Conserved immunomodulatory transcriptional networks underlie antipsychotic-induced weight gain

Rizaldy C. Zapata1,3, Besma S. Chaudry1,3, Mariela Lopez Valencia1, Dinghong Zhang1, Scott A. Ochsner2, Neil J. McKenna2 and Olivia Osborn1✉

© The Author(s) 2021

Although antipsychotics, such as olanzapine, are effective in the management of psychiatric conditions, some patients experience excessive antipsychotic-induced weight gain (AIWG). To illuminate pathways underlying AIWG, we compared baseline blood gene expression profiles in two cohorts of mice that were either prone (AIWG-P) or resistant (AIWG-R) to weight gain in response to olanzapine treatment for two weeks. We found that transcripts elevated in AIWG-P mice relative to AIWG-R are enriched for high-confidence transcriptional targets of numerous inflammatory and immunomodulatory signaling nodes. Moreover, these nodes are themselves enriched for genes whose disruption in mice is associated with reduced body fat mass and slow postnatal weight gain. In addition, we identified gene expression profiles in common between our mouse AIWG-P gene set and an existing human AIWG-P gene set whose regulation by immunomodulatory transcription factors is highly conserved between species. Finally, we identified striking convergence between mouse AIWG-P transcriptional regulatory networks and those associated with body weight and body mass index in humans. We propose that immunomodulatory transcriptional networks drive AIWG, and that these networks have broader conserved roles in whole body-metabolism.

INTRODUCTION
Antipsychotic drugs are effective medications for the treatment of psychiatric disease but have significant side effects, including antipsychotic-induced weight gain (AIWG) [1, 2]. AIWG has been shown to increase the risk of both developing metabolic syndrome [3] and of mortality [4]. Approximately 20% of patients treated with a broad range of APs gain clinically significant amounts of weight (>7% of their baseline weight) [1]. Notably, the incidence of diabetes among second-generation antipsychotic users is four times higher than age-matched, race-matched, and sex-matched controls [5]. Metabolic side effects are also the most commonly reported reason for noncompliance with second generation antipsychotic medications [6]. Although almost all antipsychotics result in some degree of weight gain [1], the extent of weight gain varies between individuals [7, 8]. Some individuals can gain a pound per week of treatment, while others are relatively refractory to weight gain [1, 9–13].

Olanzapine is one of the most clinically effective antipsychotic drugs but also results in highly significant weight gain in many patients [1, 2, 9, 13, 14]. Therefore, there is a need to determine which patients are less susceptible to the metabolic side effects of antipsychotics and would be good candidates for drugs such as olanzapine, and conversely, identifying patients that should be prescribed alternative antipsychotics with less weight gain liabilities. Although olanzapine is known to target multiple receptors including serotonergic (5-HT2 and 5-HT3), dopaminergic (D2, D3, and D4), muscarinic (M1–5), α2 adrenergic and histaminergic H1 receptors, the underlying biological mechanisms responsible for AIWG are incompletely understood. Previous studies have shown that the effect of olanzapine on AIWG can be effectively modeled in mice [15, 16]. Here, we used gene expression profiling in a mouse model to illuminate signaling pathways predisposing to AIWG, and to identify genes with potential as blood-based biomarkers of AIWG in humans.

MATERIALS AND METHODS

Mouse studies
Antipsychotic-induced food intake and weight gain study. Forty female C57BL/6J mice (stock #000664) were purchased from Jackson Laboratory at nine weeks of age. After seven days of acclimation to experimental conditions (12:12 light–dark, 20–21 °C, 50% humidity), blood was collected and total RNA isolated from blood using Mouse RiboPure Blood RNA Isolation Kit (AM1951, Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. All mice were transitioned to a 45% high-fat diet (HFD) compounded with 54 mg/kg olanzapine (Research Diets, Inc., D09092903) for 14 days. The olanzapine dose selected results in mouse plasma levels (21 ± 5 ng/mL) that are similar to the levels observed in humans treated with olanzapine (10–50 ng/mL) [17]. All 40 mice were singly housed throughout the study, and food intake was measured daily, and body weight was measured every other day for 14 days. At the end of the study, mice were anesthetized and sacrificed. Hypothalami were dissected and stored at –80 °C for qPCR analysis. Food intake was analyzed for all 40 mice. We then classified the top five weight gainers as weight gain “prone” mice (AIWG-P) and the five least weight gainers as weight gain “resistant” mice (AIWG-R). Sample sizes were selected based on previous studies [15]. No randomization or blinding was applied in these studies. All experiments were approved by and conducted in accordance with the University of California, San Diego IACUC.
**High-fat diet-induced weight gain study.** Baseline blood was collected from C57BL6 mice and total RNA isolated using Mouse RiboPure Blood RNA Isolation Kit (AM19151, Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Starting body weight was recorded before mice were transitioned from normal chow to a 45% high-fat diet (HFD) for 14 days, at which point body weight was recorded and body weight gain calculated. Mice were then divided into high-fat diet-induced weight gain-prone (HFWG-prone) and HFWG-resistant groups.

