Brief communication

MOLECULAR METABOLISM

Male-lineage transmission of an acquired metabolic phenotype induced by grand-paternal obesity

Jennifer E. Cropley [1,2,,8], Sally A. Eaton [1,2,8], Alastair Aiken [1], Paul E. Young [1], Eleni Giannoulatou [1], Joshua W.K. Ho [1,2], Michael E. Buckland [3], Simon P. Keam [4], Gyorgy Hutvagner [4], David T. Humphreys [1], Katherine G. Langley [5], Darren C. Henstridge [5], David I.K. Martin [1,6], Mark A. Febbraio [7], Catherine M. Suter [1,2,*]**

ABSTRACT

Objective: Parental obesity can induce metabolic phenotypes in offspring independent of the inherited DNA sequence. Here we asked whether such non-genetic acquired metabolic traits can be passed on to a second generation that has never been exposed to obesity, even as germ cells.
Methods: We examined the F1, F2, and F3 *a/a* offspring derived from F0 matings of obese prediabetic A^{vy}/a sires and lean *a/a* dams. After F0, only lean *a/a* mice were used for breeding.
Results: We found that F1 sons of obese founder males exhibited defects in glucose and lipid metabolism, but only upon a post-weaning dietary challenge. F1 males transmitted these defects to their own male progeny (F2) in the absence of the dietary challenge, but the phenotype was largely attenuated by F3. The sperm of F1 males exhibited changes in the abundance of several small RNA species, including the recently reported diet-responsive tRNA-derived fragments.
Conclusions: These data indicate that induced metabolic phenotypes may be propagated for a generation beyond any direct exposure to an inducing factor. This non-genetic inheritance likely occurs via the actions of sperm noncoding RNA.

Keywords Paternal effects; Epigenetic inheritance; Noncoding RNA; Sperm RNA

1. INTRODUCTION

Offspring phenotypes can be affected by parental health or parental environmental exposures, independent of variation in the inherited DNA sequence. Examples of such non-genetic transgenerational effects occur with a broad range of stressors, from dietary stress and toxin exposure to psychological stress or trauma (reviewed in [1]). In some instances, induced phenotypes can be observed across multiple generations, but whether such observations represent true inheritance of an acquired trait, or are merely a residuum of the original exposure, is not clear. This distinction has adaptive significance for organisms living in a changing environment, particularly given the potential for environmentally-induced epigenetic states to respond to selection [2,3].

In those cases in which induced phenotypes have been observed over more than one generation, determination of true inheritance is confounded by alternative scenarios, which can be very difficult to dissect. Persistence of an induced phenotype into the grand-offspring of compromised mothers can generally be attributed to the direct exposure of developing offspring germ cells to the initial stressor. Such a scenario underpins several recent reports of multi-generational programming by parental metabolism. For example, male mice exposed to a poor intrauterine environment can transmit metabolic defects to their own offspring [4–6]. Another potential confounder is 'serial' programming of the induced phenotype: that is, when an induced phenotype in F1 programs defects in F2, then those defects in F2 program F3, *et cetera*: in this scenario an induced phenotype can theoretically be propagated indefinitely, with no requirement for

[1]Molecular, Structural and Computational Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, 2010, Australia [2]Faculty of Medicine, University of New South Wales, Kensington, NSW, 2052, Australia [3]Brain and Mind Research Institute, University of Sydney, Sydney, NSW, 2006, Australia [4]Faculty of Engineering and Information Technology, Centre of Health Technologies, University of Technology Sydney, Ultimo, NSW, 2007, Australia [5]Cellular and Molecular Metabolism Laboratory, Baker IDI Diabetes and Heart Research Institute, Melbourne, VIC, 3004, Australia [6]Children's Hospital Oakland Research Institute, Oakland, CA, 94609, USA [7]Diabetes and Metabolism Division, Garvan Institute of Medical Research, Darlinghurst, NSW, 2010, Australia

[8] Jennifer E. Cropley and Sally A. Eaton contributed equally to this work.

*Corresponding author. Molecular, Structural and Computational Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, 2010, Australia. Tel.: +61 2 9295 8720. E-mail: c.suter@victorchang.edu.au (C.M. Suter).

**Corresponding author. Faculty of Medicine, University of New South Wales, Kensington, NSW, 2052, Australia. Tel.: +61 2 9295 8619. E-mail: j.cropley@victorchang.edu.au (J.E. Cropley).

