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Supplementary Information for

Kleine Levin syndrome is associated with birth difficulties and genetic variants in the TRANK1 gene loci.

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This PDF file includes:

Figures S1 to S3
Legends for Datasets S1 to S15

Other supplementary materials for this manuscript include the following:

Datasets S1 to S15

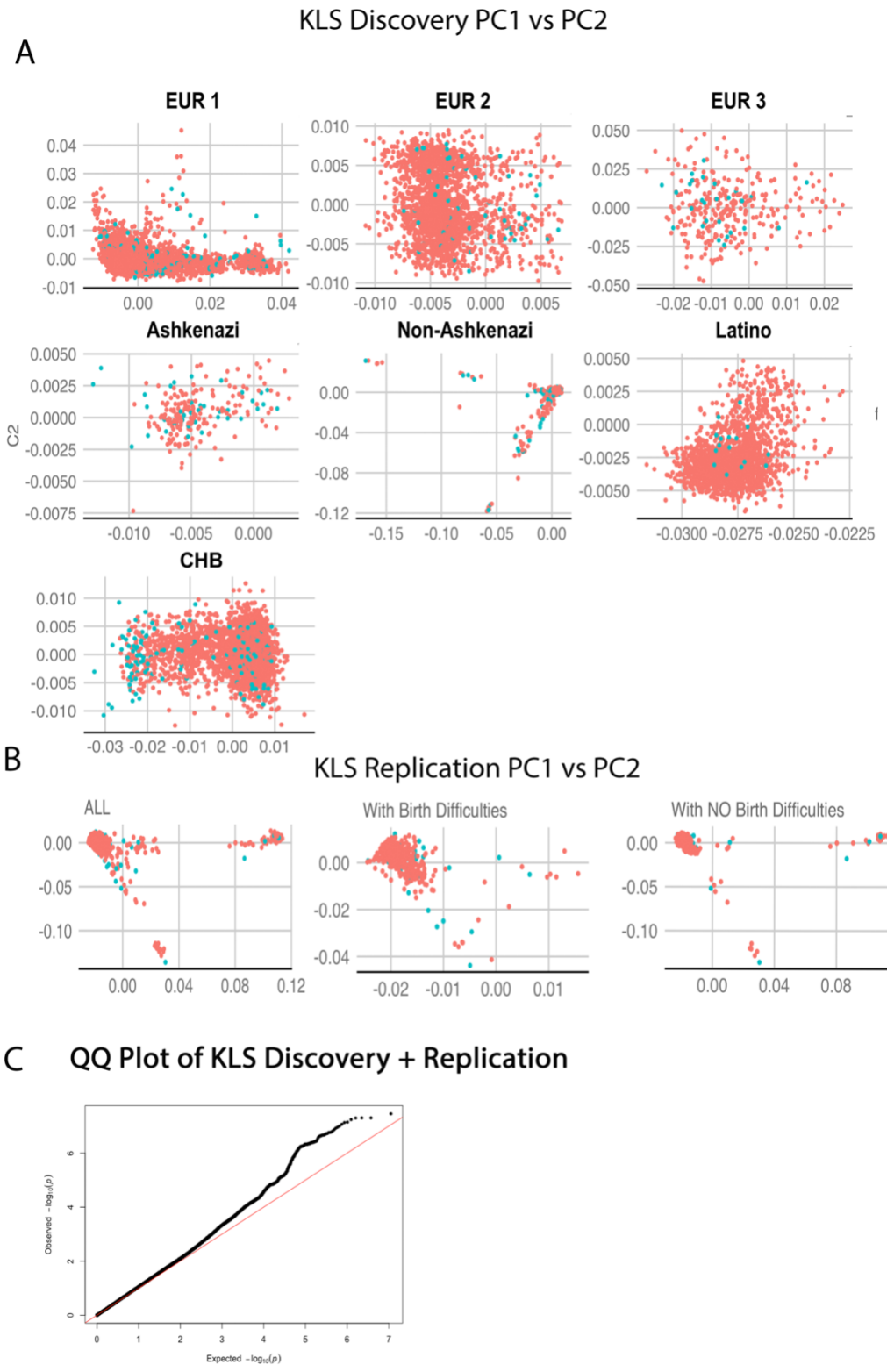

Fig. S1.A: The first 2 genetic principle components matching of case-controls in KLS discovery and **(B)** replication sub -cohorts stratified by presence of birth difficulties. **(C)** QQplot of the total KLS case-controls combined discovery + replication genetic association p-values.

