The fat mass and obesity-associated (FTO) gene was placed center stage when common intronic variants within the gene were robustly associated with human obesity. Murine models of perturbed Fto expression have shown effects on body weight and composition. However, a clear understanding of the link between FTO intronic variants and FTO activity has remained elusive. Two recent reports now indicate that obesity-associated SNPs appear functionally connected not with FTO but with two neighboring genes: IRX3 and RPGRIPL1. Here, we review these new findings and consider the implications for future analysis of GWAS hits.

When first cloned in 1999, Fto was postulated to have a role in programmed cell death and development. It was identified in the study of the “fused toe” (Ft) mouse, a model organism created by insertional mutagenesis that had a 1.6 Mb deletion on chromosome 8 (van der Hoeven et al., 1994). Mice heterozygous for the Ft mutation developed fusion of the first to fourth toes of the forelimbs and thymic hyperplasia (Peters et al., 1999). This deletion eliminated six genes (Peters et al., 2002) and three members of the Iroquois gene family (Irx3, Irx5, and Irx6) that form the IrxB cluster, as well as three other genes (Fts, Ftm, and Fto), which, at the time, were poorly characterized. Fts was initially called “Ft1,” being the first of the three to be identified; the second proved to be an elusive sequence to characterize technically, so it was labeled the Fantom (Ftm); the third gene was named “Fatso” (Fto) because of its large size (Fischer et al., 2009).

Setting a theme that plays throughout the studies of Fto biology, subsequent studies of the Ft mouse were taken up in trying to determine which of the affected genes in the larger deleted locus were responsible for the component parts of the diverse phenotypes seen in the animal. Furthermore, these initially mysterious genes underwent name changes to reflect evolving knowledge. Ftm was found to be a novel basal body protein of cilia involved in Hedgehog (Hh) signaling (Vierkotten et al., 2007). Sequence analysis revealed Ftm to be highly homologous to RPGRIPL1 (retinitis pigmentosa GTPase regulator interacting protein 1, a gene encoding a protein with a role in photoreceptor cells of the eye), and it was renamed Rpgrip1-like (Rpgrip1l). In mice, inactivation of Rpgr1l causes a multiorgan syndromic phenotype with cerebral, renal, and hepatic defects (Vierkotten et al., 2007), while mutations in RPGRIPL1 are the cause of a group of developmental disorders such as Joubert syndrome type B and Meckel syndrome (Delous et al., 2007).

With the coming of age of technologically advanced genetic association studies, Fto underwent a more subtle, but no less significant, name change, with the roots of the labeling acronym reflecting its new association with obesity and metabolism.

**A Star Is Born**

In the first half of 2007, three independent studies demonstrated a strong association between genetic variance within FTO and human obesity. The gene name morphed into “fat mass and obesity associated,” and the metabolic community had, at last, an example of common genetic variance with robust evidence of association with obesity in the general population.

The Frayling et al. (2007) study initially set out to search for type 2 diabetes mellitus susceptibility genes. In doing so, they identified a common variant in the FTO gene that, indeed, predisposed to diabetes but through an effect on body mass index (BMI). This effect on BMI was driven entirely through fat mass and, although not detectable at birth, was fully present by the age of 7 years and persisted through adulthood (Frayling et al., 2007). The major signal for association with BMI was a cluster of SNPs in the first intron of FTO. All BMI-associated SNPs were highly correlated with each other, but SNP rs9939609, having the highest genotyping success rate, was studied further. The 16% of adults who were homozygous for the risk allele A weighed close to 3 kg more and had 1.67-fold increased odds of obesity when compared with those not inheriting a risk allele (Frayling et al., 2007).

Complementing and confirming these findings, a contemporaneous report by Dina et al. (2007) took a different approach to discover this association, initially setting out to analyze the distribution of 48 neutral SNPs in a case-control obesity cohort collected from French individuals of European ancestry. While 47 of the 48 showed uniform distribution, the T allele of SNP rs1121980 (located within the first intron of FTO) was strongly associated with severe adult obesity. Building on this observation, Dina et al. demonstrated that several SNPs in the FTO locus were highly associated not only with severe obesity in other adult, European populations but also with severe childhood obesity.

The last of this triumvirate undertook a genome-wide association study in the genetically isolated population of Sardinia to identify genetic variants associated with obesity-related quantitative traits (Scuteri et al., 2007). Again, a number of common variants in FTO were associated with BMI, hip circumference, and total body weight.

