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COMMENTARY

Opportunism: a panacea for implementation of whole-genome sequencing studies in nutrigenomics research?

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Abstract Observational studies have consistently shown associations between mild deficiencies in folate and vitamin B_{12} with increased risk of a myriad of common diseases. These findings have invariably translated into null outcomes in intervention trials due in part to our ignorance of the specific genomic and environmental factors that underpin population variability in requirements to these B-vitamins. Although genome-wide association studies have shed initial light on the genetic architecture of variability in status of these vitamins, particularly vitamin B_{12}, the causal mechanisms remain uncharacterised. A recent study by Grarup et al. (PLoS Genet 9(6):e1003530, 2013) used next-generation whole-genome sequencing to gain further insight into the genetic architecture of vitamin B_{12} and folate status in the general population. Their study represents the analysis of approximately ten times greater number of genetic variants and nearly four times the number of individuals compared to the largest previous GWAS study of these B-vitamins. In light of this, we purport that although the study may be viewed as the *state of the art* in the roadmap to personalised or precision nutrition, the lack of insight provided by the study serves as a cautionary reminder of the importance of study design, particularly when leveraging large-scale data, such as those from whole-genome sequences. We believe that the precedent set by such large-scale "proof of principle" type projects will wrongly enforce a negative outlook for nutrigenomics research and present alternative study designs, which although less opportunistic are far more likely to be informative and yield novel results.

Keywords Whole-genome sequencing · Nutrigenomics · Vitamin B_{12} · Folic acid · Heritability

There is tremendous scope for translation of findings from nutritional science research into informed dietary recommendations, public health-measures, and medical applications to maintain and improve overall health across the population (Muller and Kersten 2003). To realise the full potential of the concept *health-science nutrition,* there is an urgent need to develop the evidence-base through powerful and informative studies that harness genomic data that aim to dissect the complex interplay of diet, genomics, and human health. Such an approach has the potential to facilitate identification of novel biomarkers that can be used to rationalise the nutritional needs of the population and serve as a powerful adjunct to current health interventions in different strata of the population. To this end, a recent study by Grarup et al. (2013) may be viewed by some as the *state of the art* in the roadmap to personalised—"precision"—nutrition, but the lack of insight provided by the study serves as a cautionary reminder of the importance of study design, particularly when leveraging large-scale data, such as those from whole-genome sequences.

Previous observational studies have consistently shown associations between mild deficiencies in vitamins B_9 (folate), B_{12} and total homocysteine (tHcy), an integrated marker of 1-carbon metabolism, with increased risk of a myriad of common diseases, including vascular disease, different cancers, osteoporosis, and neurodegenerative conditions (Wald et al. 2002; Casas et al. 2005; Den Heijer

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et al. 2005; Nelen 2001; Morris 2003; van Meurs et al. 2004). These findings have invariably translated into null outcomes in intervention trials (Clarke et al. 2010). Perhaps the biggest factor in governing disparities between findings from observational and interventional studies is the current *one-size-fits-all* approach to intervention trials, which fundamentally ignore population variability in status and requirements to these B-vitamins. Emerging evidence shows this variation to be underpinned in large part by genomic factors (Andrew 2013; Cotlarciuc et al. 2011).

Recently, genome-wide association studies (GWAS) have shed initial light on the genetic architecture of variability in vitamins B_9, B_{12}, and tHcy status in populations of European and Chinese ancestry (Hazra et al. 2009; Lin et al. 2012; Tanaka et al. 2009; van Meurs et al. 2013). These studies have identified a number of loci harbouring common variants of low effect influencing B_{12} levels and tHcy, although surprisingly they have been unsuccessful in identifying novel variants affecting folate status. The study by Grarup et al. uses two disparate datasets from Iceland and Denmark. The first dataset consists of whole-genome sequence (WGS) data from 1,176 reference Icelanders ascertained for WGS based on having various neoplasmic, cardiovascular, and psychiatric conditions, which are imputed into 25,960 and 20,717 chip-genotyped Icelanders with serum B_{12} and folate measurements, respectively. The second dataset consists of whole-exome sequence data generated in 1,000 Danish cases with type 2 diabetes (BMI $>$ 27.5 and hypertension) and in 1,000 matched controls. Altogether, up to 22.9 million sequence variants were analysed in combined samples of 45,576 and 37,341 individuals with serum vitamin B_{12} and folate measurements, respectively. The study marks a much anticipated transition from traditional array-based GWAS, which leverage the degree of linkage disequilibrium (LD) in the human genome to study common SNPs that tag common haplotypes in the population, towards a less biased and closer scrutinisation of the impact of rarer ($<$5 %) and rare ($\leq$1 %) sequence variants on common traits including multifactorial diseases, drug response, and micro-/macronutrient disposition.