**RNA sequencing**

The RNA extracted from blood was sequenced at the UCSD Institute for Genomic Medicine. The quality of the RNA was assessed using the Tapestation 2200 (Agilent) and Libraries prepared using TruSeq Library prep kits (Illumina), and then run on the Tapestation high-sensitivity DNA assay kits to ensure the correct library size. Libraries were quantified using the Qubit® 2.0 Fluorometer, pooled, and run on the Illumina NovaSeq 6000 (Illumina). Reads were mapped to the mouse transcriptome using Bowtie2 as previously described [22, 23]. Consensomes are gene lists ranked by PANTHER Gene Ontology analysis. Total RNA was isolated using Trizol (Invitrogen, Carlsbad, USA) and purified using RNAeasy (Qiagen) according to the manufacturer’s protocol. RNA concentration and quality were measured on a NanoDrop (NanoDrop Technologies, Rockland, DE, USA). CDNA was prepared and qPCR performed with Perfecta FastMix (95073-05 K, VWR) and specific primers (Supplementary file section 1). Gene expression was normalized usingPgk1 and Hprt as the housekeeping genes using the ΔΔCt method. Data were analyzed by Student’s t-test. Normal distribution was tested using Shapiro-Wilk test prior to proceeding with a Student’s t-test with Welch’s correction. The Kruskal-Wallis test was used to compare percentage between experimental groups. p < 0.05 was considered statistically significant using Graphpad Prism.

**RESULTS**

**Olanzapine treatment results in variation in weight gain in mice**

Although all mice gained weight in response to olanzapine/HFD feeding, the extent of weight gain varied from ~1 g to ~7 g over the course of 14 days of treatment (Fig. 1A). We identified the five mice that gained the most weight (‘prone’, AIWG-P) and compared them with five mice at the other end of the spectrum that were ‘resistant’ to weight gain (AIWG-R). The average weight gain of the AIWG-P group was 6.3 g compared with 1.3 g in the AIWG-R group (Fig. 1B). Throughout the study, the AIWG-P mice ate approximately 0.5 g (19% more food) per day than the AIWG-R group (average ‘AIWG-P’ = 3.1 g/day vs ‘AIWG-R’ = 2.6 g/day) (Fig. 1C, D). After 14 days of treatment, the mice were sacrificed, and tissues dissected. As expected, AIWG-P mice had significantly higher gonadal white adipose fat mass (gWAT) (Fig. 1E) and greater liver mass (Fig. 1F) than the AIWG-R group.

Given that olanzapine is known to induce orexigenic gene expression in the hypothalamus [15], we next compared hypothalamic expression of appetite-regulating genes in the AIWG-P and AIWG-R groups. Hypothalamic expression of Aggr (agouti-related neuropeptide), Hcrt (hypocretin neuropeptide precursor), and Mch (melanin-concentrating hormone) was strongly elevated in the AIWG-P cohort relative to the AIWG-R cohort. In contrast, expression of the anorexigenic genes Pomc (proopiomelanocortin) and Crtt (cocaine and amphetamine-related transcript) was lower in the AIWG-P relative to the AIWG-R cohort (Fig. 1G).

