Fixed human-specific neuro-regulatory single nucleotide mutations manifest staggering breadth of associations with physiological processes, morphological features, and pathological conditions of Modern Humans

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Running title: Global impact of fixed human-specific neuro-regulatory mutations in Modern Humans

Key words: human phenotypic uniqueness; human-specific regulatory sequences; human-specific traits; fixed neuro-regulatory human-specific single nucleotide mutations.

Abstract

Despite recent remarkable advances in identification and characterization of human-specific regulatory DNA sequences, their global impact on physiological and pathological phenotypes of *Homo sapiens* remains poorly understood. Gene set enrichment analyses of 8,405 genes linked with 35,074 human-specific (hs) neuro-regulatory single-nucleotide changes (SNCs) revealed the staggering breadth of significant associations with morphological structures, physiological processes, and pathological conditions of Modern Humans. Significantly enriched traits include more than 1,000 anatomically-distinct regions of the adult human brain, many different types of human cells and tissues, more than 200 common human disorders and more than 1,000 records of rare diseases. Thousands of genes connected with neuro-regulatory hsSNCs have been identified in this contribution, which represent essential genetic elements of the autosomal inheritance and offspring survival phenotypes. A total of 1,494 hsSNCs-linked genes have associated with either autosomal dominant or recessive inheritance and 2,273 hsSNCs-linked genes have been associated with premature death, embryonic lethality, as well as pre-, peri-, neo-, and post-natal lethality phenotypes of both complete and incomplete penetrance. Therefore, thousands of heritable traits and critical genes impacting the offspring survival appear under the human-specific regulatory control in genomes of Modern Humans. Interrogations of the Mouse Genome Informatics (MGI) database revealed readily available mouse models tailored for precise experimental definitions of regulatory effects of neuro-regulatory hsSNCs on genes causally affecting thousands of defined mammalian phenotypes and hundreds of common and rare human disorders. These observations highlight the remarkable translational opportunities afforded by the discovery of genetic regulatory loci harboring hsSNCs that are fixed in humans, distinct from other primates, and located in differentially-accessible (DA) chromatin regions during human brain development.

Introduction

DNA sequences of coding genes defining the structure of macromolecules comprising the essential building blocks of life at the cellular and organismal levels remain highly conserved during the evolution of humans and other Great Apes (Chimpanzee Sequencing and Analysis Consortium, 2005; Kronenberg et al., 2018). In striking contrast, a compendium of nearly hundred thousand candidate human-specific regulatory sequences (HSRS) has been assembled in recent years (Glinsky et al., 2015-2019; Kanton et al., 2019), thus validating the idea that unique to human phenotypes may result from human-specific changes to genomic regulatory sequences defined as “regulatory mutations” (King and Wilson, 1975). The best evidence of the exquisite degree of accuracy of the contemporary molecular definition of human-specific regulatory sequences is exemplified by the identification of 35,074 single nucleotide changes (SNCs) that are fixed in humans, distinct from other primates, and located within differentially-accessible (DA) chromatin regions during the human brain development in cerebral organoids (Kanton et al., 2019). Therefore, this type of mutations could be defined as fixed neuro-regulatory human-specific single nucleotide changes (hsSNCs). However, only a small fraction of identified DA chromatin peaks (600 of 17,935 DA peaks; 3.3%) manifest associations with differential expression in human versus chimpanzee cerebral organoids model of brain development, consistent with the hypothesis that regulatory effects on gene expression of these DA chromatin regions are not restricted to the early stages of brain development. Annotation of SNCs derived and fixed in modern humans that overlap DA chromatin regions during brain development revealed that essentially all candidate regulatory human-specific SNCs are shared with the archaic humans (35,010 SNCs; 99.8%) and only 64 SNCs are unique to modern humans (Kanton et al., 2019). This remarkable conservation on the human lineage of human-specific SNCs associated with human brain development sows the seed of interest for in-depth exploration of coding genes expression of which may be affected by genetic regulatory loci harboring human-specific SNCs. The GREAT algorithm (McLean et al., 2010, 2011) was utilized to identify 8,405 hsSNCs-linked genes expression of which might be affected by 35,074 human-specific SNCs located in DA chromatin regions during brain development. Comprehensive gene set enrichment analyses of these genes revealed the staggering breadth of associations with physiological processes and pathological conditions of *H. sapiens*, including more than 1,000

anatomically-distinct regions of the adult human brain, many human tissues and cell types, more than 200 common human disorders and more than 1,000 rare diseases. It has been concluded that hsSNCs-linked genes appear contributing to development and functions of the adult human brain and other components of the central nervous system; they were defined as genetic markers of many tissues across human body and were implicated in the extensive range of human physiological and pathological conditions, thus supporting the hypothesis that phenotype-altering effects of neuro-regulatory hsSNCs are not restricted to the early-stages of human brain development. It seems highly likely that hsSNCs and associated genes affect wide spectra of traits defining both physiology and pathology of Modern Humans by asserting human-specific regulatory impacts on thousands essential mammalian phenotypes.

Results

Identification and characterization of putative genetic regulatory targets associated with human-specific single nucleotide changes (SNCs) in in differentially accessible (DA) chromatin regions during brain development

To identify and characterize human genes associated with 35,074 human-specific single nucleotide changes (SNCs) in differentially accessible (DA) chromatin regions during human and chimpanzee neurogenesis in cerebral organoids (Kanton et al., 2019), the GREAT algorithm (McLean et al., 2011) have been employed. These analyses identified 8,405 genes with putative regulatory connections to human-specific SNCs (Figure 1) and revealed a remarkable breadth of highly significant associations with a multitude of biological processes, molecular functions, genetic and metabolic pathways, cellular compartments, and gene expression perturbations (Supplemental Table Set S1). To ascertain patterns of genomic associations between neuro-regulatory human-specific SNCs and putative target genes, the GREAT analyses were performed at different proximity placement distances defined by the single nearest gene maximum extension ranging from 10 Kb to 1 Mb (Figure 1). It has been observed that from 92% of all hsSNCs-linked genes are located within 200 Kb distances from their putative regulatory loci (Figure 1A). Since the size of more than 90% of topologically-associating domains (TADs) in human genomes is 200 kb or more (Dixon et al., 2012), these findings indicate

that a marked majority of neuro-regulatory hsSNCs and their putative target genes would be placed in human genomes within the boundaries of the same TAD.

It has been noted that particularly striking numbers of significant associations were uncovered by the GREAT algorithm during the analyses of two databases:

1) The Human Phenotype Ontology containing over 13,000 terms describing clinical phenotypic abnormalities that have been observed in human diseases, including hereditary disorders (326 significant records with binominal FDR Q-Value < 0.05);

2) The MGI Expression Detected ontology referencing genes expressed in specific anatomical structures at specific developmental stages (Theiler stages) in the mouse (370 significant records with binominal FDR Q-Value < 0.05).

These observations support the hypothesis that biological functions of genes under the putative regulatory control of human-specific SNCs in DA chromatin regions during brain development are not limited to the contribution to the early stages of neuro- and corticogenesis. Collectively, findings reported in the Supplemental Table Set S1 argue that genes expression of which is affected by human-specific SNCs may represent a genomic dominion of putative regulatory dependency from HSRS that is likely to play an important role in a broad spectrum of physiological processes and pathological conditions of Modern Humans.

Identification of hsSNCs-linked genes expression of which distinguishes thousands of anatomically distinct areas of the adult human brain, various regions of the central nervous system, and many different cell types and tissues in the human body

To validate and extend these observations, next the comprehensive gene set enrichment analyses were performed employing the web-based Enrichr API protocols (Chen et al., 2013; Kuleshov et al., 2016), which interrogated nearly 200,000 gene sets from more than 100 gene set libraries. The results of these analyses are summarized in the Table 1 and reported in details in the Supplemental Table Set S2. Genes expression of which were placed during evolution under the regulatory control of ~ 35,000 human-specific SNCs

demonstrate a staggering breadth of significant associations with a broad spectrum of anatomically distinct regions, various cell and tissue types, a multitude of physiological processes, and a numerous pathological conditions of *H. sapiens*.