The study claims to have identified five new loci (*CD320, TCN2, ABCD4, MMAA,* and *MMACHC*) associated with serum B_{12} status, one new loci (*FOLR3)* associated with folate levels, and confirmed all previous associations from previous GWAS for these traits (including *TCN1, FUT6, FUT2, CUBN, CLYBL, MUT*, and *MTHFR*). Conditional analyses revealed additional independent signals at *CUBN, TCN1, TCN2*, and *MTHFR*. Collectively these variants are estimated to explain 6.3 and 1 % of the observed population variance in serum B_{12} and folate levels, respectively. However, despite the impeccable technical execution of the study, it is vital to recognise that the application of *state of the art* technology will never be a substitute for informative study design. The key question raised by the study is: can WGS studies succeed in identifying novel loci of higher effect size that were previously overlooked by GWAS?

WGS-based studies have principally been used so far to identify mutations of high penetrance that underlie Mendelian diseases. Published WGS-based studies of common traits are still rare, although this will no doubt change quickly over the coming months. Grarup et al. principally set out to obtain a less biased view of the genetic architecture of population variation in vitamin B_{12} and folate levels and in doing so (1) delineate the contribution of common and rarer genetic variation to the traits under study, and (2) better characterise the *genetic architecture* for these traits. To put their effort in perspective, this study represents analysis of approximately ten times greater number of genetic variants and nearly four times the number of individuals compared with the biggest previous study of these B-vitamins (Hazra A et al. unpublished). On close scrutiny, the panel of 1,176 reference Icelanders consists of older adults (average age = 63 years) with high B_{12} (409 pmol/l (range: 305–555 pmol/l) and very high folate 22.6 nmol/l (range: 15.3–38.7 nmol/l) status. The Danish participants however are younger (average age = 46.2–49.2) and have significantly lower but normal B_{12} (280–369 pmol/l) and folate (8.5–15.9 nmol/l) status. These characteristics in combination with the "ascertainment" scheme for the Icelandic WGS study, based on having various neoplastic, cardiovascular, and psychiatric conditions—i.e. individuals affected by diseases thought to be most amenable to risk modification through B-vitamin supplementation yet exhibiting high B_{12} and folate status—undoubtedly represent the least useful ascertainment strategy for WGS of Vitamin B_{12} and folate status. The fact that the study by Grarup et al. adds so little insight into the likely role of rare(r), more penetrant sequence variants on population variation in B_{12} and folate status serves to illustrate this particular shortcoming of the study.

Future WGS studies should be designed and implemented with a view to (1) maximise power to detect rare(r) variants of moderate effect size and also (2) to facilitate interpretation of identified associations between these genetic variants and single plasma/serum measures of a metabolite. As a case in point, of the five "novel" loci identified by Grarup et al. for B_{12}, only *CD320* (minor allele frequency of 3 % in the Icelandic population) failed to reach genomewide significance in our meta-analysis of previous GWAS (based on common genetic variants) for B_{12}, and both *MMAA* and *MMACHC*, previously shown to harbour rare sequence variants associated with defects of the cobalamin metabolic pathway—cblA and cblC, respectively—have also been shown by us to harbour

common variants associated with both total plasma B_{12} (Hazra et al. unpublished) and homocysteine levels (van Meurs et al. 2013). If the so-called *missing heritability* is predominantly underpinned by rare(r) variants of moderate effect size (Bodmer and Bonilla 2008; Yang et al. 2010), a more fruitful and undoubtedly more efficient strategy is to apply WGS to individuals with extreme phenotypes (Emond et al. 2012). In the case of folate and B_{12}, but quantitative traits in general, this approach is easily justifiable (Lanktree et al. 2010; Plomin et al. 2009) and is likely to provide novel insight, as well as affording the ability to test the likely role of rarer sequence variants, as these alleles, should they exist at all, are likely to be enriched in one or both phenotype extreme groupings. Under this design, incorporation of data on nutrient intakes into the analysis protocol will also overcome a significant limitation of current GWAS of metabolite levels. Whilst a genetic-pronged approach can identify and has identified genetic loci associated with micronutrient status, such an approach will not be sensitive to identify loci that are "responsive" to a dynamic microenvironment, i.e. dietary intake. There are now a number of studies that have highlighted the importance of dietary intake in mediating associations between genetic variation and diverse phenotypes (Zheng et al. 2013; Roke et al. 2013; Kaput 2008). Such studies show that careful assessment and use of dietary intake can greatly facilitate our interpretation of associations between genetic variation and a single plasma/serum measure of a metabolite. We would suggest that results from such studies will also facilitate the design and implementation of future intervention trials.

Time will tell whether opportunism will bode well for next-generation WGS studies of common traits/diseases. Judging by the results from the study by Grarup et al., however, we think the likely answer is no and that there is a danger that GWAS will now be replaced by a rash of uninformative WGS studies. If the missing heritability for common human traits is predominantly underpinned by rare(r) genetic variants, simply performing WGS for large, unselected population samples—the opportunistic approach—is unlikely to provide useful insight. More importantly, the precedent set by such large-scale WGS "proof of principle" type projects will wrongly enforce a negative outlook for Medical Genetic research, when naively anticipated results fail to materialise in the absence of planned study design.

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