The significant challenges were clear from the beginning. FTO was widely expressed throughout the body (Frayling et al., 2007; Dina et al., 2007; Gerken et al., 2007), and, at the time, nothing was known about function or potential pathways. All of these...
initial reports made it clear that there was no ready mechanism to explain how the predisposing intronic variants affected function or expression of FTO. Indeed, right from the start, the possibility that the underlying mechanism involved neighboring genes or other, as-yet- unidentified, more distant genes was raised. However, hot on the heels of these initial findings, there followed a report that gave some important clues to FTO biology. Bioinformatics analysis indicated that FTO shared sequence motifs with Fe(II)- and 2-oxoglutarate-dependent oxygenases, with assays using recombinant murine Fto showing that the protein could catalyze Fe(II)- and 2OG-dependent DNA demethylation, the preferred substrate being 3-methyl- thymine in DNA. Fto was also found to be localized to the nucleus and highly expressed in hypothalamic regions with critical roles for the control of energy balance in a nutritionally dependent manner (Gerken et al., 2007).

Subsequently, genome-wide association studies (GWASs) for obesity-related traits in a myriad of European ancestry populations confirmed that multiple SNPs at the FTO locus were associated with BMI (Graff et al., 2013;Bradfield et al., 2012; Lindgren et al., 2009; Meyre et al., 2009; Scherag et al., 2010; Spijker et al., 2010; Thorleifsson et al., 2009; Wheeler et al., 2013; Willer et al., 2009). Many other reports found the association of FTO SNPs with BMI in non-European-derived populations, including most populations of Asian ancestry, as well as Hispanic/Latino populations and Pima Indians (reviewed by Loos and Yeo, 2014).

Studies in populations of African ancestry, in which the FTO gene shows significant differences in allele frequency and linkage disequilibrium (LD) patterns, have proven to be insightful. Although several of the previously identified variants appear not to have significant association with BMI in these populations, other intronic variations around the FTO locus (such as rs3751812 and rs9941349) show strong evidence of association (Adeyemo et al., 2010; Hassanein et al., 2010). Furthermore, the most significant value in the report by Peters et al. (2013) came from a SNP (rs56137030) that had not been highlighted in previous studies. A recent meta-analysis by Monda et al. (2013) of data derived from populations of African ancestry also provided support for shared BMI loci across populations.

The association of FTO SNPs with obesity-related traits in children and adolescents was also extended (Bradfield et al., 2012; Meyre et al., 2009; Scherag et al., 2010). While SNPs in FTO did not influence birth weight (Hori koshi et al., 2013; Jess et al., 2008; Kipeläinen et al., 2011a), longitudinal studies revealed that the effect on body weight appeared during early childhood, reaching its peak at young adulthood (Graff et al., 2013; Hardy et al., 2010; Sovio et al., 2011).

This initial wave of association studies was soon followed by reports considering whether FTO SNPs associated with more specific regulators for energy homeostasis, such as food intake or physical activity.

Data pointing to a link with food intake began to emerge. Obesity-associated FTO SNPs were found to be associated with increased energy intake (Cecil et al., 2008; Speakman et al., 2008; Timpson et al., 2008), increased intake of dietary fat (Park et al., 2013; Timpson et al., 2008) or protein (Sonestedt et al., 2009), increased appetite and reduced satiety (Wardle et al., 2008, 2009), and loss of control over eating (Tanofsky-Kraff et al., 2009). This link with food intake was not seen in every study; for example, a report by Stutzmann et al. (2009) failed to find an association of the FTO rs 1421085 C allele with eating behavior traits in a large European cohort of children and adults. However, a recent GWAS of macronutrient intake in more than 70,000 individuals identified the BMI-increasing allele of FTO SNPs as highly significantly associated with increased protein intake (Tanaka et al., 2013).

Other studies have consistently shown that FTO SNPs are not associated with physical activity levels (Ahmad et al., 2010; Franks et al., 2008; Speakman et al., 2008; Vimaleswaran et al., 2009). However, low physical activity has been reported to accentuate the effect of FTO risk alleles on obesity in French adults (Cauchi et al., 2009), with a large-scale meta-analysis of published and unpublished data of more than 200,000 adults and 20,000 children (Kipeläinen et al., 2011b) concluding that the association of FTO SNPs with the odds of obesity are reduced by close to a third in physically active adults. Phenotypic variability in BMI for a given FTO genotype was also reported by Yang et al.’s (2012) report highlighting the potential of environmental modifiers to influence the “genetic burden” of the FTO risk alleles.