Of particular interest is the apparent significant enrichment of human-specific SNCs-associated genes among both up-regulated and down-regulated genes, expression of which discriminates thousands of anatomically distinct areas of the adult human brain defined in the Allen Brain Atlas (Supplemental Figure S1; Supplemental Table Set S2). Notably, genes expressed in various thalamus regions appear frequently among the top-scored anatomical areas of the human brain (Supplemental Figure S1; Supplemental Table Set S2). These observations support the hypothesis that genetic loci harboring human-specific SNCs may exert regulatory effects on structural and functional features of the adult human brain, thus, likely affecting the development and functions of the central nervous system in Modern Humans. Consistent with this idea, the examination of the enrichment patterns of human-specific SNCs-associated genes in the ARCHS4 Human Tissues' gene expression database revealed that top 10 most significantly enriched records overlapping a majority of region-specific marker genes constitute various anatomically-distinct regions of the central nervous system (Supplemental Figure 1; Supplemental Table Set S2). However, results of gene set enrichment analyses convincingly demonstrate that inferred regulatory effects of genetic loci harboring human-specific SNCs are not restricted only to the various regions of the central nervous system, they appear to affect gene expression profiles of many different cell types and tissues in the human body (Table 1; Supplemental Table Set S2).

Identification and characterization of hsSNCs-linked genes expression of which is altered during aging of humans, rats, and mice

Genes altered expression of which is implicated in the aging of various tissues and organs of humans, rats, and mice are significantly enriched among 8,405 genes associated with human-specific regulatory SNCs (Supplemental Figure S2; Supplemental Table Set S2). Aging of the hippocampus was implicated most frequently among genes manifesting increased expression with age, while among genes exhibiting aging-associated decreased expression the hippocampus and frontal cortex were identified repeatedly (Supplemental Figure S2). Overall, twice as many significant association records were observed among aging-

associated down-regulated genes compared to up-regulated genes (Table 1). Collectively, these observations indicate that genes changes in expression of which were associated with aging in mammals, in particular, hippocampal and frontal cortex aging, represent important elements of a genomic dominion that was placed under regulatory control of genetic loci harboring human-specific SNCs.

Identification of hsSNCs-linked genes implicated in development and manifestations of hundreds physiological and pathological phenotypes and autosomal inheritance in Modern Humans

Interrogations of the Human Phenotype Ontology database (298 significantly enriched records identified), the Genome-Wide Association Study (GWAS) Catalogue (241 significantly enriched records identified), and the database of Human Genotypes and Phenotypes (136 significantly enriched records identified) revealed several hundred physiological and pathological phenotypes and thousands of genes manifesting significant enrichment patterns defined at the adjusted p value < 0.05 (Supplemental Figure S3; Table 1; Supplemental Table Set S2). Interestingly, 645 and 849 genes implicated in the autosomal dominant (HP:0000006) and recessive (HP:0000007) inheritance were identified amongst genes associated with human-specific regulatory SNCs (Supplemental Figure S3; Supplemental Table Set S2). Notable pathological conditions among top-scored records identified in the database of Human Genotypes and Phenotypes are stroke, myocardial infarction, coronary artery disease, and heart failure (Supplemental Figure S3).

A total of 241 significantly enriched records (Table 1) were documented by gene set enrichment analyses of the GWAS catalogue (2019), among which a highly diverse spectrum of pathological conditions linked to genes associated with human-specific regulatory SNCs was identified, including obesity, type 2 diabetes, amyotrophic lateral sclerosis, autism spectrum disorders, attention deficit hyperactivity disorder, bipolar disorder, major depressive disorder, schizophrenia, Alzheimer's disease, malignant melanoma, diverticular disease, asthma, coronary artery disease, glaucoma, as well as breast, prostate and colorectal cancers (Supplemental Figure S3; Supplemental Table Set S2). These observations indicate that thousands of genes putatively associated with genetic regulatory loci harboring human-specific SNCs affect risk of developing numerous pathological conditions in Modern Humans.

Identification of hsSNCs-linked genes expression of which is altered in several hundred common human disorders

Gene set enrichment analyses-guided interrogation of the Gene Expression Omnibus (GEO) database revealed the remarkably diverse spectrum of human diseases with the etiologic origins in multiple organs and tissues and highly heterogeneous pathophysiological trajectories of their pathogenesis (Supplemental Figure S4; Supplemental Table Set S2). Overlapping gene sets between disease-associated genes and human-specific SNCs-linked genes comprise of hundreds of genes that were either up-regulated (204 significant disease records) or down-regulated (240 significant disease records) in specific pathological conditions, including schizophrenia, bipolar disorder, various types of malignant tumors, Crohn's disease, ulcerative colitis, Down syndrome, Alzheimer's disease, spinal muscular atrophy, multiple sclerosis, autism spectrum disorders, type 2 diabetes mellitus, morbid obesity, cardiomyopathy (Supplemental Figure S4; Supplemental Table Set S2). These observations demonstrate that thousands of genes expression of which is altered in a myriad of human diseases appear associated with genetic regulatory loci harboring human-specific SNCs.

Identification of hsSNCs-linked genes implicated in more than 1,000 records classified as human rare diseases

Present analyses demonstrate that thousands of genes associated with human-specific regulatory SNCs have been previously identified as genetic elements affecting the likelihood of development a multitude of common human disorders. Similarly, thousands of genes expression of which is altered during development and manifestation of multiple common human disorders appear linked to genetic regulatory loci harboring human-specific SNCs. Remarkably, interrogations of the Enrichr's libraries of genes associated with Modern Humans' rare diseases identified 473, 603, 641, and 1,116 significantly enriched records of various rare disorders employing the Rare Diseases GeneRIF gene lists library, the Rare Diseases GeneRIF ARCHS4 predictions library, the Rare Diseases AutoRIF ARCHS4 predictions library, and the Rare Diseases AutoRIF Gene lists library, respectively (Supplemental Figure S5; Supplemental Table Set S2). Taken together, these observations demonstrate that thousands of genes associated with hundreds of human rare disorders appear linked with human-specific regulatory SNCs.

Gene ontology analyses of putative regulatory targets of genetic loci harboring human-specific SNCs

Gene Ontology (GO) analyses identified a constellation of biological processes (GO Biological Process: 308 significant records) supplemented with a multitude of molecular functions (GO Molecular Function: 81 significant records) that appear under the regulatory control of human-specific SNCs (Supplemental Figure S6; Supplemental Table Set 2). Consistently, both databases identified frequently the components of transcriptional regulation and protein kinase activities among most significant records. Other significantly enriched records of interest are regulation of apoptosis, cell proliferation, migration, and various binding properties (cadherin binding; sequence-specific DNA binding; protein-kinase binding; amyloid-beta binding; actin binding; tubulin binding; microtubule binding; PDZ domain binding) which are often supplemented by references to the corresponding activity among the enriched records, for example, enriched records of both binding and activity of protein kinases.

Interrogation of GO Cellular Component database identified 29 significantly enriched records, among which nuclear chromatin as well as various cytoskeleton and membrane components appear noteworthy (Supplemental Figure S6). Both GO Biological Process and GO Cellular Component database identified significantly enriched records associated with the central nervous system development and functions such as axonogenesis and axon guidance; generation of neurons, neuron differentiation, and neuron projection morphogenesis; cellular components of dendrites and dendrite's membrane; ionotropic glutamate receptor complex. In several instances biologically highly consistent enrichment records have been identified in different GO databases: cadherin binding (GO Molecular Function) and catenin complex (GO Cellular Component); actin binding (GO Molecular Function) and actin cytoskeleton, cortical actin cytoskeleton, actin-based cell projections (GO Cellular Component); microtubule motor activity, tubulin binding, microtubule binding (GO Molecular Function) and microtubule organizing center, microtubule cytoskeleton (GO Cellular Component).

