BSPED Research and Innovation Award 2018

Epigenetic regulation of thyroid function: examining the potentially modifiable effect of differential PAX-8 methylation on thyroid function in children in The Gambia

Background

Thyroid hormones contribute to a wide range of physiological processes and influence a variety of outcomes related to cognition, growth, bone, cardiovascular, and metabolic health[1]. Variability of thyroid function within the normal population reference range is associated with a range of phenotypic traits including blood pressure, lipids, obesity, cardiovascular mortality, bone mineral density (BMD) and cancer risk[3].

PAX8 (paired-box 8) protein is one of four known thyroid transcription factors (TTF) involved in thyroid development and function (others include NKX2-1, FOXE1 and HHEX)[13]. These transcription factors are known to regulate expression of thyroid specific genes related to thyroid hormone production and storage such as TPO (thyroid peroxidase), Tg (thyroglobulin) and the sodium-iodide symporter[13,14], and are also important for development and differentiation of the thyroid gland[15].

Epigenetic processes, including DNA methylation, histone modification, chromatin remodelling and RNA-based mechanisms can affect gene expression[21]. Metastable epialleles (MEs) are epigenetic loci that demonstrate systemic (concordant across tissues) methylation patterns within individuals with significant interindividual variation[24]. The methylation patterns are thought to be established early in embryonic development[25] and are not driven by genetic variation[24,25]. They may also be influenced by maternal diet around conception[26–30], and putative human MEs with stable methylation signatures have been linked to disease-related phenotypes[26,27]. This positions MEs as potential epigenetic mediators in the developmental origins of health and disease.

PAX8 is a putative human metastable epiallele with evidence of systemic interindividual variation and sensitivity to periconceptional environment[24,26,28,31]. Furthermore, there is evidence that *PAX8* methylation patterns in leucocytes and thyroid are concordant in children[32]. Data from The Gambia showed that leucocyte *PAX8* methylation is higher in those conceived in the rainy (or 'hungry') season[26,31]. In Bangladesh, significantly higher methylation at the *PAX8* gene was reported in offspring following gestational (at least 7 months of pregnancy) famine exposure[28]. A set of interlocking pathways, collectively known as one-carbon metabolism, provide methyl groups for methylation reactions including DNA methylation, and these are dependent on multiple nutritional factors that act as substrates and essential co-factors[33,34]. Seasonally-driven variations in maternal circulating levels of one-carbon metabolites have been reported in the Gambia[35]. Maternal folate (a one-carbon

metabolite) supplementation has been associated with differential *PAX8* methylation in offspring with methylation differences seen in adulthood[36] and maternal preconception micronutrient supplementation was nominally associated with differential methylation at *PAX8* in Gambian children[37].

Epigenetic influence on thyroid function or development has been little explored. The *PAX8* gene is a prime candidate for study due to its key role in both thyroid gland development and in the regulation of the differentiated thyroid gland, and is additionally of particular interest due to its apparent epigenetic sensitivity to the early environment.

Methods and Results

Using a recall-by-epigenotype design, 118 Gambian children were recruited from the top and bottom quintiles for *PAX8* methylation measured in banked peripheral blood DNA taken at 2-years of age from a longitudinal Gambian cohort study. Participants were assessed for thyroid volume, function (free T3, free T4, TSH and Tg), urinary iodine, and body composition and bone measures by whole body DXA scan at age 5-8 years. In multiple linear regression models adjusted for relevant covariates, the low *PAX8* methylation group was associated with a 0.61cm^3 [SE=0.15] or 21% increase in thyroid volume ($p<0.0001$) and a 0.85 pmol/L [0.24] increase in free T4 (a change equivalent to 8.4% of the normal range, $p<0.001$), though not free T3, TSH or Tg. Increased free T4 was associated with a decrease in all measures of overall body fat including log fat mass index (β=-0.04 [0.02], p=0.033), log total fat mass (β=-0.05 [0.02], p=0.041) and log fat lean ratio (β=-0.05 [0.02], p=0.045), and with a decrease in log total-body-less-head bone mineral density (β= -0.008 [0.004], p= 0.044) after adjustment for relevant covariates. No fat or bone-related measures were independently associated with *PAX8* methylation group.

Infant *PAX8* methylation measurements and nutritional biomarker data from maternal blood taken from early pregnancy were available for 303 children from the longitudinal Gambian cohort study. Logit *PAX8* methylation z-score was significantly lower in boys (β= -0.24 [0.09], p=0.005) and was nominally associated with 4 maternal one carbon metabolites: homocysteine (standardised β=-0.11 [0.05], p=0.05), cysteine (β=-0.16 [0.05], p=0.003), B12 (β=-0.1 [0.05], p=0.05) and the vitamin B6 vitamer, PLP (β=-0.12 [0.06], p=0.03). The association with cysteine remained significant after correction for multiple testing. *PAX8* methylation was shown to be stable in samples from Gambian children taken at age 7 and 17 years ($R=0.76$, $p<2.2\times10^{-16}$). We observed a strong relationship between *cis*-genotype and *PAX8* methylation variability suggesting sensitivity to environmental exposures may be under partial genetic control.

An inverse relationship between *PAX8* antisense (PAX8-AS1) expression and methylation at the analysed region was seen in non-malignant thyroid tissue samples from The Cancer Genome Atlas. However no association was observed in thyroid tissue samples from the Genotype-Tissue Expression Project.

Conclusions

A maternal early nutrition-sensitive DNA methylation signature at the *PAX8* gene measured in Gambian children at 2 years of age was significantly associated with thyroid volume and free T4 at 5-8 years of age. Free T4 was in turn associated with body fat and bone mineral density at 5-8 years of age. These findings may have significant implications for early embryonic programming of thyroid-related health and disease.

Benefit to applicant

This grant provided a fantastic opportunity to explore early life programming of thyroid function and development. This work has formed a substantial part of my PhD. I began my PhD solely exploring epigenetic regulation of *POMC* and its association with obesity and body composition but this grant has allowed me to widen my research skills and knowledge base. This work led directly to a collaboration with the molecular and human genetic department at Baylor College of Medicine, Texas, USA. The research has recently been submitted for consideration by *Cell Metabolism*. My hope is that this work will be the basis of future research studies examining early embryonic programming of thyroid function and I am actively exploring potential research opportunities and collaborations.

Benefit to department

The MRCG at LSHTM Nutrition theme have produced several high impact publications demonstrating a seasonal and nutritional effect on offspring DNA methylation[1–5]. This study is the first of it's kind from the group to demonstrate a link between DNA methylation at region of the genome sensitive to maternal early nutrition and phenotype. This is an important step to establish the link between environmental exposures, epigenetic changes and phenotype and is essential to develop future interventions.

Benefit to endocrinology

Epigenetic effects on thyroid function are little studied. This study provides insights in to how DNA methylation at the *PAX8* gene (which may be potentially modifiable by targeted maternal nutritional interventions) influences a range of thyroid function measures. By understanding more about epigenetic regulation of thyroid function, we can gain insights into the pathogenesis of diseases like thyroid dysgenesis and congenital hypothyroidism.