religious and cultural differences rather than by genetic differences.

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