Analyses of human and mouse databases of the Kyoto Encyclopedia of Genes and Genomes (KEGG; Supplemental Figure S7) identified more than 100 significantly enriched records in each database (KEGG 2019 Human (2019): 129 significant records; KEGG 2019 Mouse: 106 significant records). Genes associated with human-specific regulatory SNCs were implicated in a remarkably broad spectrum of signaling pathways

ranging from pathways regulating the pluripotency of stem cells to cell type-specific morphogenesis and differentiation pathways, for example, melanogenesis and adrenergic signaling in cardiomyocytes (Supplemental Figure S7). Genes under putative regulatory control of human-specific SNCs include hundreds of genes contributing to specific functions of specialized differentiated cells (gastric acid secretion; insulin secretion; aldosterone synthesis and secretion), multiple receptor/ligand-specific signaling pathways, as well as genetic constituents of pathways commonly deregulated in cancer and linked to the organ-specific malignancies, for example, breast, colorectal, and small cell lung cancers (Supplemental Figure S7). Other notable entries among most significantly enriched records include pathways of the axon guidance; dopaminergic, glutamatergic, and cholinergic synapses; neuroactive receptor-ligand interactions; and AGE-RAGE signaling pathway in diabetic complications (Supplemental Figure S7; Supplemental Table Set 2).

Structurally, functionally, and evolutionary distinct classes of human-specific regulatory sequences (HSRS) share the relatively restricted elite set of common genetic targets

It has been suggested that unified activities of thousands candidate HSRS comprising a coherent compendium of genomic regulatory elements markedly distinct in their structure, function, and evolutionary origin may have contributed to development and manifestation of human-specific phenotypic traits (Glinsky, 2019). It was interest to determine whether genes previously linked to other classes of HSRS, which were identified without considerations of human-specific SNCs, overlap with genes associated in this contribution with genomic regulatory loci harboring human-specific SNCs. It was observed that the common gene set comprises of 7,406 coding genes (88% of all human-specific SNCs-associated genes), indicating that structurally-diverse HSRS, the evolutionary origin of which has been driven by mechanistically-distinct processes, appear to favor the regulatory alignment with the relatively restricted elite set of genetic targets (Supplemental Figure S8; Supplemental Table Set S3).

Previous studies have identified stem cell-associated retroviral sequences (SCARS) encoded by human endogenous retroviruses LTR7/HERVH and LTR5_Hs/HERVK as one of the significant sources of the evolutionary origin of HSRS (Glinsky, 2015-2019), including human-specific transcription factor binding sites (TFBS) for NANOG, OCT4, and CTCF (Glinsky, 2015). Next, the common sets of genetic regulatory targets

were identified for genes expression of which is regulated by SCARS and genes associated in this study with human-specific regulatory SNCs (Supplemental Figure S8). It has been determined that each of the structurally-distinct families of SCARS appears to share a common set of genetic regulatory targets with human-specific SNCs (Supplemental Figure S8). Overall, expression of nearly half (4,029 genes; 48%) of all genes identified as putative regulatory targets of human-specific SNCs is regulated by SCARS (Supplemental Figure S8; Supplemental Table Set S3). Consistent with the idea that structurally-diverse HSRS may favor the relatively restricted elite set of genetic targets, the common gene set of regulatory targets for HSRS, SCARS, and SNCs comprises of 7,833 coding genes or 93% of all genes associated in this contribution with human-specific regulatory SNCs (Supplemental Figure S8; Supplemental Table Set S3).

To gain insights into mechanisms of SCARS-mediated effects on expression of 4,029 genes linked to human-specific regulatory SNCs, the numbers of genes expression of which was either activated (down-regulated following SCARS silencing) or inhibited (up-regulated following SCARS silencing) by SCARS have been determined. It was observed that SCARS exert the predominantly inhibitory effect on expression of genes associated with human-specific regulatory SNCs, which is exemplified by activated expression of as many as 87% of genes affected by SCARS silencing (Supplemental Figure S8; Supplemental Table Set S3). These findings indicate that when SCARS-associated networks are active during the human preimplantation embryogenesis, they exert a dominant effect on gene expression, whereas when SCARS are silenced during the postimplantation embryonic development and in the adulthood, regulatory impact of human-specific regulatory SNCs is prevalent.

Identification of 2,273 genes associated with human-specific SNCs and implicated in premature death and embryonic, prenatal, perinatal, neonatal, and postnatal lethality phenotypes

Interrogation of MGI Mammalian Phenotype databases revealed several hundred mammalian phenotypes affected by thousands of genes associated with genomic regulatory regions harboring human-specific SNCs: the MGI Mammalian Phenotype (2017) database identified 749 significant enrichment records, while the MGI Mammalian Phenotype Level 4 (2019) database identified 407 significant enrichment records (Supplemental Figure S9; Supplemental Table Set S2). Strikingly, present analyses identified a total of 2,273 genes that

appear under the regulatory control of genetic loci harboring human-specific SNCs and mutations of which result in phenotypes of premature death, embryonic lethality, as well as prenatal, perinatal, neonatal, and postnatal lethality of both complete and incomplete penetrance. A significant fraction of these 2,273 genes, which collectively could be defined based on their mutation phenotypes' patterns as an offspring survival genomic dominion, were implicated in the autosomal dominant (389 genes) and recessive (426 genes) inheritance in Modern Humans. Based on these observations, it has been concluded that thousands of genes within the genomic dominions of putative regulatory dependencies from human-specific SNCs represent the essential genetic elements of the mammalian offspring survival phenotypes.

Genes linked with neuro-regulatory hsSNCs represents intrinsic genetic elements of developmentally and physiologically distinct human-specific genomic regulatory networks (GRNs)

It was of interest to determine whether hsSNCs-linked genes are represented among genes previously identified of human-specific GRNs operating in developmentally and physiologically distinct human tissues and cells. Importantly, human-specific GRNs utilized in these analyses were defined employing vastly different experimental, analytical, and computational approaches that were applied within the broad range of experimental settings (Glinsky, 2019). Specifically, the interrogated human-specific GRNs include the following data sets: i) Great Apes' whole-genome sequencing-guided identification of human-specific insertions and deletions (Kronenberg et al., 2018); ii) genome-wide analysis of retrotransposon's transcriptome in postmortem samples of human dorsolateral prefrontal cortex (Guffanti et al., 2018); iii) shRNA-mediated silencing of LTR7/HERVH retrovirus-derived long non-coding RNAs in hESC (Wang et al., 2014); iv) single-cell expression profiling analyses of human preimplantation embryos (Glinsky et al., 2018); v) network of genes associated with regulatory transposable elements (TE) operating in naïve and primed hESC (Theunissen et al., 2016; Pontis et al., 2019); vi) pluripotency-related network of genes manifesting concordant expression changes in human fetal brain and adult neocortex (Glinsky, 2017); vii) network of genes governing human neurogenesis in vivo (Nowakowski et al., 2017); viii) network of genes differentially expressed during human corticogenesis in vitro (van de Leemput et al., 2014). Thus, selected for these analyses human-specific GRNs appear to function in a developmentally and physiologically diverse spectrum of human cells that are biologically and anatomically

highly relevant to manifestations of human-specific phenotypes ranging from preimplantation embryos to adult dorsolateral prefrontal cortex (Supplemental Table Set S3).

Significantly, in all instances a highly significant enrichment of hsSNCs-linked genes has been observed (Supplemental Table Set S3). These observations are consistent with the hypothesis that neuro-regulatory hsSNCs and associated genes represent important components of the exceptionally broad range of human-specific GRNs operating in the wide spectra of developmental and physiological contexts reflecting species-defining human-specific phenotypes.