Model Organisms and Fto

The phenotype of mice with a global germline loss of Fto was first reported in 2009 (Fischer et al., 2009). Fto null mice suffered from a high perinatal mortality and a postnatal period characterized by reduction in both body length and body weight, the latter being a result of reduced fat and lean mass. They were reported to have both an increase in food intake and an increase in metabolic rate. Later analyses of several independent murine models of Fto loss also confirmed this pattern of postnatal growth retardation, but one report has shown that, when corrected for alteration in body composition, there was no difference in energy expenditure in mice globally lacking Fto (McMurray et al., 2013).

In 2010, Cox and colleagues in Harwell, UK, generated mice globally expressing additional copies of the Fto gene (Church et al., 2010). This ubiquitous overexpression of Fto increased body and fat mass, with the obese phenotype more marked on a high-fat diet. Food intake was significantly increased, but energy expenditure and physical activity were unaltered in these mice.

Reports of more specific regional and temporal Fto perturbation soon emerged. Gao et al. (2010) used a conditional allele to delete Fto in the nervous system. Their findings that this resulted in similar phenotypes as that of the whole body deletion pointed to Fto having a crucial role in the central nervous system to regulate postnatal growth. Following from the finding that Fto expression in the arcuate nucleus of the hypothalamus was nutritionally regulated (Gerken et al., 2007; Stratigopoulos et al., 2011; Poritsanos et al., 2011), we targeted this region with stereotactically delivered adeno-associated virus and demonstrated that overexpression decreased food intake while knockdown of Fto increased food intake (Tung et al., 2010).

McMurray et al. (2013) also used a conditional allele to globally delete Fto at 6 weeks of age. Development in the weeks after was characterized by a loss of lean mass but an increase in fat mass (McMurray et al., 2013). Intriguingly, regional deletion of Fto in mediobasal hypothalamus of adult mice did affect feeding behavior but did not affect body composition, which is
suggestive of Fto expressed in nonhypothalamic sites having a role in controlling lean mass.

Thus, although many questions remained unanswered, from these animal data at least, it seemed plausible that Fto had a role in the regulation of body composition and energy balance.

**Novel Functions, Unexpected Roles**
How then does one to try and link these murine and association studies with a common molecular mechanism, particularly one involving the demethylase activity reported by Gerken et al. (2007)? The family of demethylase enzymes had long been known to have a role in the repair of methyl lesions in DNA and RNA (Ougland et al., 2004; Lee et al., 2005) but had little to link them directly to control of metabolism and body composition. Further studies followed that confirmed that FTO catalyzed the demethylation of 3-methylthymine in single-stranded DNA, as well as 3-methyluracil (3meU) (Gerken et al., 2007; Jia et al., 2008) and 6-methyl adenosine (6meA) (Jia et al., 2011) in single-stranded RNA.

The crystal structure of FTO was resolved and showed an N-terminal catalytic domain and a C-terminal domain of unknown function (Han et al., 2010). The specificity for single-stranded nucleic acids appeared to come from an L1 loop, not present in other members of the AlkB family, that acted to hinder double-stranded nucleic acids from entering the catalytic pocket (Han et al., 2010).

The finding that 6meA, the most common modified nucleoside found in messenger RNA (mRNA) (Desrosiers et al., 1974), was demethylated by FTO, with 50-fold greater affinity than 3meU (Jia et al., 2011), gave rise to a hypothesis in which FTO influenced metabolism by posttranscriptional modification of RNA message. This idea was featured in two studies in 2013 (Hess et al., 2013; Karra et al., 2013) that explored how FTO’s putative demethylase action might affect complex human phenotypes.