We next prepared RNA from blood taken at baseline from individuals subsequently assigned to the AIWG-P and AIWG-R cohorts and conducted RNA sequencing (Fig. 2A). We identified 558 genes that were significantly differentially expressed (FC > ± 1.25, p < 0.05) at baseline between the AIWG-P and AIWG-R cohorts (Fig. 2B and Supplementary file section 2), of which 389 were elevated in AIWG-P > AIWG-R (AIWG-P gene set), and 169 were elevated in AIWG-R > AIWG-P (AIWG-R gene set). Panther Gene Ontology (GO) analysis [25] of the AIWG-P gene set indicated enrichment of genes mapping to a number of metabolic processes with known relevance to lipid metabolism (Fig. 3; Supplementary file section 3). For example, the overlapping porphyrin (GO:0006778; q = 8e-8) and tetracyrrole (GO:00033013; q = 3e-5) metabolic process pathways encompass genes encoding enzymes with characterized roles in energy metabolism, including Hmox1 [26, 27], Alas1, and Alad [28]. Similarly, mitochondrial autophagy (GO:0000422; q < 4e-3) is a fundamental process in cellular energy metabolism that has been linked to a spectrum of signature disorders of the metabolic syndrome, including type 2 diabetes and obesity [29–32]. Of particular interest given the well-characterized connection between immunity, inflammation and obesity [33, 34] was the enrichment for the immune system (GO:0002376; q < 1e-2) process (Fig. 3).
Fig. 1  Weight gain and food intake in response to olanzapine treatment. A. Individual daily weight gain in all 40 mice. B Average weight gain. C Average daily food intake. D Average food intake across 14 days of treatment. E gWAT. F Liver weight. G Hypothalamic gene expression in top five most weight gain ‘prone’ mice and five most ‘resistant’ to weight gain. *p < 0.05 determined by students t-test and corrected with 2-stage set-up method of Banjamini Krieger and Yekutieli for multiple comparisons with FDR = 0.10. Data are expressed as mean ± standard error.

Fig. 2  RNA sequencing analysis displays differential gene expression between ‘prone’ and ‘resistant’ cohorts. A. Principal component analysis. B Heat map of differentially expressed genes between cohorts.
AIWG transcriptional regulatory networks are enriched for immunomodulatory and inflammatory signaling nodes

Although the Panther GO analysis illuminated processes in which AIWG-P genes were functionally involved, it was much less informative on the extent to which genes in the AIWG-P and AIWG-R sets had common upstream transcriptional regulators. The Signaling Pathways Project (SPP) curates archived ‘omics’ datasets to compute consensus-regulatory signatures, or consensusomes, which rank genes based on measures of the strength of their transcriptional regulatory relationships with specific upstream signaling pathway nodes [22]. As such, SPP represents a reduced-bias environment within which to identify high-confidence transcriptional targets (HCTs) of these nodes. We recently described HCT intersection analysis, in which we computed coronavirus infection HCTs against human signaling node HCTs to identify candidate host pathways mediating the transcriptional response to CoV infection [23]. To gain insight into signaling pathway nodes whose gain or loss of transcriptional function contributes to AIWG, we next applied HCT intersection analysis to compute intersections between the AIWG-P and AIWG-R gene sets and HCTs for mouse signaling pathway nodes. We interpreted the extent and significance of these intersections as evidence for loss or gain of function of signaling nodes in weight gain propensity or resistance.

Figure 4 shows node HCT intersections (q < 0.01) with the AIWG-P and AIWG-R gene sets; the full set of intersections is provided in Supplementary file section 4. All q-values cited in the following section were generated using the R GeneOverlap package as previously described [23]. Strikingly, node HCT intersections were strictly partitioned between the AIWG-P and AIWG-R gene sets such that no nodes had HCT intersections with both gene sets. Consistent with the Panther GO analysis (Fig. 3), and the well-documented evidence connecting inflammation, the immune system, and metabolic disease [29, 31, 32, 35], we observed HCT intersections for numerous immunomodulatory and inflammatory nodes that were specific to the AIWG-P gene set. The two most robust AIWG-P intersections were for Tal1/SCL (2e–48) and Gata1 (1e–36), which play critical roles in the development of hematopoietic lineages with fundamental roles in immune processes [36, 37]. Other prominent immunomodulatory nodes with AIWG-P intersections included members of the AP-1 ([21339212]; Jun, 4e-7; Jund, 4e-4; Fos, 7e-3), interferon-regulatory ([38]; Irf1, 7e-3; Irf9, 2e-2; Irf2, 4.9e-2), STAT ([38]; Stat2, 2e-2; Stat3, 3e-2; Stat1, 3e-2), C/EBP ([39]; Cebpa, 3e-4; Cebpb, 6e-3; Cebpδ, 2e-2), and SMAD ([40]; Smad1, 3e-2) transcription factor families. Given the wealth of studies connecting circadian rhythms and metabolic dysfunction, and our recent observations of the importance of circadian rhythms in AIWG [41], we were interested to note significant intersections of the AIWG-P gene set with HCTs of several transcription factors with characterized roles in circadian biology, including Nr3c1/GR (q = 5e-3; [42]), MAX (4e-2 [43]), and members of the estrogen-related receptor (Esrra, q = 4e-4, Esrrb, 2.4e-2 [44]) and C/EBP (Cebpa, 3e-4; Cebpb, 6e-3; Cebpδ, 2e-2; [45]) families.

