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Supplemental Data

The Human-Specific *BOLA2* Duplication Modifies Iron Homeostasis and Anemia Predisposition in Chromosome 16p11.2 Autism Individuals

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Supplemental Figures

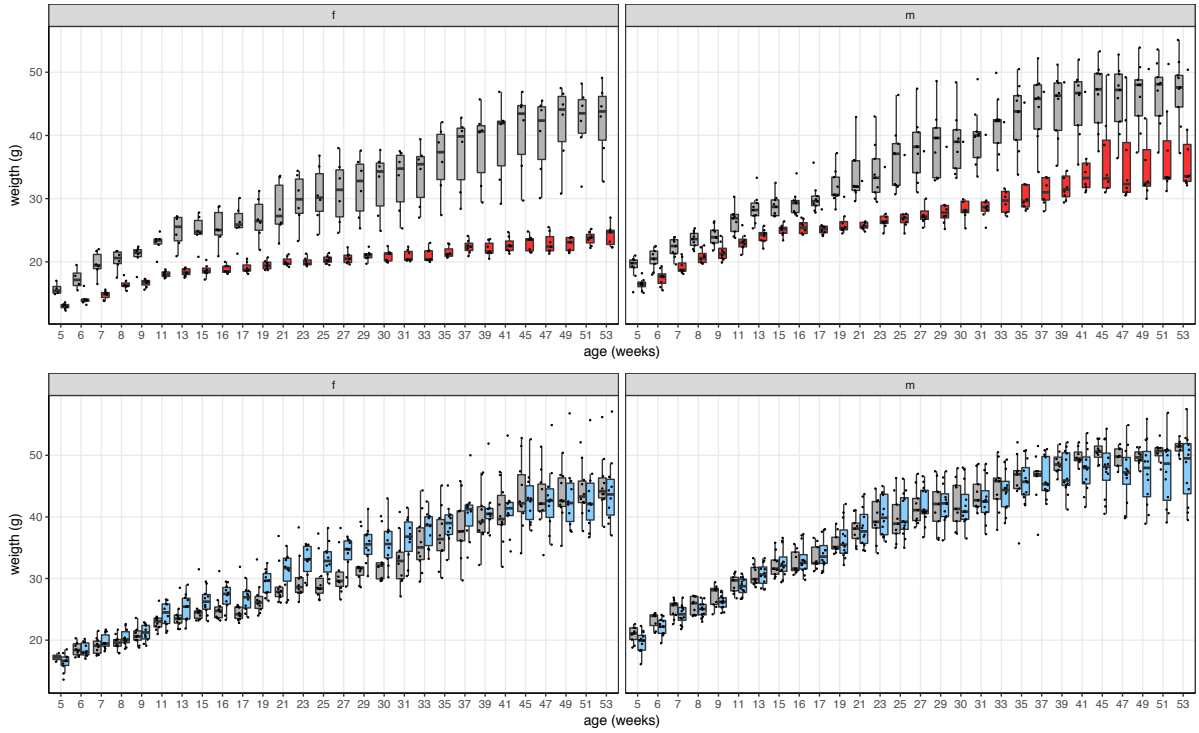

Figure S1. Longitudinal body weight profiles of *Del/+* (*top*) and *Dup/+* (*bottom*) female (*left*) and male (*right*) mouse models and wild-type littermates. The genotype color code is: Del/+ (red), Dup/+ (light blue), wild-type (grey). Note that the x-axis scale is not continuous.

Figure S2. SMRT sequencing platform reads of a neomycin cassette-excised *Bola2*$^{-/-}$ male were aligned to mm10. Within the *Bola2* locus on Chromosome 7 shown here, please note the nine spanning reads, all indicating the deletion. PBSV calls a homozygous deletion of 351 bp (cyan box).

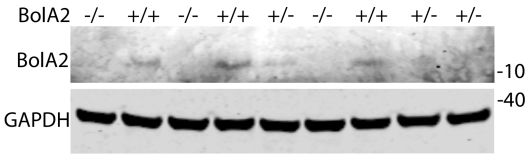

Figure S3. Western blot of liver samples from *Bola2*$^{+/+}$, *Bola2*$^{+/-}$, and *Bola2*$^{-/-}$ mice with an anti-BOLA2 antibody performed as described in Frey et al.[5]

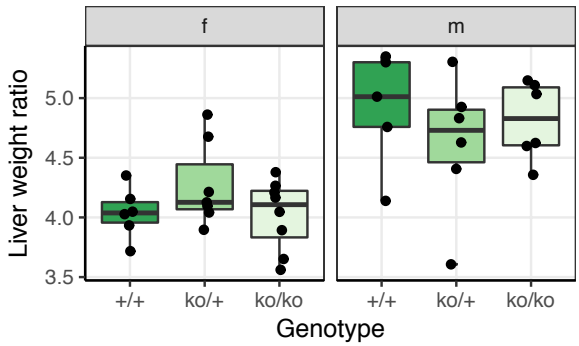

Figure S4. Liver weight normalized to body weight (mg/g) of *Bola2*$^{-/-}$, *Bola2*$^{+/-}$, and wild-type littermates from the Swiss cohort at 31 weeks of age.