**FTO and Dopamine**
Hess et al. (2013) described a role for Fto in controlling the dopaminergic circuitry within the midbrain. The idea to explore a putative Fto-dopamine link came about because the phenotype of the Fto null mouse closely matched the description of mice lacking dopamine receptor type 2 (D2R) (Sibley, 1999). Their studies found that Fto null mice responded differently to controls animals after administration of cocaine, a drug that can inhibit the dopamine transporter and, thereby, increase synaptic dopamine levels. In response to cocaine, Fto-deficient mice failed to show an increase in locomotor activity; had less c-fos activation in the ventral tegmental area, caudate putamen, and nucleus accumbens; and had a blunted increase in extracellular dopamine. Further studies, in which pharmacological agents known to act through the dopaminergic system were administered to a range of genetically engineered animals, confirmed that Fto loss impairs D2R-like signaling in a cell-autonomous manner.

An elegant component to this story combined methylated RNA immunoprecipitation with bioinformatics to try and link these novel findings with previous data on the demethylase action of FTO. By identifying transcripts containing m6A in midbrains and striatum from control and Fto-deficient mice, Hess et al. (2013) found that the transcripts selectively methylated in Fto null mice were disproportionately linked to synaptic transmission and cell signaling. Furthermore, in the absence of Fto, both mRNA and protein of key regulators of dopaminergic neuron activity showed increased methylation.

The identification of FTO-specific adenosine methylation on a subset of mRNA involved in dopamine signaling pathway was the first in vivo demonstration of FTO as a functional m6A demethylase. These observations also provided the first indication that FTO may play a tissue- or even cell-type-specific role by regulating specific subsets of mRNA relevant to the physiological function of the tissue, and this might help explain how a single protein can appear to have such widespread influence over a range of seemingly disparate functions.

**Ghrelin and the Brain: Is FTO the Missing Link?**
The findings of the Hess et al. (2013) study gave Fto a potential role in the reward systems. Following shortly afterward, a study by Karra et al. (2013) pursued a mechanistic link between FTO SNPs and gut peptides. They hypothesized that FTO variants might not only influence circulating levels of hormones known to have a role in appetitive behavior but might also affect the neuronal response to food cues.

Their studies focused on small numbers of young, normal-weight volunteers divided into two matched groups on the basis of their genotype at the rs9939609 locus, either high risk (AA) or low risk (TT). Compared to TT subjects, after a test meal, the AA group was reported to have a less marked fall in acyl ghrelin and an attenuated suppression in a visual analog hunger score.

Analysis of mRNA from peripheral blood cells after a period of fasting showed that subjects with the AA genotype had, in comparison to the TT group, a 1.5-fold increase in FTO expression, a 2.5-fold increase in ghrelin precursor GHRL mRNA abundance, and a reduction in m6A methylation of ghrelin mRNA. fMRI analysis showed between-genotype differences in a number of brain regions recognized to have roles in energy homeostasis and reward related activity.

A further potential link to ghrelin was suggested by data from in vitro studies where FTO overexpression increased not only ghrelin and ghrelin O-acyltransferase mRNA but also total and acyl ghrelin concentrations in cell lysates. FTO overexpression was also reported to reduce m6A methylation of ghrelin mRNA.

These data led Karra et al. (2013) to the suggestion that the association of FTO risk allele with food intake may be mediated through ghrelin, with the risk allele increasing FTO expression, which, in turn, reduces m6A ghrelin mRNA demethylation and, thereby, alters ghrelin production. The differential responses on fMRI observed in subjects with SNP were certainly striking because such phenomena have only been demonstrated previously in situations with drastic physiological differences (De Silva et al., 2012), such as comparing lean versus obese individuals or fed versus fasted state, or in the exogenous administration of appetite-modulating hormones (e.g., ghrelin, PYY, GLP-1, and leptin).

While this work is of interest, it is worth noting that leucocytes are not recognized as the natural home for understanding gut hormone biology, and the sizeable changes in mRNA levels in white cells were not reflected in differences in the amount of circulating active acyl-ghrelin seen in the fasted state. Although there are measurable relative changes in white cell levels, the absolute contribution from these cells to total biological active
ghrelin may not be large. It remains to be determined how, if at all, risk genotype affects ghrelin expression and production in the stomach and whether what is happening in a leucocyte bears any relationship to what might be happening within the antrum of the stomach. Additionally, it is worth noting that several studies have failed to reveal any influence of the FTO genotype on mRNA level of FTO; although mRNA expression of FTO in adipose tissue has been shown to be greater in obese individuals, this difference did not extend to FTO expression in blood cells. The fact that mice globally lacking Fto have unchanged levels of acylated ghrelin in both the fed and fasted states also suggests that Fto levels contribute little to circulating levels of ghrelin.