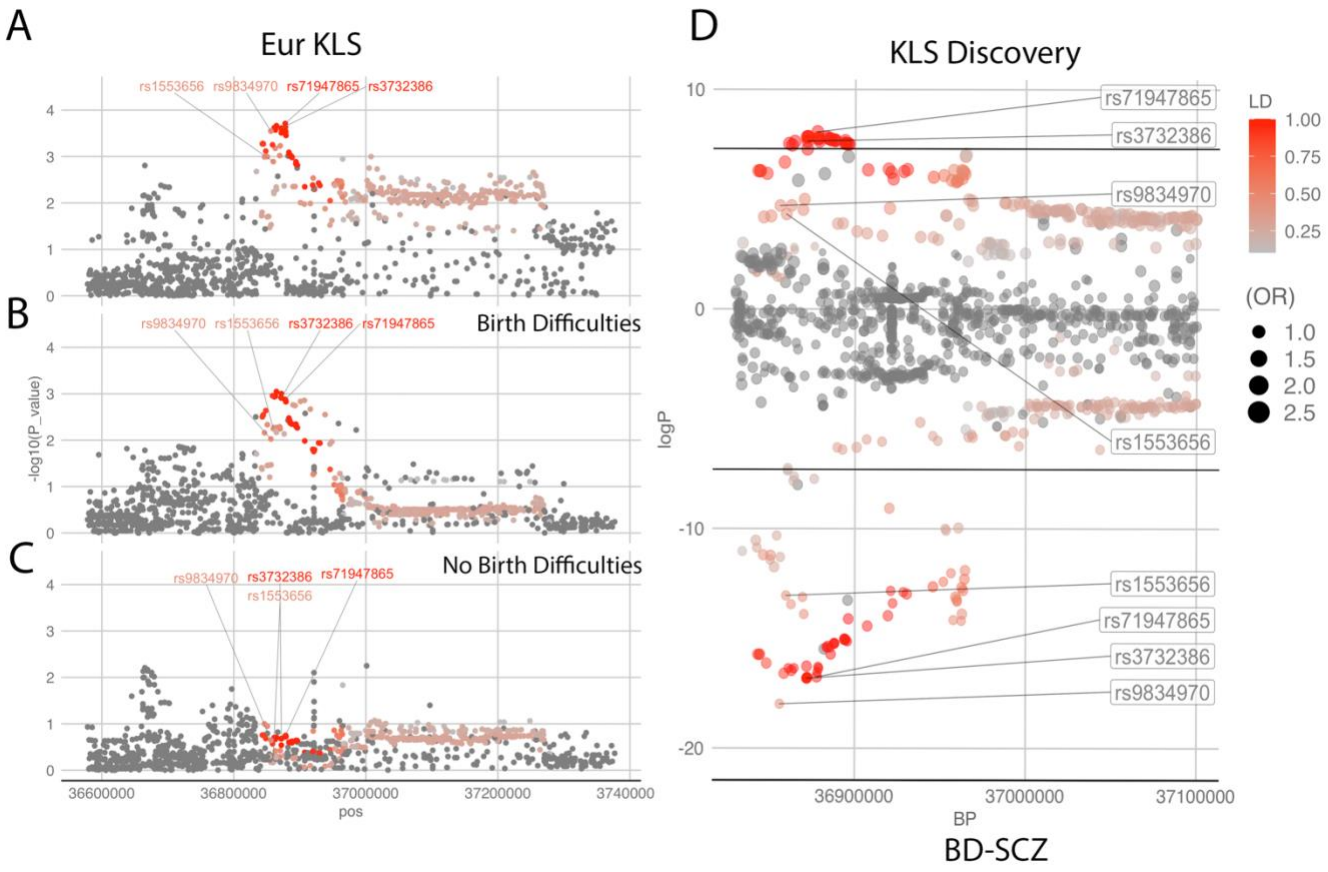

Fig. S2. (A-C): TRANK1 regional plots in the European origin KLS meta-analysis and sub-cohorts with and with our birth difficulties. **(D)** – TRANK1 loci plots with KLS discovery in the positive fraction contrasted with Bipolar Disorder-Schizophrenia (BD-SCZ) GWAS summary stats in the negative fraction(1) (Ruderfer DM et al. 2018), horizontal lines indicate GWAS significance threshold (p=5e-8).