Candidate drivers of AIWG transcriptional programs are enriched for genes supporting body fat mass and weight gain in mice

As candidate transcriptional modulators of AIWG, we next hypothesized that nodes with q < 0.05 HCT intersections with the AIWG-P gene set would be enriched for genes whose disruption was associated with underweight or slow weight gain phenotypes in mice. To investigate this further, we assembled a list of genes that (i) were annotated by the Monarch [46], IMPC [47], or MPMP [48] initiatives to the Mammalian Phenotype Ontology phenotypes “decreased total body fat mass (DBFM)” or “slow postnatal weight gain (SPWG)” (Supplementary file section 5). Consistent with their potential participation in signaling pathways mediating AIWG, we found that nodes with significant (q < 0.05) HCT intersections with the AIWG-P gene set were robustly enriched (20/59; OR, 3.0, p = 9.7e-7) for genes whose disruption is associated with either of the two phenotypes (Fig. 5; Supplementary file section 4, DBMP/SPWG column).

Cross-species markers of AIWG predisposition are classical antiviral inflammatory effectors with conserved upstream-regulatory nodes

We next wished to determine whether the pathways driving AIWG are conserved between mice and humans. To do this, we compared our blood AIWG-P gene set with a set of 155 genes that were significantly differentially expressed in baseline blood between two groups of subjects segregated on the basis of AIWG-P vs AIWG-R after three months of antipsychotic treatment [20] (Supplementary file section 6). We identified four transcripts in common between the mouse AIWG-P gene set and human AIWG-P FC > 1.25 genes, namely, Rhd, Ifit1, Ifitm3, and Rsad2 (OR = 3.2 p = 0.04, hypergeometric test; Table 1). Rhd encodes the highly immunogenic erythrocyte Rh factor antigen of the Rh blood group system [49]. The three other genes—Ifit1, Ifitm3, and Rsad2 (Viperin)—are classic interferon-inducible effectors that oppose DNA and RNA virus infection through a variety of mechanisms, including disruption virus entry [50] and inhibition of viral replication [51–53]. Interestingly, adipose tissue expression of Rsad2 is increased in obesity and its genetic ablation results in decreased fat mass due to increased thermogenesis [54].

Given that expression levels of these four transcripts were conserved between mice and humans with respect to predisposition to AIWG-P, we speculated that their upstream signaling nodes would be similarly conserved. Figure 6 compares percentile rankings of the four cross-species’ AIWG-P genes in human and mouse ChIP-Seq consensomes for which at least one of both the human and mouse orthologs is a HCT (the full list of percentile rankings for all four genes is in Supplementary file section 7). This in silico analysis also recapitulates previous in vitro studies identifying transcriptional regulatory connections between Stat2 and Ifit1 [55], Stat1 and Rsad2 [56], and Tal1, Gata1, and Rhd [57]. We observed striking cross-species conservation of the regulatory relationships of the four genes with several of the key immunomodulatory nodes previously identified in the HCT intersection analysis. Moreover, we noted a clear demarcation...
between the nodes conserved upstream of the canonical interferon-stimulated genes *Ifi1*, *Ifitm3*, and *Rsad2* on the one hand, and *Rhd* on the other. Specifically, nodes upstream of the conserved interferon-stimulated genes comprised members of the STAT and interferon-regulatory families. This was in sharp contrast to the close cross-species-regulatory relationship of *Rhd* with *Tal1* and *Gata1*, which is consistent with the genomic co-occupancy of these factors [58], as well as their fundamental requirement in the development of hematopoietic lineages [36, 37].

---

**Table 1.** Candidate biomarkers of AIWG in mouse and human studies.

| Symbol | Name                                      | Mouse | Human |
|--------|-------------------------------------------|-------|-------|
| Ifi1   | Interferon Induced Protein With Tetratricopeptide Repeats 1 | 1.32  | 1.72  |
| Ifitm3 | Interferon-induced transmembrane protein 3 (*IFITM3*) | 1.25  | 1.28  |
| Rhd    | Rh blood group, D antigen                 | 1.94  | 1.45  |
| Rsad2  | Radical S-Adenosyl Methionine Domain Containing 2 | 1.54  | 2.56  |

Four genes (*IFI1*, *IFITM3*, *RHD* and *RSAD2*) are elevated in AIWG-P individuals relative to AIWG-R individuals.