Identification of the experimentally trackable models for molecular definitions of regulatory effects of human-specific SNCs on expression of genes associated with thousands of mammalian phenotypes and human diseases

To identify all genes linked with human-specific regulatory SNCs that are associated with defined mammalian phenotypes and human diseases with one or more mouse models, the analyses have been carried out utilizing the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/). These analyses identified 125,938 Mammalian Phenotype Ontology records and 1,807 Human Disease Ontology records associated with 5,730 and 1,162 human-specific regulatory SNCs-linked genes, respectively (Supplemental Table Sets S4 and S5). Remarkably, genes linked with human-specific regulatory SNCs have been associated with a majority (61%) of all human diseases with one or more mouse models (967 of 1,584 human disease ontology terms; Supplemental Table Set S4). Similarly, human-specific SNCs-linked genes have been associated with 71% of all Mammalian Phenotype Ontology terms (9,190 of 12,936 records; Supplemental Table Set S5). These observations identify readily available mouse models for experimental interrogations of regulatory effects of human-specific SNCs and other types of HSRS on genes causally affecting thousands of defined mammalian phenotypes and hundreds of common and rare human disorders.

Discussion

In recent years, elucidation of genetic and molecular mechanisms defining the phenotypic uniqueness of Modern Humans attained a significant progress in illuminating the potentially broad role of thousands human-

specific regulatory sequences (HSRS) in contrast to the relatively modest impact of human-specific changes of a limited number of coding genes (Kronenberg et al., 2018; Glinsky, 2019; Kanton et al., 2019). The macromolecules comprising the essential building blocks of life at the cellular and organismal levels remain highly conserved during the evolution of humans and other Great Apes. Identification and initial structural-functional characterization of nearly hundred thousand candidate HSRS (Kronenberg et al., 2018; Glinsky, 2019; Kanton et al., 2019; this contribution) validate the idea that unique to human phenotypes may result from human-specific changes to genomic regulatory sequences defined as “regulatory mutations” (King and Wilson, 1975). Technological advances enabled the exquisite degree of accuracy of molecular definition of 35,074 SNCs that are fixed in humans, distinct from other primates, and located in DA chromatin regions during human brain development (Kanton et al., 2019). Notably, 99.8% of candidate regulatory hsSNCs that overlap DA chromatin regions during brain development are shared with the archaic humans while only 64 hsSNCs are unique to Modern Humans. The conservation on the human lineage of a vast majority of regulatory hsSNCs associated with early stages of human brain development suggest that coding genes expression of which is regulated by hsSNCs may have a broad effect on human-specific traits beyond embryonic development. This concept has been substantiated by the multiple lines of evidence acquired and reported in the present contribution.

Employing the GREAT algorithm (McLean et al., 2010, 2011), 8,405 genes have been identified that are linked to hsSNCs via genomic proximity co-localization analysis, indicating that expression of these hsSNCs-linked genes might be affected by hsSNCs located in DA chromatin regions during brain development. Comprehensive gene set enrichment analyses of these 8,405 genes revealed the staggering breadth of associations with physiological processes and pathological conditions of *H. sapiens*. Most significantly enriched records include more than 1,000 anatomically-distinct regions of the adult human brain, many human tissues and cell types, more than 200 common human disorders and more than 1,000 rare diseases.

Based on the reported above observations, it has been concluded that genes linked to neuro-regulatory hsSNCs appear contributing to development, morphological architecture, and biological functions of the adult human brain, other components of the central nervous system, and many tissues and organs across human

body. They were implicated in the extensive range of human physiological and pathological conditions, thus supporting the hypothesis that phenotype-altering effects of neuro-regulatory hsSNCs are not restricted to the early-stages of human brain development. Results of the analyses utilizing the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/) revealed that neuro-regulatory hsSNCs-associated genes affect wide spectra of traits defining both physiology and pathology of Modern Humans, perhaps, reflecting the global scale of human-specific regulatory impacts on thousands essential mammalian phenotypes. Significantly, outlined herein analytical approaches and reported end-points provide readily available access to mouse models for precise molecular definitions of regulatory effects of neuro-regulatory human-specific SNCs and other types of HSRS on genes causally affecting thousands of defined mammalian phenotypes and hundreds of common and rare human disorders.

Methods

Data source and analytical protocols

Candidate human-specific regulatory sequences and African Apes-specific retroviral insertions

A total of 94,806 candidate HSRS, including 35,074 neuro-regulatory human-specific SNCs, detailed descriptions of which and corresponding references of primary original contributions are reported elsewhere (Glinsky et al., 2015-2019; Kanton et al., 2019). Solely publicly available datasets and resources were used in this contribution. The significance of the differences in the expected and observed numbers of events was calculated using two-tailed Fisher's exact test. Additional placement enrichment tests were performed for individual classes of HSRS taking into account the size in bp of corresponding genomic regions.

Data analysis

Categories of DNA sequence conservation

Identification of highly-conserved in primates (pan-primate), primate-specific, and human-specific sequences was performed as previously described (Glinsky, 2015-2019). In brief, all categories were defined by direct and reciprocal mapping using LiftOver. Specifically, the following categories of candidate regulatory sequences were distinguished:

- Highly conserved in primates' sequences: DNA sequences that have at least 95% of bases remapped during conversion from/to human (Homo sapiens, hg38), chimp (Pan troglodytes, v5), and bonobo (Pan paniscus, v2; in specifically designated instances, Pan paniscus, v1 was utilized for comparisons). Similarly, highly-conserved sequences were defined for hg38 and latest releases of genomes of Gorilla, Orangutan, Gibbon, and Rhesus.
- Primate-specific: DNA sequences that failed to map to the mouse genome (mm10).
- Human-specific: DNA sequences that failed to map at least 10% of bases from human to both chimpanzee and bonobo. All candidate HSRS identified based on the sequence alignments failures to genomes of both chimpanzee and bonobo were subjected to more stringent additional analyses requiring the mapping failures to genomes of Gorilla, Orangutan, Gibbon, and Rhesus. These loci were considered created *de novo* human-specific regulatory sequences (HSRS).

To infer the putative evolutionary origins, each evolutionary classification was defined independently by running the corresponding analyses on all candidate HSRS representing the specific category. For example, human-rodent conversion identify sequences that are absent in the mouse genome based on the sequence identity threshold of 10%). Additional comparisons were performed using the same methodology and exactly as stated in the manuscript text.

Gene set enrichment and genome-wide proximity placement analyses

Gene set enrichment analyses were carried-out using the Enrichr bioinformatics platform, which enables the interrogation of nearly 200,000 gene sets from more than 100 gene set libraries. The Enrichr API (January 2018 through January 2020 releases) (Chen et al., 2013; Kuleshov et al., 2016) was used to test genes linked to HSRS of interest for significant enrichment in numerous functional categories. In all tables and plots (unless stated otherwise), in addition to the nominal p values and adjusted p values, the "combined score" calculated by Enrichr is reported, which is a product of the significance estimate and the magnitude of enrichment (combined score $c = log(p) * z$, where p is the Fisher's exact test p-value and z is the z-score deviation from the expected rank). When technically feasible, larger sets of genes comprising several thousand entries were analyzed. Regulatory connectivity maps between HSRS and coding genes and additional functional enrichment analyses were performed with the GREAT algorithm (McLean et al., 2010; 2011) at default

settings. Genome-wide Proximity Placement Analysis (GPPA) of distinct genomic features co-localizing with HSRS was carried out as described previously (Glinsky, 2015-2019).

Mammalian Phenotype Ontology and Human Disease Ontology analyses

To validate and extend findings afforded by the gene set enrichment analyses and to identify all genes linked with human-specific regulatory SNCs that are associated with defined mammalian phenotypes as well as implicated in development of human diseases with one or more mouse models, the additional analyses have been carried out utilizing the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/).

