Author’s response to reviews

Title: New directions in childhood obesity research: how a comprehensive biorepository will allow better prediction of outcomes.

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Author’s response to reviews: see over
Dear BMC

We are grateful to the editorial committee and reviewers for their careful review of this manuscript.

Each comment has been addressed, as described below, with amendments made to the manuscript with tracked changes as requested.

With kind regards

Dr Matthew Sabin
Reviewer: James R Sowers

Reviewer's report:

This review discusses a strategy to study the genetics of obesity in children.

There should be included:

1. A better discussion of current knowledge of genetics and epigenetic factors implicated in obesity.

The following section has been added to the background:

“Numerous genes have been identified through genome wide association studies (GWAS) and candidate gene approaches that appear to be associated, either directly or indirectly, with the regulation of body weight [9]. It is likely that, through a process of natural selection, these genes have become more prevalent due to the evolutionary advantage that they offer by promoting energy storage to survive periods of food deprivation. Within our obesogenic environment, however, these genetic susceptibility traits are now associated with an increased risk of obesity and associated metabolic diseases, such as Type 2 diabetes.

The majority of genes identified in monogenic cases of obesity appear to be involved in the central regulation of energy intake. In this regard, the most strongly replicated candidate gene has been the melanocortin 4 receptor, which
ha been suggested to be responsible for up to 6% of cases of severe, early onset obesity [10], but also likely contributes to population variations in body fat, adipose tissue distribution, some metabolic traits and childhood weight gain [11]. Genes involved with energy utilization have also been implicated in common obesity, with replicated associations shown for genes encoding β-adrenergic receptors 2 and 3, hormone-sensitive lipase, and mitochondrial uncoupling proteins 1, 2, and 3 [12]. The FTO gene is another example of a key gene that appears to be responsible for population-wide variations in body weight and composition [13], and represents just one of many genes identified in recent times [14]. This is a rapidly progressing field of research, in terms of both the identification of new genes and the role that they play in both adult and early onset weight gain [15-17].

The role of epigenetics in body weight regulation is less clear, however, and remains the focus of intense interest [18]. Epigenetic factors include several different classes of modified nucleotides and proteins that interact to regulate the activity state of underlying DNA sequence. Such factors are usually heritable throughout cell division and play a pivotal role in specifying cell fate and function. The methylation of specific CpG dinucleotides (the most widely studied epigenetic mark) is known to affect gene expression and to be sensitive to environmental disruption, including dietary change [19-23]. Epigenetic profile is modifiable during critical developmental periods [23-26] and is involved in several obesity-related metabolic pathways [26, 27]. Epigenetic analyses, including DNA methylation profiling, thus offers a compelling new paradigm for
how early nutrition may impact upon later obesity [28]] and, although studies assessing the role of DNA methylation in human obesity are rare [29,30], the challenge is to now develop specific studies aimed at investigating how environmental exposures interact with underlying genetic determinants to dysregulate gene expression and lead to metabolic disorders [31].”

2. A diagram illustrating the proposed collection of biological material for genetic and epigenetic analysis.

We are uncertain as to the kind of diagram being requested. Table 2 outlines the proposed collection of biosamples within COBRA, with details on biospecimen fractions & number/volume of stored aliquots. A figure outlining recruitment to COBRA, and the process relating to collection of data and samples, has been added as Figure 1.

The following sentence has been added on page 17; “The process of recruitment, along with data and sample collection, is shown as a flowchart in Figure 1.”

3. It would be helpful to delineate potential statistical approaches that are to be used.

The following sections of text have been added to page 20:
“The proportion of participants with specific adverse health problems will be reported with 95% confidence intervals. Allele frequencies and presence of environmental factors of interest will also be summarised. Logistic regression models and Cox proportional hazards models for binary and time to event outcomes respectively, will be fitted to identify the risk and protective factors (predictor variables) for health problems and long-term disease (outcomes) for obese children.

Logistic and Cox regression models will be fitted to test the hypothesis that the effect of specific alleles on the risk of developing adverse health problems and long-term diseases is modified by the presence of select environmental factors (e.g. is the size of the odds ratio (or hazard ratio) between a particular gene and a specific health problem dependent upon whether the environmental risk factor is present). In these models the gene and environment variables (both categorical) will be used in the model as predictor variables in addition to a variable that represents the interaction between the gene and environment variables. The p-value for the gene-by-environment interaction variable will be used to quantify evidence against the hypothesis that there is no effect modification (interaction).

Where the effects of the gene on the disease are not in opposing directions within the categories of the environmental exposure, evidence for a gene-disease effect will be quantified using a simultaneous (or “joint”) hypothesis test [62] of the gene and gene-by-environment interaction effects. There is recent
evidence that this joint test, as opposed to a test of the gene variable alone in a simple marginal gene-disease model, improves power for detecting a gene effect - even in the presence of a degree of misclassification in the environmental measures [62]. The results from the interaction tests, supplemented by the joint tests where appropriate, will clarify the nature of the specific gene-disease associations."

4. Do the authors plan to do micro-RNA analysis?

This is a good question. Micro-RNA analysis may be very interesting to undertake but, as no specific plans are in place for this at the moment, we have not discussed its methodology. The manuscript is simply aimed at emphasising the major strengths of a Biorepository - its flexibility, capacity and mission to support diverse and novel analyses and techniques as new questions arise over time.