---

**Fig. 4** ChiP-Seq high-confidence transcriptional target (HCT) intersection analysis of the AIWG-P and AIWG-R gene sets and mouse signaling nodes. *q*-values were generated using the GeneOverlap analysis package in R as previously described (Ochsner et al. 2020) [23]. White cells represent *q* > 5e-2 intersections. Shown are *q* < 0.01 intersections; full numerical data are provided in Supplementary file section 4. The universe for the intersection was set at a conservative estimate of the total number of transcribed genes in the mouse genome (30,000; [71]).
Cross-species markers of AIWG are specific to drug-induced weight gain and are not markers of predisposition to diet-induced weight gain

To determine if the levels of these four genes were specific predictors of drug or diet-induced weight gain, we conducted an additional control study in mice. We analyzed blood-based gene expression of these four genes before exposure to HFD for two weeks (Supplemental Fig. 1A). Mice exposed to HFD for two weeks gained between 2.9 g and 10.8 g each, with the HFWG-P group gaining an average of 9.5 ± 0.86 and HFWG-R group gaining just 4.9 ± 1.34 (Supplemental Fig. 1B). Importantly, there were no significant differences in gene expression of Ifit1, Ifitm3, Rd, or Rsad2 in baseline blood samples between these two groups (Supplemental Fig. 1C), suggesting that these genes are specifically markers of drug-induced weight gain and not diet-induced weight gain.

Conservation of immunomodulatory transcriptional networks predisposing to AIWG-P in mice and to weight gain and elevated body mass index in humans

The conservation of AIWG-P genes and their transcriptional regulators in the context of the mouse and human AIWG studies led us to consider whether the AIWG-P transcriptional regulatory network might be more broadly conserved in whole-body fat metabolism in humans. To investigate this further we retrieved a set of 157 human genes whose expression levels in blood were shown to have a strong positive correlation with weight and body mass index (BMI) in humans (designated HWG/BMI genes; Supplementary file section 8; [59]). Consistent with the connection between the AIWG-P gene set and downstream inflammatory processes (Fig. 3), pathway analysis of this gene set had indicated enrichment of inflammatory genes [59]. When we overlaid mouse orthologs of the HWG/BMI genes on the AIWG-P and AIWG-R gene sets, the high degree of overlap of mouse orthologs of the HWG/BMI genes with the AIWG-P gene set (40/157; OR = 30, p = 5e-41) was in striking contrast to the complete absence of overlap with the AIWG-R gene set (0/169; p = 1) (Fig. 7A; Supplementary file section 2, column “Mm HWG/BMI”). As shown in Table 2, genes common to the AIWG-P and HWG/BMI gene sets map to a broad range of functional categories, classes, and families annotated according to SPP convention as previously described. Of particular note, given known connections between the ubiquitin–proteasome system, innate immunity and obesity [60–62] is the substantial overrepresentation (6/40; OR = 11.4, p = 1e-5, hypergeometric test) of genes encoding enzymes in the E2 ubiquitin-conjugating and E3 ubiquitin ligase classes.

To evaluate whether this strong identity between mice and humans extended to upstream transcriptional drivers of these gene sets, we next identified nine human nodes with significant (q < 0.05) HCT intersections with the HWG/BMI gene set (Supplementary file section 9). The gene encoding the top-ranked node in

---

**Fig. 6** Transcriptional regulatory relationships between cross-species AIWG-P genes and immunomodulatory transcription factors are highly conserved between mice and humans. Shown are percentile rankings of the four cross-species AIWG-P genes in human and mouse ChIP-Seq consensomes for which at least one of both the human and mouse orthologs is a HCT. Refer to the Supplementary file for the human AIWG-P and AIWG-R gene sets (section 6) and the full list of mouse and human percentile rankings for the four genes (section 7).