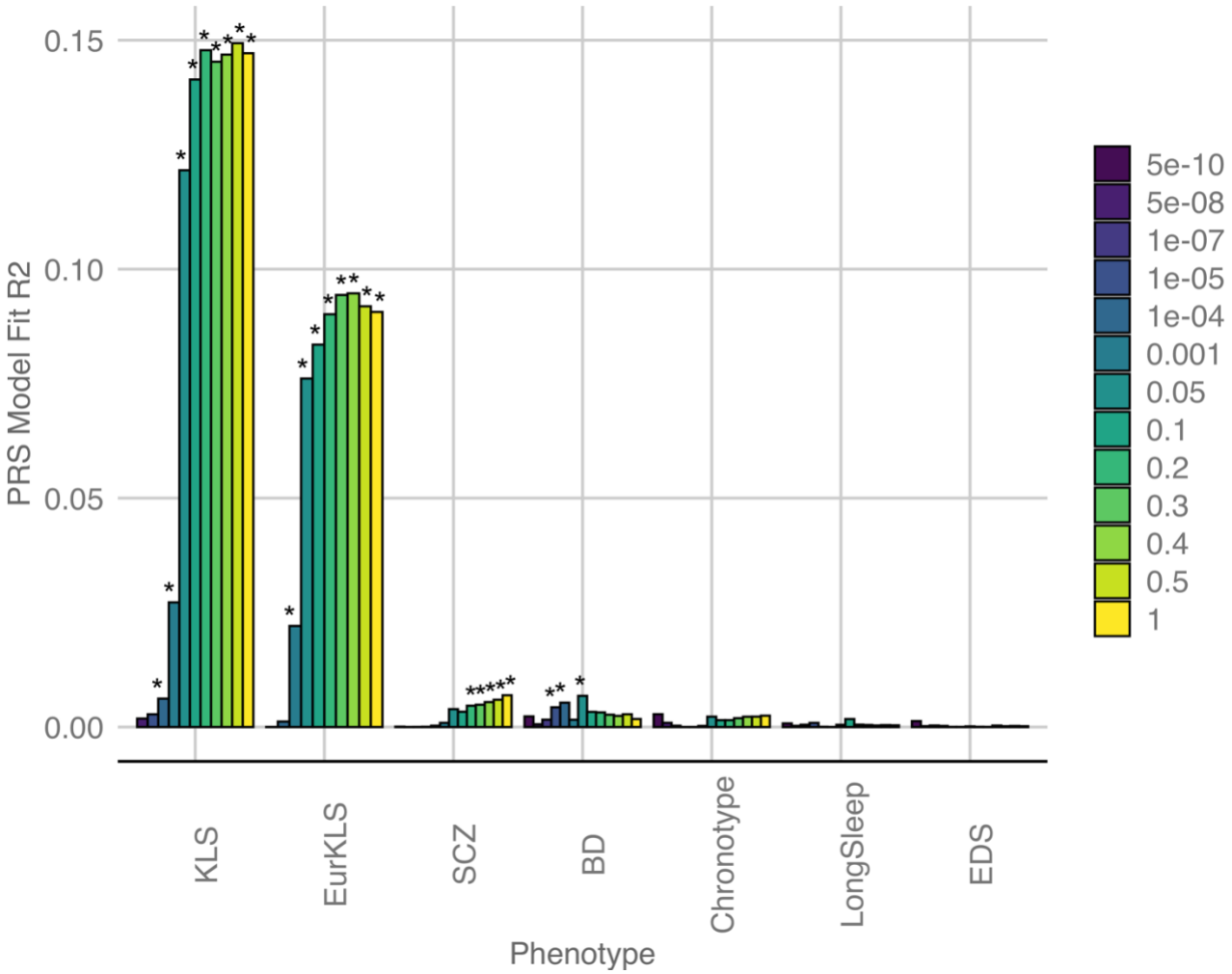

Fig. S3. PRS (polygenic risk scores) constructed from various other GWAS studies i.e. bipolar disorder, schizophrenia, circadian chronotype, sleep duration and excessive daytime sleepiness, evaluating their predictive performance on the KLS follow up cohort. EurKLS – PRS built on Caucasian discovery and validated in Caucasian replication KLS samples. The y-axis is the % variance explained by the PRS while the x-axis represents the p-value thresholds. Inset are marked the Pvalue of the fit. The * on the top of the bars represent the empirical p value ($p<0.05$) associated with the test of p-value threshold.

Dataset S1: The total cohort used in the analysis split by sub-cohort and genotyping array used grouped by KLS cases and controls.

Dataset S2: Variants in the TRANK1 loci associated with KLS in worldwide discovery cohort GWAS meta-analysis in 673 KLS cases and 15341 control individuals and in a Follow-up replication cohort of 171 KLS cases and 1956 controls. The effect allele was B*.