Loss-of-Function Mutations in Humans

Despite all these emerging data, there remained the recurring uncertainty of what these findings brought to our greater understanding of human disease, the primary driver for undertaking the genetic studies in the first place.

Important data on the potential consequences of FTO loss in humans came from a study of a consanguineous family in which affected individuals presented with a previously unreported autosomal-recessive syndrome characterized by polymalformation and childhood mortality (Boissel et al., 2009). Genome-wide autozygosy screening identified a unique region of shared homozygosy on chromosome 16q12. Further genotype and haplotype analyses reduced the critical region to a 6.5 Mb interval encompassing 28 genes. After sequencing coding regions and splice junctions of all known and putative genes in this linked region, the only mutation found was a homozygous single-nucleotide variation at complementary DNA position 947 within the FTO gene. Affected individuals carried a homozygous R316Q mutation in FTO, with loss of this highly conserved arginine residue rendering FTO enzymatically null in two different in vitro assays; the first based on the conversion of the 2-oxoglutarate to succinate and the second based on the ability of FTO to demethylate 3-methylthymine in DNA. The affected individuals homozygous for a catalytically inactive FTO also had, like Fto null mice, an early growth retardation phenotype but, unlike the Fto null mice, also had developmental abnormalities in the central nervous or cardiovascular systems. These differences are not readily explained, and the molecular mechanisms whereby the mutant FTO leads to the severe phenotype observed these patients remain unknown. However, it may be that the point mutation in FTO has deleterious gain-of-function effects and/or dominant-negative interference with other biochemical processes that are not seen with the complete deficiency seen in the Fto null mouse.

What of other members of this extended family? Fto<sup>+/−</sup> mice were considered to be resistant to diet-induced obesity (Fischer et al., 2009), so could heterozygous carriage of this deleterious R316Q mutation actually protect against obesity? This also remains uncertain because, although this report comments that none of the parents of the affected children were obese, detailed phenotypic data on the extended family has not been reported.

Other studies have gone on to investigate whether nonsynonymous variants of FTO might be enriched in either lean or obese subjects. Meyre et al. (2010) sequenced entire FTO coding regions in both subjects with severe obesity and in individuals with lifelong leanness. Interestingly, nonsynonymous mutations were equally common in both the obese and lean cohorts. Furthermore, heterozygous mutations that severely impaired enzymatic activity of FTO were found in both lean and obese subjects who were otherwise clinically unremarkable.

Other studies in lean and obese cohorts of children brought similar findings. In both African American and Chinese Han populations, variants were identified in FTO, but the overall frequencies were similar in case and control, with none conferring risk of obesity (Deliard et al., 2013; Zheng et al., 2013).

Taken in isolation, these human data fall some way short of a compelling case for FTO being the candidate mediator of the obesity association. Murine data indicate that an increase in Fto expression could potentially lead to obesity, but, to date, only deleterious point mutations have been described in obese patients. However, it may be that there are as-yet-uncharacterized patients in other cohorts with genetically deleterious mutations leading to complete loss of FTO. For example, such an individual may, if one were to phenocopy the mouse model of Fto deficiency, present initially as a “failure to thrive” and thereafter be noted to be of small stature.

An Interesting Neighborhood

As outlined earlier, the initial FTO intronic associations, followed by identification of association SNPs in intron 3 (Tönjes et al., 2010) and intron 8 (Adeyemo et al., 2010), set in train a plethora of studies based around Fto biology. However, other groups took a different path, with the Leibel group, in particular, choosing to focus on Rpgrip1 as being the potential mechanistic link (Stratigopoulos et al., 2008, 2011). They initially took an in silico bioinformatics approach to focus on two intronic SNPs (rs17817449 and rs8050136) and predicted that they were located in a putative binding site for the transcription factor CUTL1 via a single regulatory site in the first intron of FTO. Initial in vitro data were promising. In chromatin immunoprecipitation of DNA from human fibroblasts using a CUTL1-specific antibody, a 90-base-pair fragment that included rs8050136 was precipitated. Furthermore, small interfering RNA-mediated reduction of Cut1 by 70% resulted in FTO expression decreasing by 90% and Rpgrip1L by 65%.

