OBESITY: A stochastic basis for metabolic phenotypes

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STANDFIRST: It has long been recognised that some phenotypic variation in mammals cannot be explained by known genetic or environmental variables. Here, the authors show that the absence of Nnat expression is associated with polyphenism in mice with the same genotype. Broadly consistent effects are also found in humans.

Phenotypic variation that occurs even when both inter-individual genetic and environmental differences are controlled suggests there are additional dimensions that contribute to trait variation. The underpinnings of this "unexplained phenotypic variation" (UPV)[1] are likely multifactorial and the effects are frequently disregarded as random biological noise. However, model organisms show that such variation can originate even from single loci being regulated in a probabilistic manner to establish an "on" or "off" gene expression state[2]. The implication of such bimodal regulation is that it can give rise to predictable outcomes, which may be important in the context of human disease[2,3]. One of the challenges of identifying mediators of UVP is accounting for complex gene-environment interactions,

including environmental impacts during development, as well as modelling the complexities of gene-gene and gene-environment modifier effects[3]. Given the paucity of mechanistic understanding, it is not surprising that the contribution of UVP to trait variation in human populations is still enigmatic and somewhat controversial. Trait discordance between monozygotic twins does, however, suggest it is present, an interesting consideration given that genome-wide association studies of complex traits most often explain only a fraction of heritability.

In this issue of Nature Metabolism, the team led by Andrew Pospisilik[4] builds on their previous work where they characterised the effects of the epigenetic modifier, *Trim28*, as a factor that buffers against UPV[5]. Using a mouse model, they identify the maternally imprinted gene, *Nnat*, as a suppressor of alternative phenotypes in isogenic mice. They then extrapolate these findings to humans to show that expression levels of *Nnat* correlate with specific metabolic subtypes. Interestingly, they describe how genetic ablation of *Nnat* expression in an inbred, presumably isogenic background results in mice that demonstrate two distinct, but reproducible phenotypic states: one that is indistinguishable from wild-type and the other characterised as "overgrowth." The second population had increased lean and fat mass that was measurably different before the animals reached maturity. Even though *Nnat* expression was found to be downregulated in *Trim28* haploinsufficient mice[5], compound mutants demonstrate three distinct phenotypes, showing that the mechanisms by which each gene buffers against UPV are different. Remarkably, this shows the potential for a given genome to deliver three discrete and reproducible phenotypic outcomes that are probabilistically determined.

Using this model, Pospisilik and colleagues[4] delve into the physiological origins of the overgrowth phenotype, establishing that it is not due to alterations in growth hormone/insulin-like growth factor signalling, but is associated with elevated plasma insulin levels. Excess circulating insulin was due to increased proliferation of pancreatic beta-cells rather than altered functionality. In an elegant set of experiments, the authors convincingly demonstrate that the overgrowth phenotype is driven by

hyperinsulinemia. Transcriptomic analysis of pancreatic islets prior to observable phenotypic differences identified a set of genes regulated by histone deacetylase (HDAC) responsible for the switch to a hyperproliferative state, a finding confirmed *ex vivo*. Collectively, these findings show that *Nnat* functions to buffer against activation of HDAC-driven beta-cell hyperplasia in early life.

To identify if a similar probabilistic, bimodal phenotype can arise in humans in the absence of known genetic variation, the authors[4] assessed phenotypic discordance between monozygotic twins to identify two distinct patterns of variation, one with reciprocal fat and lean mass difference between twins and the other with coordinated lean and fat mass increase in the heavier twin, phenocopying the *Nnat* mice that demonstrated overgrowth. Transcriptomic analysis of adipose tissue revealed that *Nnat* expression differences between cotwins correlated with differences in lean and fat mass exclusively in the latter group, with lower *Nnat* expression in the heavier cotwin. No phenotype associations were observed with *Trim28* expression levels. In addition to *Nnat*-coupled fat and lean mass variation in this subpopulation, discordance in insulin levels was most pronounced in this group and also tightly correlated with body mass index, supporting a similar physiological basis for discordance in twins as in the distinct bistable phenotypes of *Nnat*-deficient mice. The unique classes of UPV observed in the twins was further reflected in unique patterns of between-twin variation in DNA methylation. Interestingly, only the class of variation associated with variable *Nnat* expression showed reproducible changes in regional methylation and these were enriched for proximity to metabolic disease loci, consistent with their observations in mice[4].

Finally, the authors show that the gene expression signature from adipose tissue defined using twins discordant for the “overgrowth”-like phenotype can be used to identify metabolic subtypes in unrelated individuals. Using single cell data to deconvolute the *Nnat*-associated signature suggests that it derives from increased adipose tissue inflammation. This signature is observed in childhood and is not strictly coupled to obesity but is associated with a higher obesity incidence. Importantly, obese individuals can be stratified into two distinct groups based on these molecular profiles.

This work highlights that mammalian phenotypes can be probabilistically determined in the absence of obvious genetic or environmental factors and that mechanisms exist to limit the resulting UPV[4-6]. This phenomenon can be a driver of metabolic outcomes in both mice and humans. Importantly, through a detailed molecular and phenotypic characterisation of the bistable phenotypes suppressed by *Nnat* in mice, Pospisilik and colleagues[4] provide mechanistic insight into an insulin-driven overgrowth. The presence of a similar molecular signature in humans raises the interesting question of whether this can be used clinically to stratify disease risk and treatment approaches. Another intriguing question is, how does genetic variation and environment influence outcomes? Strain background differences in mice have been reported to modify both the bimodal distribution and metabolic changes in paternally-inherited null allele animals[7]. Could a genome-wide association study for *Nnat*-dependent molecular signatures identify modifiers in humans? As the bistable phenotype in mice is detectable by weaning, it stands to reason that any environmentally induced modulation of the axis would be limited to earlier exposures. As *Nnat* expression levels can be regulated by nutrient status in adult tissues, it would also be interesting to query whether the *Nnat* axis responds to developmental programming[8,9]. While this work has many strengths, a key question that remains is, what is the "trigger" or "threshold" that underlies the observed bimodality? The observation that the *Nnat* and *Trim28* effects are independent in the same *in utero* context highlights the complexity of trying to deconvolute the contribution of multiple axes that could be independently regulated. Regardless, the work led by Dr. Pospisilik makes an important step forward in understanding the origin of UPV.

Conflict of interest: The authors declare no competing interests.

Understanding the origins of unexplained phenotypic variation through mouse models. Nnat is an imprinted gene expressed only from the paternal allele. The authors mate wild-type (WT) dams with

Nnat -/- sires, and analyse the Nnat +/-p offspring, in addition to a WT control arm (the WT mice are congenic to the Nnat +/-p mice). By the age of 4 weeks, a subgroup of Nnat +/-p mice start to diverge from WT mice, while the others do not. The divergent Nnat +/-p mice ('heavy') start gaining lean and fat mass ultimately resulting in an overgrowth phenotype, whereas the rest of the Nnat +/-p mice ('light') follow a growth trajectory similar to WT. Although the initial trigger that sets in motion the molecular pathways ultimately resulting in 'heavy' mice is unknown, the underlying physiology is pancreatic beta cell hyperplasia, leading to high circulating insulin driving the overgrowth relative to the light mice or WTs.

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