**Fig. 7** Conservation of immunomodulatory pathways supporting AIWG in mice and weight gain and elevated body mass index in humans. A. Volcano plot of differential gene expression between AIWG-P and AIWG-resistant mice. The AIWG-P gene set (AIWG-P/ AIWG-R FC > 1.25, p < 0.05) is indicated in red and the AIWG-R gene set (AIWG-P/ AIWG-R FC < 0.75, p < 0.05) is indicated in blue. p > 0.05 genes are colored gray. A hypergeometric test was performed on the overrepresentation in the AIWG-P gene set of mouse orthologs of the human HWG/BMI gene set, indicated in yellow. Refer to the Supplementary file for the specific intersecting genes (section 2, Mm HWG/BMI column) and the full human HWG/BMI gene set (section 8). Box acronyms: INT, intersection; OR, odds ratio; p, p-value of hypergeometric test. B In total, 699 pathway nodes included in the AIWG-P HCT intersection analysis (Supplementary file section 4) were plotted as log odds ratio (log OR) against log10(–log10 P). A double-log procedure was required due to the large p-value range. Nodes with significant (q < 0.05) HCT intersections with the AIWG-P gene set are colored gray with an odds ratio of 30 (HYPERGEO. TEST = 5e-41) was in striking contrast to drug-induced weight gain and not diet-induced weight gain.
this analysis, TAL1, has been identified as a human BMI-associated GWAS locus, and knockout of the Drosophila ortholog results in decreased percent body fat [63]. Of these nine nodes, mouse orthologs for seven had been included in the earlier mouse HCT intersection analysis (Supplementary file section 4, column q < 0.05 INT HWG/BMI). Reflecting a strong conservation between the AIWG-P and HWG/BMI gene sets at the transcriptional regulatory level, mouse orthologs of five of these seven nodes (Tal1, Gata1, Zmiz1, Cebpd, and Smad1; OR 6.3, \(p = 2e-4\), hypergeometric test) had \(q < 0.05\) intersections with the mouse AIWG-P gene set (Fig. 7B). Notably, of these five nodes, all but Zmiz1 have well documented roles in the inflammatory and immunomodulatory signaling. Moreover, Tal1/TAL1 and Gata1/GATA1 were the top-ranked nodes in both analyses (Fig. 7B). Collectively, these data indicate that inflammatory and immunomodulatory signaling pathways contributing to weight gain and BMI are strongly conserved between mice and humans at the levels of both transcriptional drivers (pathway nodes) and effectors (pathway node genomic targets).

**DISCUSSION**

In this study, we found that immunomodulatory transcriptional effectors and their upstream regulatory drivers strongly predispose

---

**Table 2.** Functional categorization of genes in common between the mouse AIWG-P gene set and the human HWG/BMI gene set.

| Category                  | Class                  | Family                      | Symbol |
|---------------------------|------------------------|-----------------------------|--------|
| Enzymes                   | Dehydratases           | Carbonic anhydrases (CA)    | Car2   |
|                           | E2 ubiquitin conjugating enzymes | Ubiquitin conjugating enzymes E2 (UBE2) | Ube2f |
|                           |                        |                             | Ube2h  |
|                           | E3 ubiquitin ligases   | Maktein (MKRN)              | Mkm1   |
|                           |                        | Membrane associated ring-CH-type finger | Marchf8 |
|                           |                        | Tripartite motif-containing (TRIM) | Trim10 |
|                           |                        |                             | Trim58  |
| Kinases                   | Membrane palmitoylated proteins |                        | Mpp1   |
| Lyases                    | Ferrochelatases        |                             | Fch    |
| Oxidases                  | Spermine oxidases      |                             | Smax   |
| Peptidases                | Cathepsins (CTS)       |                             | Ctsb   |
| Peroxidases               | Peroxiredoxins (PRDX)  |                             | Prdx2  |
| Regulatory factors        | Protein phosphatase 1 regulatory subunits |                        | Bci2l1 |
| Transferrases             | Hydroxymethylbilane synthases |                       | Hmbs   |
| Other enzymes             | Glutamate-ammonia ligases |                             | Glul   |
|                           | Glutaredoxin            |                             | Glx5   |
| Transcription factors     | E2F/FOX                |                             | E2F    |
| Co-nodes                  | Cell cycle, cell division & DNA repair | Cell cycle checkpoint proteins (RAD) | Rad23a |
|                           | CNS Proteins           | Growth arrest and DNA damage inducible | Gadd45a |
|                           | Cytoskeleton components & regulators | Dematin act binding protein | Dmtn   |
|                           | F box domain           | F-box protein               | Fbxo7  |
|                           |                        |                             | Fbxo9  |
| Family with sequence similarity | Family with sequence similarity member |                         | Fam104a |
|                           | Globalins              | Hemoglobin subunit theta    | Hbq1b  |
| Membrane proteins         | Transmembrane and coiled-coil domain |                        | Tmcc2  |
| Receptor associated factors | GABA type A receptor associated protein like |                      | Gabarap2 |
|                           | Glutamate ionotropic receptor NMDA type subunit associated | Grina   |
| Ring finger proteins      | Ring finger protein    |                             | Rnf10  |
| Stress response factors   | DnaJ heat shock protein (Hsp40) member |                      | Dnaj4  |
| Transporters & transport proteins | Solute carrier superfamily member |                    | Slc25a37 |
|                           |                        |                             | Slc48a1 |
|                           |                        |                             | Slc4a1  |
| Other co-nodes            | BCL2 interacting protein like |                             | Bnip3l |
|                           | Cellular repressor of E1A stimulated genes |                         | Creg1  |
|                           | FUN14 domain containing |                             | Fundc2 |
|                           | PITH domain containing  |                             | Pithd1 |
mice to AIWG. In validation of our findings, we showed that genes encoding these upstream regulatory drivers are enriched for genes whose disruption in mice is associated with reduced body fat mass and slow postnatal weight gain. We proceeded to identify a minimal, cross-species AIWG-P signature that has the potential to determine the likelihood of AIWG in individual patients. We then demonstrated robust conservation between AIWG-P effectors and transcriptional drivers and those associated with weight gain and body mass index in humans. Collectively, our results provide mechanistic insight into the known links between inflammation, immunomodulation, obesity, and the metabolic syndrome [33, 34], as well as the extent to which pathways driving these connections are conserved between mice and humans.