Reviewer: Alison Ventura

Reviewer's report:

Major Compulsory Revisions

None. I felt this manuscript was well written and informative.

Minor Essential Revisions

1. The authors should add more information on the longitudinal component of this biorepository. How often will follow-ups occur? Have
any follow-ups occurred yet? What information will be collected in the follow-ups? Will the investigators aim to obtain equally spaced assessments from all participants?

Specifically, and in response to each question, follow-up appointments are planned for every 3 months. Some follow-up visits have already occurred but data relating to these have not yet been analysed. Information collected at each visit include details relating to Auxology, attempts at weight management, other clinical events, and results of routine repeat clinical investigations. The aim is to obtain data from each clinic visit at 3 monthly intervals while recognising that some patients may miss some appointments. This is unlikely to be problematic however given the fact that COBRA is set up to collect data over long periods of time, and consent to recontact is included in the study protocol and in the written consent form.

To address these comments in the manuscript, and also because we have now received ethical approval potential inclusion in BioGrid, section d on Page 17 has been amended to read: “Longitudinal follow-up data are collected through clinic re-attendance up until patients reach 18 years of age. These clinic visits are scheduled at equally spaced intervals (initially 3 monthly), with routine clinical data collected (including Auxology, all details relating to attempts at weight management, other clinical events, and results of routine repeat clinical investigations). Follow-up in adulthood for the parent organisation will be enabled through participation in BioGrid (http://www.biogrid.org.au/wps/portal),
a Victorian data-linkage resource that can identify and facilitate access to data on medical visits at other participating hospitals or health administration datasets maintained by the Victorian Department of Health. As such data linkage resources may not be available for other national or international centres, and recognising that some children will fail to reattend follow-up appointments, COBRA has also incorporated a ‘consent to re-contact’ procedure should participants be lost to follow up through standard clinical contact.

We have also added the following sentence to Page 22: “Some follow-up visits have already occurred, but data relating to these have not yet been analysed.”

2. How are overweight and obesity being defined for the purposes of this biorepository? This definition should be provided to ensure continuity of the conceptualization of childhood overweight and obesity.

We apologise for this omission. The following sentences have been added on page 12, also recognising this reviewer’s point made in point number 4:

“For the purposes of recruitment, overweight and obesity is classified using the 85th and 95th percentile cut-offs of US-derived data produced by the Centres for Disease Control and Prevention (www.cdc.gov), as these data are routinely used on growth charts in Australia. When it comes to longitudinal analysis, there are a range of alternative systems for classification (e.g. International Obesity Task Force criteria, or World Health Organisation recommendations) and these
may continue to evolve over time. Therefore, the COBRA dataset will always include raw height, weight and BMI data, so that other classifications can be used flexibly and as appropriate. All children and adolescents (up to age 17.99 years) are approached, with no minimum age for recruitment recognising that, realistically, very few (if any) children are referred and routinely managed through specialist weight management services before the age of 2 years.”

3. How will the investigators measure and account for any treatment these overweight children may undergo while also enrolled in the COBRA longitudinal study? I imagine that if these are treatment-seeking children, they will be subject to interventions that may influence their health outcomes. How will their participation in and adherence to these interventions be accounted for?

We feel that this has now been addressed in the response to Point 1 (above), in that clinical data (including attempts at weight management by different means) will be collected at baseline and follow-up visits. This may allow identification of factors associated with success in controlling weight and avoidance of long-term disease, as outlined in the third specific question that can be addressed by COBRA (described on page 11).

4. The authors state children will be followed until age 18; is there a minimum age for the sample recruited?
There is no minimum age although, realistically, very few if any children are referred and managed through specialist weight management services before the age of 2 years. To address this in the manuscript, the text has been amended as described in point 2 above.

5. Within the environmental measures, please provide more detail as to what is assessed by the "parent questionnaire" and "child questionnaire."
I think Table 1 and the text regarding these questionnaires could be better linked and clarified. Is data being collected for both parents or just one?

All COBRA parent and child questionnaires, with details relating to which parent completes the parent-surveys, have now been uploaded as supplementary material, with the following sentence added on page 15:
“Parent and child questionnaires are available as supplementary material to this manuscript.”

Discretionary Revisions

1. The authors mention in the limitations section that the recruitment of treatment-seeking overweight and obese children may bias their sample.
Do the investigators have plans to seek out and recruit non-treatment-seeking overweight and obese children? This addition to the study protocol would greatly increase the generalizability of the biorepository.
This is an interesting point and would, as the reviewer suggests, add another dimension to the study. The difficulty, however, lies in the identification, recruitment and follow-up of overweight and obese children in families who are not treatment-seeking as, by definition, they do not present to specialist clinics and are likely to be less compliant with long-term follow-up. We have not, at present, added text to the manuscript explaining this further as it may lead to confusion relating to COBRA’s main study objectives. We would, however, be very happy to include this if the editorial committee / reviewers request it be included.

OTHER

1. We kindly request the addition of a few supplementary words on page 17. While it was originally proposed to use WAGER to house COBRA-generated data, it has become clear that other similar data repositories may be more beneficial to the international harmonisation proposed. We would therefore like to add “or similar resource” to the mention of data storage.

2. We request the addition of ‘Obesity Research Centre’ in the affiliation for Gary M Leong please.