Statistical Analyses of the Publicly Available Datasets

All statistical analyses of the publicly available genomic datasets, including error rate estimates, background and technical noise measurements and filtering, feature peak calling, feature selection, assignments of genomic coordinates to the corresponding builds of the reference human genome, and data visualization, were performed exactly as reported in the original publications and associated references linked to the corresponding data visualization tracks (http://genome.ucsc.edu/). Any modifications or new elements of statistical analyses are described in the corresponding sections of the Results. Statistical significance of the Pearson correlation coefficients was determined using GraphPad Prism version 6.00 software. Both nominal and Bonferroni adjusted p values were estimated. The significance of the differences in the numbers of events between the groups was calculated using two-sided Fisher's exact and Chi-square test, and the significance of the overlap between the events was determined using the hypergeometric distribution test (Tavazoie et al., 1999).

Supplemental Information

Supplemental information includes Supplemental Table Sets S1 – S5, Supplemental Text, and Supplemental Figures S1-S9.

Author Contributions

This is a single author contribution. All elements of this work, including the conception of ideas, formulation, and development of concepts, execution of experiments, analysis of data, and writing of the paper, were performed by the author.

Acknowledgements

This work was made possible by the open public access policies of major grant funding agencies and international genomic databases and the willingness of many investigators worldwide to share their primary research data.

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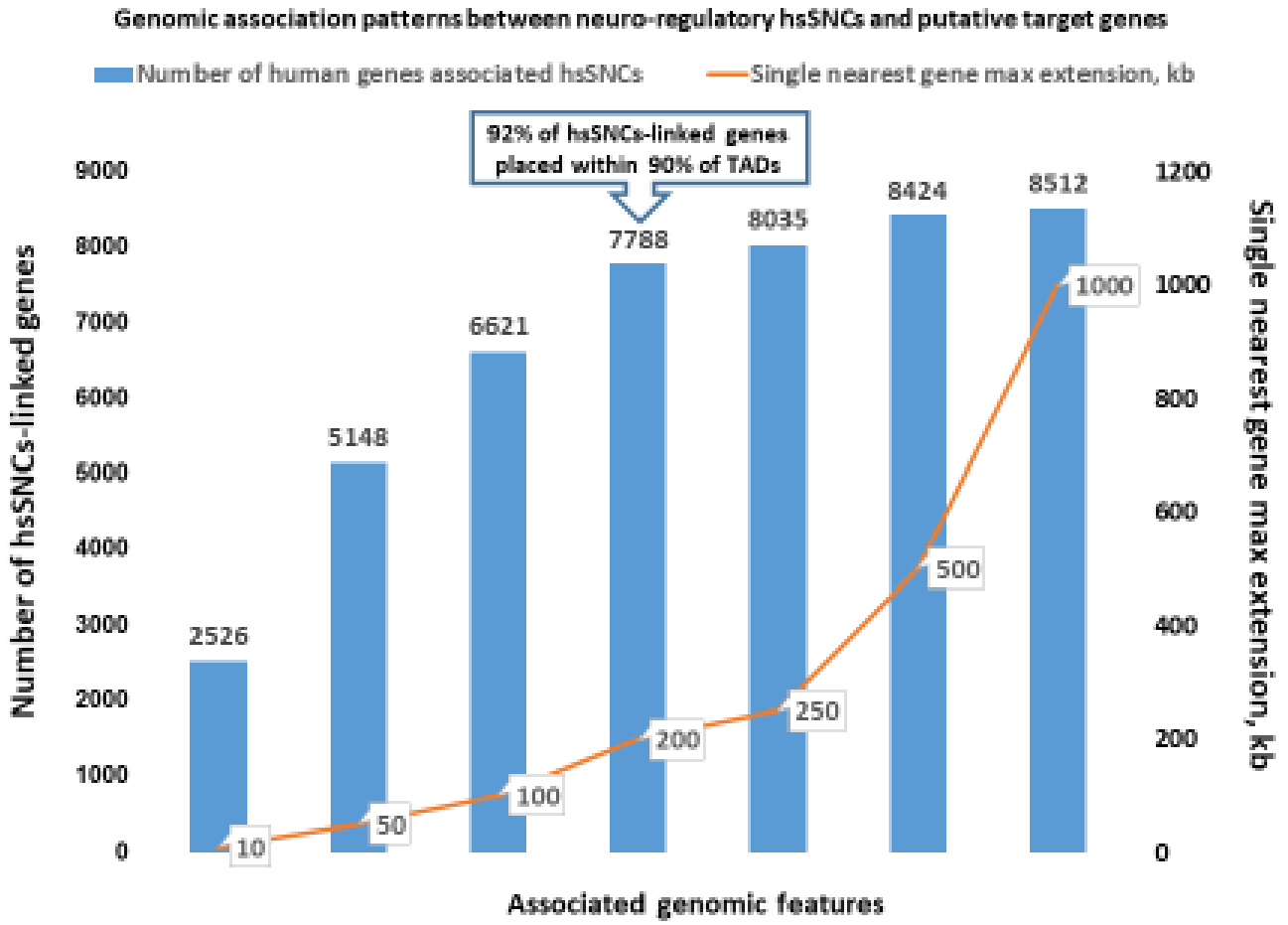
Table 1. Associations with human physiological processes and pathological conditions of 8,405 genes linked with 35,074 human-specific single nucleotide changes (SNC) within differentially-accessible (DA) chromatin regions identified during human and chimpanzee brain development in cerebral organoids.

Database	Number of significant records*
ARCHS4 Human Tissues	39
GO Biological Process 2018	392
GO Molecular Function 2018	89
GO Cellular Component 2018	33
KEGG 2019 Human	129
KEGG 2019 Mouse	106
MGI Mammalian Phenotype Level 4 2019	407
MGI Mammalian Phenotype 2017	749
Human Phenotype Ontology	298
GWAS Catalog 2019	241
Rare Diseases AutoRIF Gene Lists	1116
Rare Diseases GeneRIF Gene Lists	473
Rare Diseases GeneRIF ARCHS4 Predictions	603
Rare Diseases AutoRIF ARCHS4 Predictions	641
Aging Perturbations from GEO (Up-regulated genes)	34
Aging Perturbations from GEO (Down-regulated genes)	67
Human Brain Regions: Allen Brain Atlas (Up-regulated genes)	1218
Human Brain Regions: Allen Brain Atlas (Down-regulated genes)	1102
Disease Perturbations from GEO (Down-regulated genes)	240
Disease Perturbations from GEO (Up-regulated genes)	204
Human Database of Genotypes and Phenotype (dbGaP)	136
DisGeNET database	1313
UK Biobank GWAS v1	357

Legend: *, defined at adjusted p-value < 0.05; GEO, gene expression omnibus; GO, gene ontologies; GWAS, genome-wide association studies; ARCHS4, all RNA-seq and ChIP-seq sample and signature search; KEGG, Kyoto Encyclopedia of Genes and Genomes; MGI, mouse genome informatics;

A


Genomic association patterns between neuro-regulatory hsSNCs and putative target genes
Number of human genes associated hsSNCs
Single nearest gene max extension, kb
92% of hsSNCs-linked genes placed within 90% of TADs
Number of hsSNCs-linked genes
Single nearest gene max extension, kb
Associated genomic features
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
0
200
400
600
800
1000
1200
2526
5148
6621
7788
8035
8424
8512
10
50
100
200
250
500
1000