Combining conventional pathway analysis of AIWG-P genes with a dissection of their regulatory relationships with upstream signaling pathway nodes, our analysis affords insights into the mechanisms underlying AIWG that are unavailable through pathway analysis alone. Initial GO pathway analysis of the underlying biological functions of AIWG-P genes indicated a strong link with inflammatory processes, which are known drivers of the development of metabolic disease [33, 34]. This was corroborated and extended by the HCT intersection analysis, which illuminated robust conservation of the regulatory relationships of AIWG-P genes with numerous inflammatory nodes in both mice (Fig. 4) and humans (Fig. 6), suggesting that individuals with a primed immune system are highly susceptible to AIWG. Significantly, while genes encoding AIWG-P nodes were enriched for genes whose disruption is associated with reduced body fat mass and slow postnatal weight gain (Fig. 5), no such enrichment was observed for the AIWG-P gene set itself (data not shown). These results indicate that transcriptional drivers of AIWG-P, rather than transcriptional effectors, are more likely to have nonredundant roles in the regulation of AIWG.

Although inflammation and immunomodulation were the primary themes connecting across AIWG-P genes, other paradigms were evident. For example, the significant intersections of the AIWG-P gene set with HCTs of several transcription factors with characterized roles in circadian biology were of additional interest in light of our recent studies showing that the circadian rhythm plays an important role in AIWG [41]. This suggests that variation in expression of genes that regulate the circadian rhythm also contributes to the risk potential of AIWG. Interestingly, the AIWG-P HCT intersection for the adipogenic master regulator Pparg is relatively small (4.6e-2), suggesting either that members of this gene set are indirect Pparg targets, or that they represent a module of transcriptional effectors that predispose to weight gain via a mechanism that is largely independent of classical adipogenic pathways. In addition, although the primary motivation of this study was the characterization of markers of AIWG and their transcriptional drivers, this analysis also paves the way for future studies into the role of AIWG-P nodes with no previously reported connections to whole-body energy metabolism, including Anp32e (q = 9e-3) Six4 and Zfp281 (both q = 8e-3).

In this study, we focused on female mice as they most closely model the antipsychotic-induced weight gain observed in human patients. Notably, there is a growing body of literature that women are more susceptible to AP-induced weight gain and metabolic side effects than men [64–67]. In addition, olanzapine was dosed in the diet (54 mg/kg diet) resulting in mouse plasma levels (21.5 ± g/100 mL) that are similar to the levels observed in humans treated with olanzapine (10–50 mg/mL) [17]. In previous dosing studies in mice, olanzapine dosing above 25–100 mg/kg in the diet had equivalent effects on body weight gain [17]. Importantly, the terminal elimination half-life of OLZ in mice is approximately 3 hr [68] compared with 21–54 h in humans [69], depending on a range of factors including sex and ethnicity [70]. Therefore, to ensure exposure across the course of the day and night, we opted for diet administration, whereby self-administration ensures exposure in rodents across the day.