Dataset S3: TRANK1 region SNPs correlation in schizophrenia and bi-polar disorder GWAS studies. * indicates Genome wide significant (p<5e-8). PGC is Psychiatric Genomics Consortium.

Dataset S4: TRANK1 deletion (rs71947865) and its univariate association with KLS clinical variables. We used 2 linear additive models Model1- the additively coded minor allele genotype was fit as a function of the clinical variable, Model2 – was similar to model1 except for including ethnicity, Gender and missing data proportion. A Fisher's exact test of allelic counts was further performed for categorical variables. In these analyses 592 KLS cases were included. (Frequency % | mean ± sem was computed). BB- allele G, AB- GGGAGCCA/G, AA - GGGAGCCA/ GGGAGCCA, *Minor Allele G (deletion) was the effect allele.

Dataset S5: Birth Difficulty and developmental delay summary in the KLS cases.

Dataset S6: (A) TRANK1 SNPs in birth year stratified analysis, Fisher's exact count test was performed for case -control analyses. Minor *A allele was effect allele. **(B)** Allelic counts of TRANK1 SNPs in KLS cases compared to each birth year bin, Fisher's exact count test was performed for case only analyses. Minor A allele was effect allele.

Dataset S7: Variants in the TRANK1 loci associated with KLS in worldwide discovery cohort GWAS meta-analysis in 673 KLS cases and 15339 control individuals and Follow-up replication sub-cohorts based on presence of birth difficulties .The effect allele was B*. genomic coordinates in hg19/GRCh37.

Dataset S8: **(A)** TRANK1 SNPs association in KLS cases with birth difficulties compared to controls stratified by discovery and the replication cohort, Fisher's exact count test was performed. Minor *A allele was effect allele. **(B)** TRANK1 SNPs in birth difficulty stratified analysis in the combined discovery and replication cohort, Fisher's exact count test was performed. Minor *A allele was effect allele .

Dataset S9: Meta-analysis of Variants in the TRANK1 loci in European origin KLS cases in 2 cohorts stratified by all, presence of birth difficulties and absence of birth difficulties. Effect allele is B*. Filter on Analysis column to see associations by birth difficulties .genomic coordinates in hg19/GRCh37.

Dataset S10: Polygenic risk score analysis of KLS follow up sample in relation to other disorders including Bipolar Disorder, Schizophrenia, Circadian chronotype and Excessive daytime sleepiness.

Dataset S11: LD hub output containing LD score regression and shared SNP heritability with KLS across phenotypes, the table lists the top traits that share heritability with KLS**.**

Dataset S12: Top 2 enriched pathways in a MAGMA based gene set enrichment analysis was carried out using A total of 5346 sets including curated gene sets, KEGG pathways, Reactome pathways and GO biological processes derived from msigdb6.1 were used. Genomic coordinates in hg19/GRCh37.

Dataset S13: (A) The KLS cases and families (n=32) used in the exome sequencing studies. Controls were derived from 1000 genomes phase 3. **(B)**: Frequency and burden of all LMOD3 gene

variants derived from whole exome sequencing in 17 KLS Probands/sporadic cases and 286 European controls. Genomic coordinates in hg19/GRCh37.

Dataset S14: (A) Exome burden test restricted to rare variants with high and moderate effects grouped by reference genes within each families, probands and total cohort are listed, Columns with prefix KLS implies all the 32 cases and column prefix proband includes the proband in each family in addition to the 3 sporadic cases (n=17). genomic coordinates in hg19/GRCh37.**(B):** Exome burden test restricted to rare variants with high and moderate effects grouped by reference genes shared by at least 3 families, Columns with prefix KLS implies all the 32 cases and column prefix proband includes the proband in each family in addition to the 3 sporadic cases (n=17). genomic coordinates in hg19/GRCh37.

Dataset S15: (A) Top hits from Exome SKAT model with population covariates in 32 KLS cases and 286 controls with a focus on collapsing variants in the genes belonging to REACTOME_RORA_ACTIVATES_CIRCADIAN_EXPRESSION and REACTOME_CIRCADIAN_REPRESSION_OF_EXPRESSION_BY_REV_ERBA both of which were 5% FDR significant in the joint Discovery-Replication KLS GWAS pathway analyses. **(B)**: Top hits from Exome SKAT model with population covariates in 17 KLS probands and 286 controls with a focus on collapsing variants in the genes in pathways derived from a total of 5346 sets including curated gene sets, KEGG pathways, Reactome pathways and GO biological processes derived from msigdb6.1 were used.

References

References

1. D. M. Ruderfer *et al.*, Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. *Cell* **173**, 1705-1715. e1716 (2018).