B

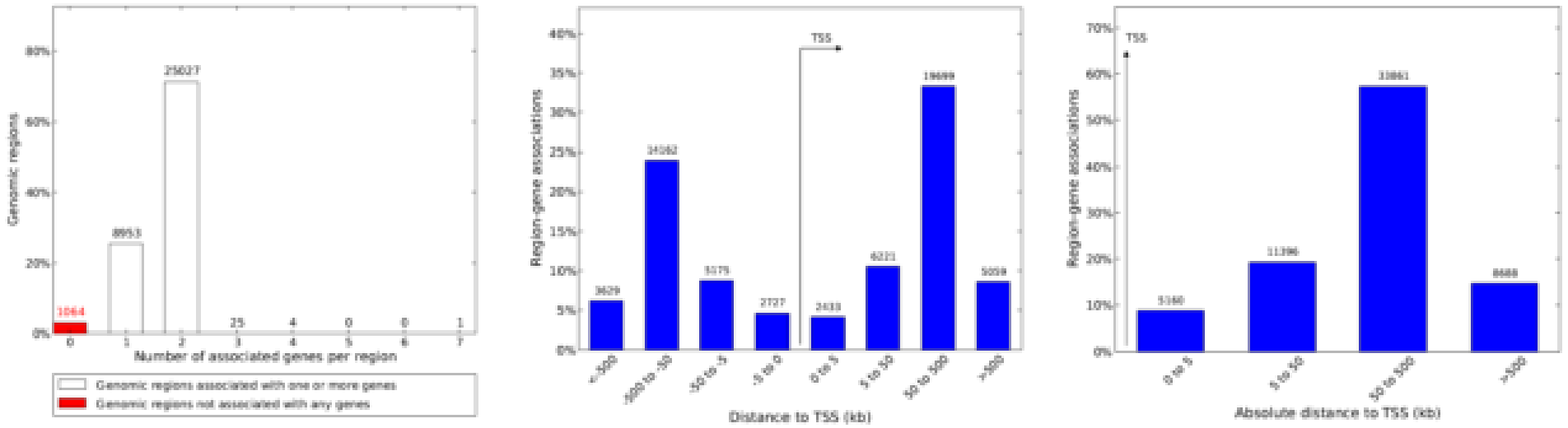
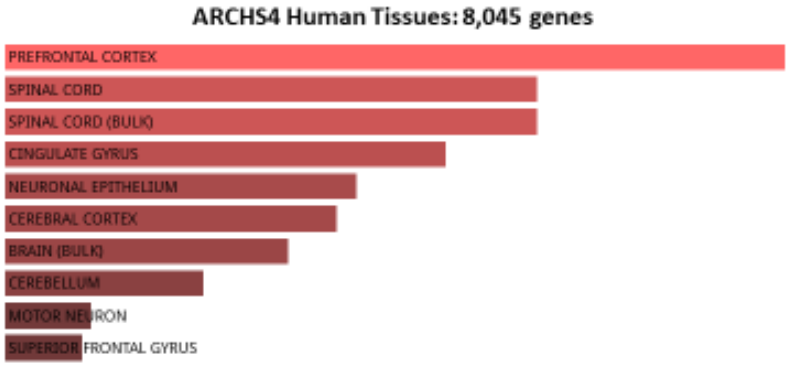


Figure 1. GREAT analysis identifies 8,405 human genes associated with 35,074 human-specific single nucleotide changes (SNCs) in differentially accessible (DA) chromatin regions during human and chimpanzee brain development in cerebral organoids.

A. Patterns of genomic associations between neuro-regulatory SNCs and putative target genes defined at different single nearest gene maximum extensions.

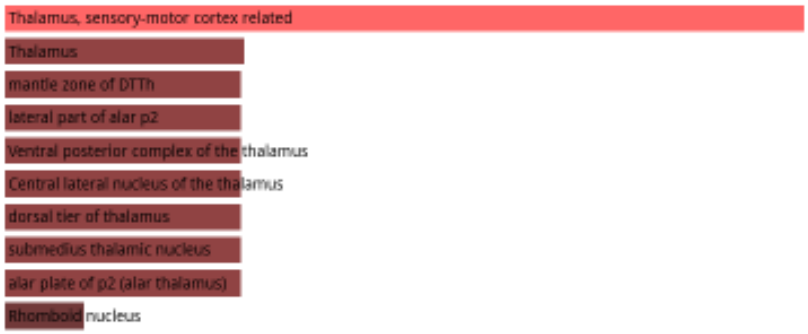
B. A total of 1,064 of all 35,074 SNCs (3%) are not associated with any genes in the human genome, while a total of 34,010 (97%) human-specific SNCs in DA regions appear associated with 8,405 human genes.

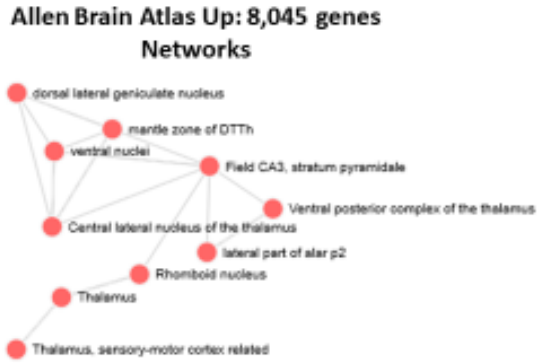
SUPPLEMENTAL FIGURES

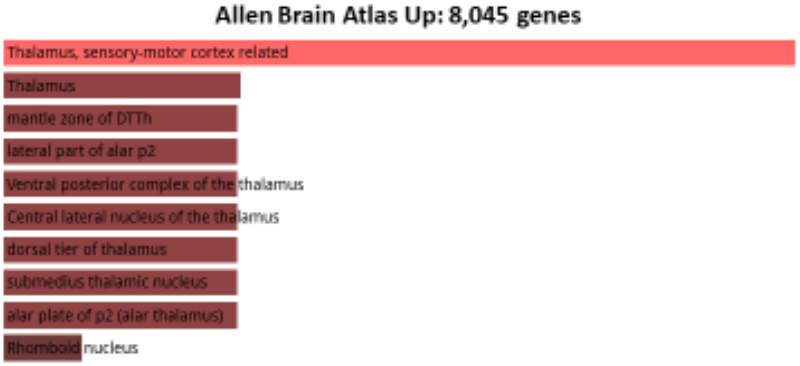


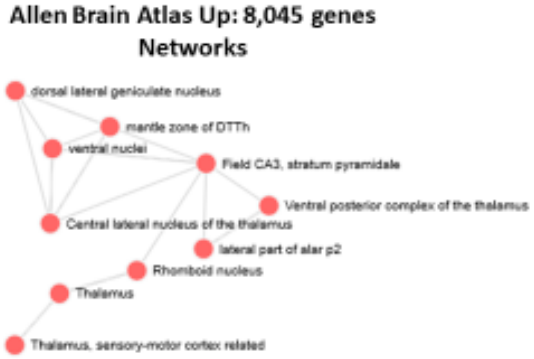
ARCHS4 Human Tissues: 8,045 genes
Top 10 of 39 significant records

Term	Overlap	P-value	Adjusted P-value
PREFRONTAL CORTEX	1348/2316	2.06686E-62	2.23E-60
SPINAL CORD	1299/2316	1.17222E-47	4.22E-46
SPINAL CORD (BULK)	1299/2316	1.17222E-47	4.22E-46
CINGULATE GYRUS	1279/2316	3.13703E-42	8.47E-41
NEURONAL EPITHELIUM	1258/2316	6.71385E-37	1.45E-35
CEREBRAL CORTEX	1253/2316	1.09815E-35	1.98E-34
BRAIN (BULK)	1241/2316	7.35673E-33	1.14E-31
CEREBELLUM	1218/2316	8.73598E-28	1.18E-26
MOTOR NEURON	1184/2316	4.19448E-21	5.03E-20
SUPERIOR FRONTAL GYRUS	1181/2316	1.4634E-20	1.58E-19