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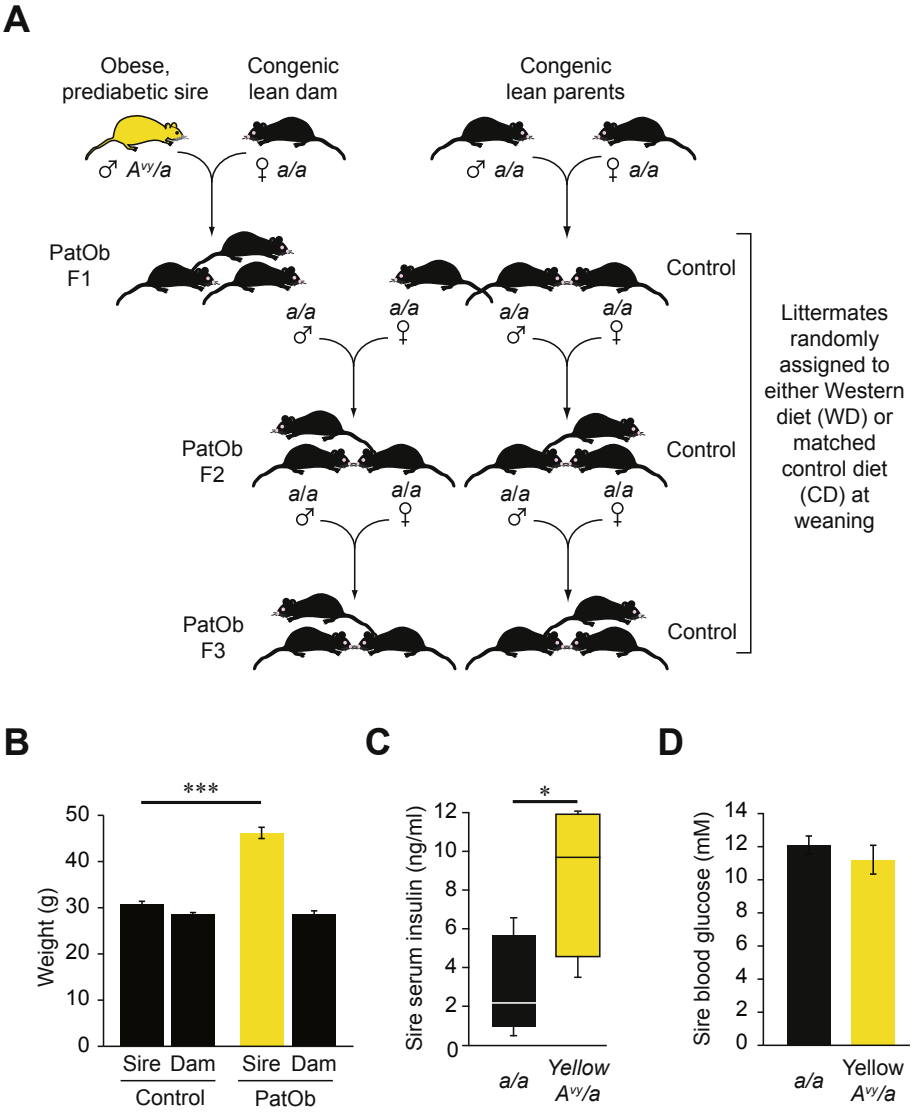

Figure 1: Experimental design and metabolic characteristics of obese founder males. (A) Schematic diagram showing breeding strategy. Obese yellow A^{vy}/a males were mated with congenic lean *a/a* dams to generate PatObF1 offspring. PatObF1 males were mated with Control F1 daughters, generated by mating lean *a/a* mice, to generate PatObF2. PatObF3 were generated by intercrossing PatObF2 males and females. For simplicity, A^{vy}/a offspring of obese yellow A^{vy}/a sires are not shown. **(B)** Weights of lean *a/a* sires and their *a/a* dams (Control, $n = 62$) and of obese yellow A^{vy}/a sires and their *a/a* dams (PatOb, n = 21) measured one week after birth of offspring. **(C)** Blood glucose of lean *a/a* and obese yellow A^{vy}/a males at 12 weeks of age ($n = 12$). **(D)** Serum insulin of lean *a/a* and obese yellow A^{vy}/a males at 12 weeks of age ($n = 5$). Error bars represent SEM; *p $<$ 0.05, ***p $<$ 0.001.

'epigenetic inheritance'. It has been proposed that serial programming resulting from a repeatedly compromised gestational environment may underlie most, if not all, examples of multigenerational maternal programming [7].

It is currently unclear whether an induced metabolic phenotype can be transmitted into successive generations without the continued influence of the stimulus; in other words, whether true, non-genetic inheritance can occur via gametes that were never exposed [8]. True inheritance of parental effects is arguably best studied through the male lineage, where confounding effects of the intrauterine environment can be largely excluded. Many paternal effects have been reported across a broad range of stressors [9]. In rodents, perturbed paternal metabolism can induce metabolic defects in first-generation offspring [5,10–12], but whether these effects can be carried into a second unexposed generation is not known. Epidemiological observations suggest it could be the case [13,14], however inheritance of something acquired is difficult to ascertain in human cohorts: genetic heterogeneity, lifestyle factors, and the necessarily retrospective nature of longitudinal studies are major confounders.

We have addressed the question of inheritance of induced metabolic traits using a congenic rodent model of obesity and pre-diabetes in which the dominant obesogenic allele can be segregated away from the offspring. In this model we find that paternal obesity induces a latent metabolic phenotype in F1 sons that is unmasked by short exposure to a Western-style high-fat diet. By breeding F1 sons maintained on a healthy control diet (those offspring exposed to paternal obesity but metabolically normal), we find that the induced but latent phenotype is inherited into a second, unexposed generation. We also find that this inheritance is associated with changes to the small RNA profile of F1 sperm, despite these sperm developing and maturing in metabolically normal environments.