In summary, we have identified transcriptional regulatory networks comprising immunomodulatory signaling nodes and their downstream effectors that predispose to AIWG. In addition, we identified a group of cross-species AIWG-P effectors whose regulatory relationships with several immunomodulatory nodes are highly conserved between mice and humans. To further validate these cross-species effectors as biomarkers in predicting AIWG, future studies will be needed involving independent replicate groups of mice and humans. In validation of our study, we found that numerous AIWG-P nodes are encoded by mouse genes with known adipose phenotypes, and that the immunomodulatory AIWG-P transcriptional networks are conserved in predisposing to weight gain and elevated body mass index in humans. These points of convergence underscore the relevance of immunomodulatory transcriptional networks identified in this study to AIWG and metabolic disease more generally across mice and humans.

REFERENCES

1. Bak M, Fransen A, Janssen J, van Os J, Drukker M. Almost all antipsychotics result in weight gain: a meta-analysis. PLoS ONE. 2014;9:e94112.
2. Spertus J, Horvitz-Lennon M, Abing H, Normand SL. Risk of weight gain for specific antipsychotic drugs: a meta-analysis. NPJ Schizophr. 2018;4:12.
3. Rojo LE, Gaspar PA, Silva H, Risco L, Arena P, Cubillos-Robles K, et al. Metabolic syndrome and obesity among users of second generation antipsychotics: A global challenge for modern psychopharmacology. Pharm Res. 2015;101:74–85.
4. Newcomer JW. Comparing the safety and efficacy of atypical antipsychotics in psychiatric patients with comorbid medical illnesses. J Clin Psychiatry. 2009;70:30–6.
5. Wofford MR, King DS, Harrell TK. Drug-induced metabolic syndrome. J Clin Hypertens. 2006;8:114–9.
6. Haddad PM, Brain C, Scott J. Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. Patient Relat Outcome Meas. 2014;5:43–62.
7. Zhang JP, Lenz C, Zhang RX, Nitta M, Maayan L, John M, et al. Pharmacogenetic associations of antipsychotic drug-related weight gain: a systematic review and meta-analysis. Schizophr Bull. 2016;42:1418–37.
8. Zhang JP, Malhotra AK. Pharmacogenetics and antipsychotics: therapeutic efficacy and side effects prediction. Expert Opin Drug Metab Toxicol. 2011;7:29–37.
9. Citrome L, McEvoy JP, Todtenkopf MS, McDonnell D, Weiden PJ. A commentary on the efficacy of olanzapine for the treatment of schizophrenia: the past, present, and future. Neuropsychiatr Dis Treat. 2019;15:2559–69.
10. Maayan L, Correll CU. Management of antipsychotic-related weight gain. Expert Rev Neurother. 2010;10:1175–200.
11. Manschreck TC, Boshes RA. The CATCH schizophrenia trial: results, impact, controversy. Harv Rev Psychiatry. 2007;15:245–58.
12. Nakhte KT, Subedar NK, Kokare DM. A role of neuropeptide CAMP in hyperphagia and weight gain induced by olanzapine treatment in rats. Brain Res. 2018;1695:45–52.
13. Newcomer JW. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs. 2005;19:1–93.
14. Jain S, Bhargava M, Gautam S. Weight gain with olanzapine: Drug, gender or age? Indian J Psychiatry. 2006;48:39–42.
15. Perez-Gomez A, Carretero M, Weber N, Peterka V, To A, Titova V, et al. A phenotypic Caenorhabditis elegans screen identifies a selective suppressor of antipsychotic-induced hyperphagia. Nat Commun. 2018;9:5272.
16. Zapata RC, Osborn O. Sensitivity of male wild type mouse strains to antipsychotic-induced weight gain. Physiol Behav. 2002;220:112859.
17. Morgan AP, Crowley JJ, Nonneman RJ, Quackenbush CR, Miller CN, Ryan AK, et al. The antipsychotic olanzapine interacts with the gut microbiome to cause weight gain in mice. PLoS ONE. 2014;9:e115225.
18. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–60.
19. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinforma. 2011;12:323.
20. Crespo-Facorro B, Prieto C, Sainz J. Altered gene expression in antipsychotic-induced weight gain. NPJ Schizophr. 2019;5:57.
COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-021-01528-y.

Correspondence and requests for materials should be addressed to O.O.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021