Having raised the possibility that both genes were regulated by CUTL1/CUX1 via a single regulatory site in the first intron of FTO, Leibel’s group looked, in more detail, at the role of this transcription factor on the different isoforms (the larger P200 and the smaller p100 isoform, generated from p200 by enzymatic cathepsin L activity) (Stratigopoulos et al., 2011).

A series of detailed biochemical and promoter analysis experiments led to a proposed mechanism whereby, together, FTO, Rpgrip1L, and CUX1 facilitate the leptin response within the hypothalamic arcuate nucleus and thereby affect food intake. The model proposed that reduced circulating leptin, as seen in negative energy balance, results in the reduced enzymatic processing of CUX1 P200 and decreased CUX1 P110 protein levels. In turn, this change in transcription factor isoform ratio decreases Rpgrip1L and FTO expression, reducing leptin signaling through an alteration in leptin receptor recruitment at the cilium.
and thereby increasing the drive to eat. The proposed model goes on to suggest that, because P110 has reduced affinity for the rs8050136 obesity-risk A allele but higher affinity for the protective C allele, individuals with the C allele would have higher FTO and RPGRIP1L expression and be relatively more leptin sensitive (Figure 1A).

Building on their previous findings, this group has more recently published a detailed metabolic phenotype of mice lacking one copy of Rpgrip1l (Stratigopoulos et al., 2014). As noted previously, homozygous mutations in Rpgrip1l led to significant developmental anomalies (Vierkotten et al., 2007), but Rpgrip1l+/C0 mice had a small but statistically significant 10% increase in body weight demonstrable by 10 weeks old (Figure 1B). Analysis of older animals showed this to be driven by an increase in fat but not lean mass. By 19 weeks old, male mice had an increase in both fat and lean mass, with absolute food intake corrected for different body compositions showing Rpgrip1l+/C0 to be hyperphagic.

They concluded that alteration in leptin receptor signaling, brought about as a direct consequence of alteration in the function of primary cilia, accounted for the increase in fat mass of Rpgrip1l+/− mice. These model organism studies are presented as further supportive evidence that individuals with obesity risk alleles at rs8050136 have reductions in both RPGRIP1L and FTO expression due to reduction on p110 at cognate CUX1 binding, causing diminished leptin signaling, increased food intake, and adiposity.

Disorders of ciliary biology have long been linked to “ syndromic obesity,” and these data from the Leibel group now raise the intriguing possibility of primary cilia having a role in common forms of obesity (Stratigopoulos et al., 2008, 2011). However, there remains still that elusive, direct connection between obesity-associated variants and expression of the gene in question: in particular, whether in humans the number of copies of the risk allele associates with changes of expression of RPGRIP1L and FTO, not only in the direction predicted by the model but also in physiologically relevant tissue.