List of Supplemental Tables

All Supplemental Tables are in a separate excel file.

Table S1. Hematological parameters of 16p11.2 CNV carriers and controls of the UK Biobank.

Table S2. *BOLA2* copy number and anemia status of 16p11.2 deletion carriers from the SVIP cohort.

Table S3. Hematological and blood iron quantifications of 16p11.2 deletion carriers from the European cohort with genotyped *BOLA2* copy number. Values in red are below the minimum value of the reference range. Individuals not considered in the statistics are grey-shaded (related family members).

Table S4. Effects of the 16p11.2 BP4-BP5 CNV and SNP rs3809627 on blood cell traits in the UK Biobank.

Table S5. Heparin-plasma iron level of *Del/+* and *Dup/+* mouse models and their wild-type littermates.

Table S6. Hematological parameters of *Del/+* mouse models and their wild-type littermates.

Table S7. Hematological parameters of *Dup/+* mouse models and their wild-type littermates.

Table S8. Heparin-plasma iron level of *Bola2*$^{+/+}$, *Bola2*$^{+/-}$, and *Bola2*$^{-/-}$ mice (Swiss cohort).

Table S9. Hematological parameters of *Bola2*$^{+/+}$, *Bola2*$^{+/-}$, and *Bola2*$^{-/-}$ mice (Swiss cohort).

Table S10. Serum iron, TIBC, Tf sat, ZPP, liver and spleen iron levels of *Bola2*$^{+/+}$, *Bola2*$^{+/-}$, and *Bola2*$^{-/-}$ mice (neo-in mice, US cohort).

Table S11. Hematological parameters of *Bola2*$^{+/+}$, *Bola2*$^{+/-}$, and *Bola2*$^{-/-}$ mice (neo-in mice, US cohort).

Table S12. Serum iron, TIBC, Tf sat, and ZPP levels of *Bola2*$^{+/+}$ and *Bola2*$^{-/-}$ mice (neo-excised mice, US cohort).

Table S13. Hematological parameters of *Bola2*$^{+/+}$ and *Bola2*$^{-/-}$ mice (neo-excised mice, US cohort).

Table S14. Normal hemoglobin and iron levels in the blood of adult humans, great apes, and mice.

Table S15. Members of the 16p11.2 Consortium.

Supplemental Methods

Genome-wide association studies (GWAS) for blood cell traits have uncovered that the variant rs3809627 mapping to the 16p11.2 BP4-BP5 region affects the RBC count (RBCc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean reticulocyte volume (MRV).[1-3] We used available SNP genotyping data of the UK Biobank (UKB) and SVIP[4] cohorts to assess the contribution of the variant rs3809627 on hematological traits and diagnosis of anemia and its relationship with the 16p11.2 BP4-BP5 CNV in the former dataset and the *BOLA2* duplicon CNV in the latter.

We reassessed the association of rs3809627 with blood cell traits in the UKB. The SNP was associated with almost all traits of red blood cells, reticulocytes, and platelets (**Table S4**), showing the strongest association with RBCc, MCV, MCH, and MRV. We next ran conditional analyses to discern the contribution of the CNV and the SNP variant. Multivariate linear regression (including both the CNV and the rs3809627 as regressors) confirmed that the (joint) effects of the CNV and the SNP on hematological parameters are independent. The effects of the SNP were smaller than those of the CNV.

We next compared the rs3809627 genotypes in deletion carriers vs control individuals and found a trend toward higher frequency of the alternative allele in deletion carriers (P = 0.1). We then compared the rs3809627 allele distribution in deletion carriers of the UKB with and without anemia and found no difference between groups. In particular, among the 18 deletion carriers referred as anemic, seven have the reference allele, nine the alternative one, and for two the genotype is not determined. Among the remaining 71 carriers, 33 have the reference allele and 33 the alternative one (for five the genotype is not determined). This result shows that the presence/absence of anemia among 16p11.2 deletion carriers is not associated with the rs3809627 genotype on the remaining allele.

We then assessed the association of rs3809627 with the diagnosis of anemia in the entire UKB and found no significant association (P = 0.1). In the conditional analysis with anemia as dependent variable, the 16p11.2 CNV was associated (P = 1e-5) while the SNP was not significantly associated (P = 0.1). This can be explained by the small effect of the SNP on blood parameters, which does not necessarily lead to anemia. For example, the hemoglobin concentration is reduced by only 0.02 g/dL.

As SNP data are available for the SVIP 16p11.2 deletion clinical cohort, we sought to run a conditional analysis to assess the effects of *BOLA2* copy number and the SNP rs3809627 on the anemia phenotype. As this SNP was not assayed, we used rs12444415 as best proxy (R^2 = 0.6). The *BOLA2* copy number remained associated with anemia (P = 0.02) while this SNP did not show any significant effect (P = 0.3). This result shows that the presence/absence of anemia among 16p11.2 deletion carriers is not associated with the genotype of the rs3809627 proxy (i.e. rs12444415) on the remaining allele in the SVIP cohort.

Supplemental References

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