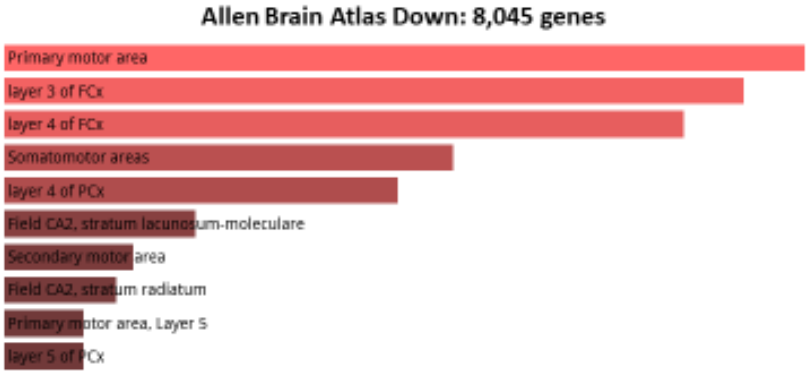


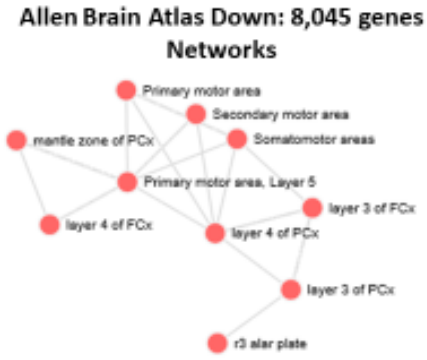


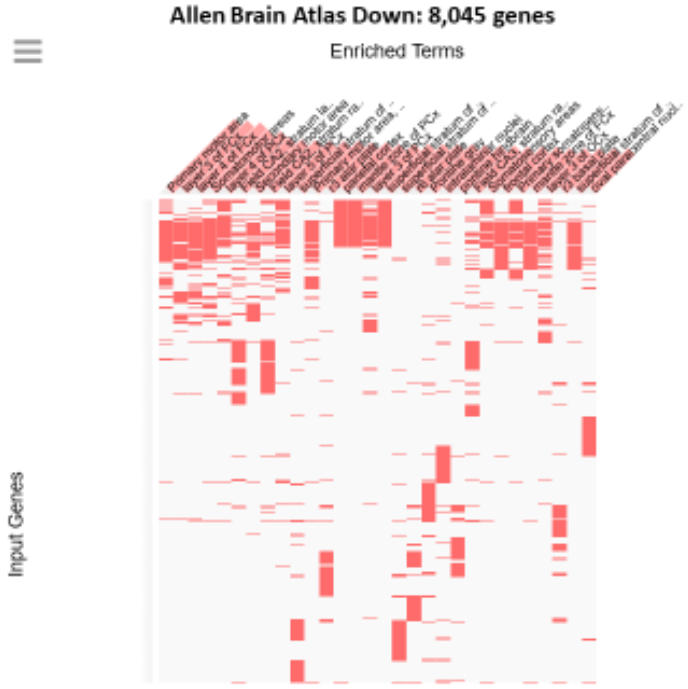


Allen Brain Atlas Up: 8,045 genes
Top 30 of 1,200 significant records

Term	Overlap	P-value	Adjusted P-value
Thalamus, sensory-motor cortex related	207/301	3.57E-21	7.82E-18
Thalamus	222/334	9.71E-20	2.42E-17
mantle zone of DTTh	204/301	9.94E-20	2.42E-17
lateral part of alar p2	204/301	9.94E-20	2.42E-17
Ventral posterior complex of the thalamus	204/301	9.94E-20	2.42E-17
Central lateral nucleus of the thalamus	204/301	9.94E-20	2.42E-17
alar plate of p2 (alar thalamus)	204/301	9.94E-20	2.42E-17
submedius thalamic nucleus	204/301	9.94E-20	2.42E-17
dorsal tier of thalamus	204/301	9.94E-20	2.42E-17
Rhomboid nucleus	227/345	2.51E-19	5.34E-17
ventral nuclei	203/301	2.92E-19	5.34E-17
Ventral group of the dorsal thalamus	203/301	2.92E-19	5.34E-17
Field CA3, stratum pyramidale	202/301	8.47E-19	1.24E-16
Ventral posteromedial nucleus of the thalamus	202/301	8.47E-19	1.24E-16
ventral posteromedial nucleus	202/301	8.47E-19	1.24E-16
intermediate stratum of DTTh	201/301	2.42E-18	2.94E-16
prosomere 2	201/301	2.42E-18	2.94E-16
central lateral nucleus	201/301	2.42E-18	2.94E-16
dorsal lateral geniculate nucleus	200/301	6.79E-18	7.84E-16
hilus of the DG	199/301	1.88E-17	2.06E-15
lateral (parvicellular) part of MD	198/301	5.13E-17	4.33E-15
Dorsal part of the lateral geniculate complex	198/301	5.13E-17	4.33E-15
Ventral posterolateral nucleus of the thalamus	198/301	5.13E-17	4.33E-15
posterior (ventral) nucleus	198/301	5.13E-17	4.33E-15
Hippocampal formation	198/301	5.13E-17	4.33E-15
intralaminar nuclei	198/301	5.13E-17	4.33E-15
intermediate stratum of DG	353/597	9.09E-17	7.38E-15
Field CA3, stratum radiatum	219/342	1.17E-16	9.12E-15
mantle zone of CA	197/301	1.38E-16	9.76E-15
Thalamus, polymodal association cortex related	197/301	1.38E-16	9.76E-15