IRX3: Another Interesting Neighbor

Irx3 was one of the initial genes identified within the Ft region but initially did not attract as much attention as its neighbors did. A report by Ragvin et al. (2010) reignited interest in its relationship with FTO. This group focused on the fact that the FTO LD block appeared to contain numerous highly conserved noncoding elements (HCNEs) and used computational analysis to determine if these potential regulatory elements might affect target genes elsewhere.
Their initial analysis suggested that the target gene of the
HCNE within FTO was IRX3 and, using sequences from the
obesity-associated FTO LD block in a zebrafish-based, green
fluorescent protein reporter assay, found that expression pat-
terns matched those of IRX3. As IRX3 is highly expressed in
pancreas, they went on to suggest that at least some of the effect
of the FTO locus on susceptibility to type 2 diabetes may be
through changes in insulin secretion.
Smemo et al. (2014) built on these observations in a compre-
hensive study bringing together evidence from murine, human,
and in vitro studies to make a compelling case for the involvem-
et of IRX3 in the association between human obesity and FTO.
They initially investigated cis-regulatory interactions between
FTO and IRX3, using chromatin conformation capture in both
embryonic (mice and zebrafish) and brain tissue from adult mice.
They found that the promoter of Irx3 strongly interacted
with the obesity-associated interval within FTO (Figure 1A).
Results from an in vivo mouse reporter assay that tested hu-
man DNA fragments from the FTO obesity-associated region,
plus data from studies using a human bacterial artificial chromo-
some (BAC) spanning the FTO locus that included its promoter
and the obesity-associated region, clearly showed that IRX3
relied on long-range regulatory input from FTO.
Building on this, a crucial data set missing from previous
studies looked at gene expression levels in the cerebellum, a
site of high expression of both FTO and IRX3. This expression
quantitative trait loci (eQTL) mapping in human brain samples
demonstrated obesity-linked SNPs such as rs9930506
were associated with IRX3 expression, but not with expression
of FTO, thereby directly linking these variants to IRX3 regulation
(Figure 1B).
Returning to mouse models, Smemo et al. (2014) reported that
Irx3 null mice had a significant reduction in body weight, with less
fat and lean mass compared to wild-type controls. Transcripti-
onal analysis of tissue indicated that there may be increased
sympathetic tone to white adipose tissue, as well as increased
activation of brown adipose, leading to increased energy
expenditure that contributes to the phenotype seen. Using Cre-lox
technology to express a dominant-negative Irx3 only within the
hypothalamus, this group determined that disrupting Irx3 func-
tion in this region alone recapitulated the metabolic phenotype
of Irx3-deficient mice, thereby supporting the notion that hypo-
thalamic Irx3 is critical in the regulation of body composition.
While this appears to be a compelling set of data, there are still
some areas of uncertainty. The cerebellum is not an area of brain
normally recognized to be involved in the control of energy
expenditure and eQTL analysis using hypothalamic tissue seems
an important next step. Furthermore, it would be very inter-
esting to determine if overexpression of Irx3, either globally or in
a more tissue-specific manner, causes obesity in a model organ-
ism. Nevertheless, it would appear that IRX3 has a strong candi-
dacy to be a major mediator of the effects of the common intronic
SNPs on human adiposity.
Discussion
The technological and computational advances that underpin
genetic association studies have brought forth a glut of new
information relevant to a whole host of different human pheno-
types, complex traits, and disease conditions. They have much
potential to reveal previously unanticipated insights, but as
powerful as the data generated are, there remains the need to
move from an association map into systems, models, and plat-
forms that can be used as tools to get to grips with the biological
architecture of the problem under scrutiny.
So, 7 years on from the initial cluster of reports, how far have
we come with the poster boy of metabolic GWASs?
The data set from animal studies make a decent case that FTO
has a role in the control of energy homeostasis and body compo-
sition. However, there is still much to learn. Whereas initial
studies understandably focused on regions of the brain that
are well characterized to have a role in energy balance, there
remains uncertainty not only as to which brain regions are most
critical but also the role Fto may play in peripheral tissues such
as skeletal muscle and fat.
Taken in isolation, the data on FTO from human studies fall
some way short from making a compelling case for FTO as a
candidate mediator of the obesity association. As described
earlier, the most deleterious mutation has been identified in a sin-
gle extended family with a multisystem phenotype that caused
significant morbidity and early mortality. To date, there appear
to be no reports of deleterious encoding IRX3 mutations being
found in populations of lean or obese; these would be a welcome
addition to the field.
Can we learn wider lessons on how to tackle the ever-growing
list of “hits” from metabolically relevant association studies?
Although murine models have proven invaluable in the study of
monogenic disorders of energy balance and appetitive behavior,
it is naive to assume that murine models alone can give us all the
answers. A comprehensive perspective that clearly lays out
many of the issues surrounding the determination of pathoge-
nicity (or otherwise) of genetic variance can be found in a recent
article by MacArthur et al. (2014), where the primacy of robust
genetic support for causation is rightly emphasized. Further-
more, the recent paper by Smemo et al. (2014), highlighting the
role of IRX3, is an exemplar of how a multitiered combination
of techniques and evidence need to be marshaled together.
The eQTL data presented are key pieces of evidence, and in
the future, when determining if noncoding SNPs are exerting a
functional impact through the alteration of gene expression, a
stronger emphasis on eQTL mapping seems a sensible step for-
ward. Material contained with the GtEX data set, for example,
where a larger number of human tissues have been used for
eQTL mapping (http://www.gtexportal.org/home/), is likely to
be a valuable resource.
Many of the strongest signals coming from GWASs spotlight
genetic loci located some distance from coding exons. Sticking
a pin in the nearest named coding sequence and studying the
consequence of loss of that gene is one very simple way to
move forward. However, if we are to learn anything from the iter-
ative process that the FTO story has been to date, then we learn
that it is only by embracing all the technologies at our disposal
will we be able to move from GWAS signals to biological under-
standing.

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