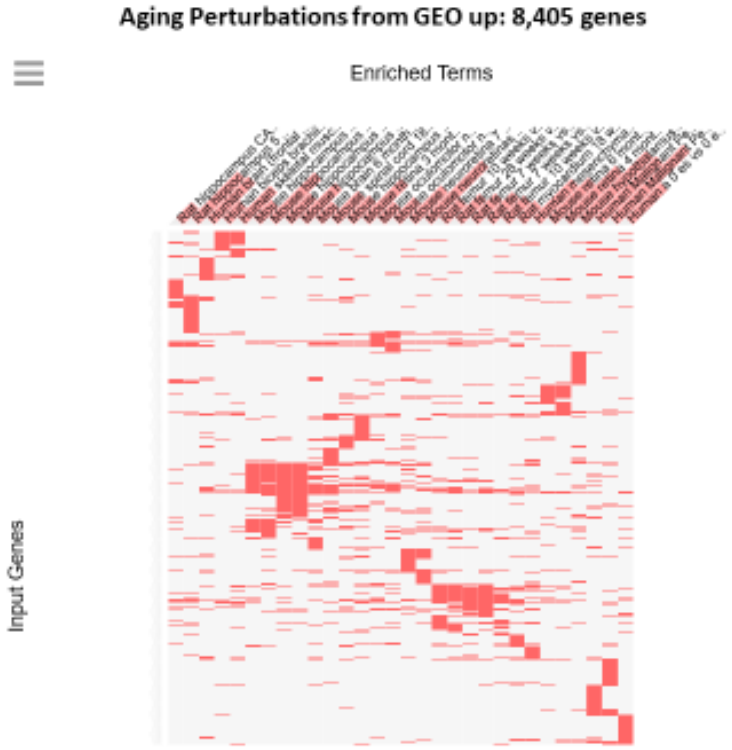




Allen Brain Atlas Down: 8,045 genes
Top 31 of 1,062 significant records

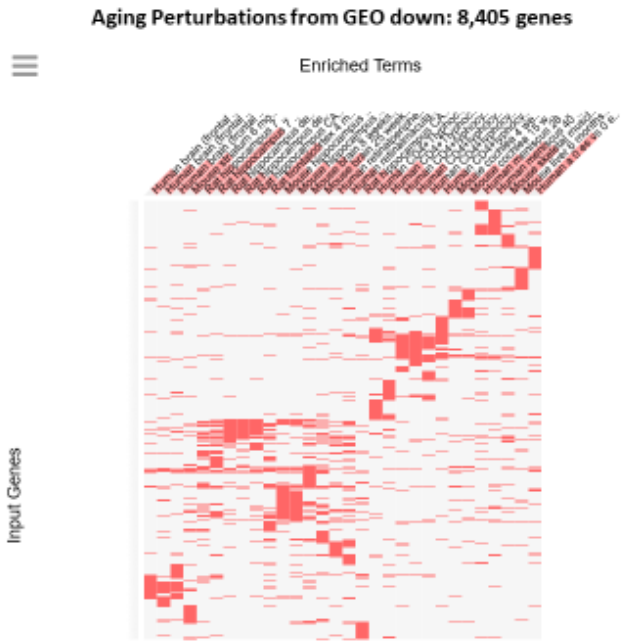
Term	Overlap	P-value	Adjusted P-value
Primary motor area	195/300	5.73E-16	1.26E-12
layer 3 of FCx	194/300	1.49E-15	1.63E-12
layer 4 of FCx	193/300	3.81E-15	2.79E-12
Somatomotor areas	189/300	1.42E-13	7.77E-11
layer 4 of PCx	188/300	3.38E-13	1.48E-10
Field CA2, stratum lacunosum-moleculare	248/426	8.01E-12	2.93E-09
Secondary motor area	183/300	2.1E-11	6.57E-09
Field CA2, stratum radiatum	262/458	2.75E-11	7.54E-09
Primary motor area, Layer 5	182/300	4.59E-11	9.16E-09
layer 5 of PCx	182/300	4.59E-11	9.16E-09
superficial stratum of m1B	182/300	4.59E-11	9.16E-09
r3 alar plate	181/300	9.92E-11	1.67E-08
parietal cortex	181/300	9.92E-11	1.67E-08
mantle zone of PCx	180/300	2.11E-10	2.44E-08
layer 3 of PCx	180/300	2.11E-10	2.44E-08
Pontine gray	180/300	2.11E-10	2.44E-08
r6 alar plate	183/300	2.11E-10	2.44E-08
superficial stratum of PCx (cortical plate/marginal zone)	180/300	2.11E-10	2.44E-08
superficial stratum of p2B	180/300	2.11E-10	2.44E-08
intralaminar nuclei	179/300	4.44E-10	4.64E-08
pontine hindbrain	182/300	4.44E-10	4.64E-08
Field CA3, stratum radiatum	211/364	4.83E-10	4.81E-08
Primary somatosensory area	178/300	9.2E-10	8.07E-08
frontal cortex	179/300	9.2E-10	8.07E-08
Somatosensory areas	178/300	9.2E-10	8.07E-08
mantle zone of FCx	178/300	1.88E-09	1.37E-07
layer 3 of OCx	177/300	1.88E-09	1.37E-07
r3 basal plate	177/300	1.88E-09	1.37E-07
superficial stratum of FCx (cortical plate/marginal zone)	178/300	1.88E-09	1.37E-07
oval paracentral nucleus	177/300	1.88E-09	1.37E-07
Primary motor area, Layer 2/3	176/300	3.79E-09	2.6E-07

Supplemental Figure S1. Identification of genes expression of which distinguishes thousands of anatomically distinct areas of the adult human brain, various regions of the central nervous system, and many different cell types and tissues in the human body.



Aging Perturbations from GEO up: 8,405 genes
Top 30 significant records

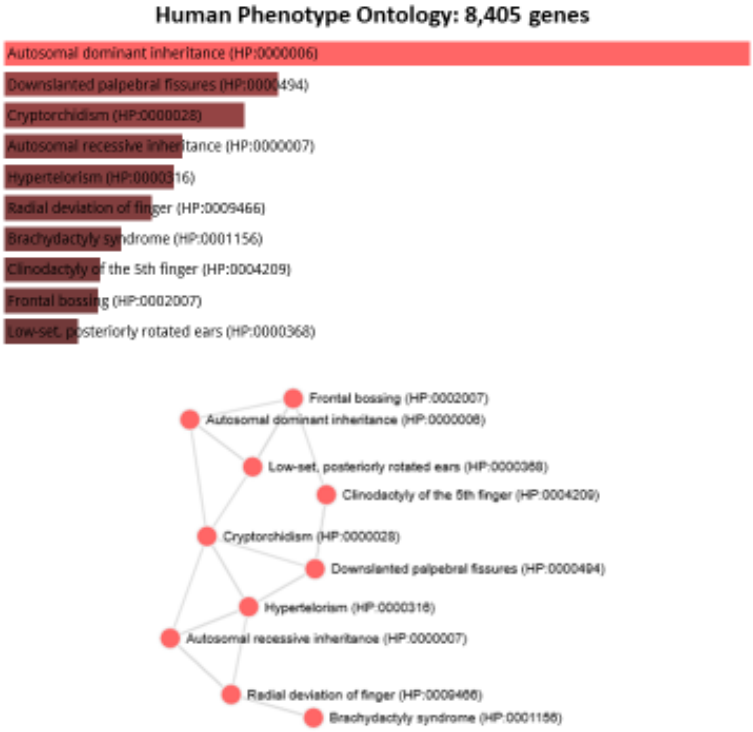
Term	Overlap	P-value	Adjusted P-value
Human_Malignant Peripheral Nerve Sheath Tumour_24 years vs 53 years_GSE17118_aging:364	197/337	6.9E-10	1.97E-07
Mouse_hypothalamus_42 days vs 182 days_GDS3895_aging:107	201/349	2.59E-09	3.7E-07
Human_Malignant Peripheral Nerve Sheath Tumour_27 years vs 61 years_GSE17118_aging:363	195/343	1.78E-08	1.5E-06
Mouse_neuroretinas_7 weeks vs 64 weeks_GSE38671_aging:211	168/289	2.1E-08	1.5E-06
Mouse_retina_4 months vs 10 months_GSE33674_aging:304	148/254	1.16E-07	6.63E-06
Rat_hippocampus_5 months vs 24 months_GSE14505_aging:346	230/429	6.82E-07	3.25E-05
Mouse_hippocampus_9 months vs 20 months_GSE48911_aging:391	243/459	1.23E-06	5.02E-05
Mouse_retina_6 months vs 10 months_GSE33674_aging:305	125/218	3.39E-06	0.000121
Human_brain (frontal cortex)_55 years vs 82 years_GSE53890_aging:229	171/313	4.05E-06	0.000129
Mouse_retina_3 months vs 16 months_GDS2654_aging:66	199/372	4.53E-06	0.00013
Human_mesenchymal stem cells (from bone marrow)_42 years vs 79 years_GSE35955_aging:293	133/238	1.03E-05	0.000255
Human_a_0 es vs 0 es_GDS5077_aging:106	172/319	1.07E-05	0.000255
Mouse_hippocampus_9 months vs 14 months_GSE48911_aging:390	254/494	1.28E-05	0.000283
Mouse_neuroretina_7 weeks vs 64 weeks_GSE38671_aging:210	171/319	1.76E-05	0.00036
Human_skeletal muscle_19 years vs 65 years_GDS4858_aging:10	140/259	5.77E-05	0.0011
Mouse_spinal cord_18 months vs 30 months_GDS1280_aging:1	172/331	0.000151	0.002697
Mouse_hippocampus_9 months vs 14 months_GSE48911_aging:384	246/494	0.000252	0.004247
Rat_femur_7 weeks vs 53 weeks_GDS509_aging:264	197/389	0.000331	0.005257
Rat_femur_28 weeks vs 54 weeks_GDS509_aging:271	193/383	0.000523	0.007575
Rat_femur_7 weeks vs 27 weeks_GDS509_aging:258	155/301	0.00053	0.007575
Mouse_hippocampus_2 months vs 15 months_GSE5078_aging:398	151/293	0.000593	0.008072
Rat_femur_10 weeks vs 30 weeks_GDS509_aging:260	123/236	0.001057	0.013739
Mouse_oculomotor nucleus_6 months vs 30 months_GDS1280_aging:6	140/273	0.001185	0.01473
Rat_femur_10 weeks vs 56 weeks_GDS509_aging:266	191/384	0.001248	0.014873
Mouse_hippocampus_9 months vs 20 months_GSE48911_aging:385	216/442	0.001959	0.022408
Human_biceps brachii muscles_24 years vs 70 years_GDS4858_aging:33	119/233	0.003152	0.034649
Rat_myocardium_18 weeks vs 22 weeks_GDS4025_aging:142	124/244	0.003271	0.034649
Rat_hippocampus CA3 region_6 months vs 25 months_GSE14724_aging:347	170/345	0.003642	0.036658
Mouse_brain_6 months vs 14 months_GSE15129_aging:313	189/387	0.003717	0.036658
Mouse_spinal cord_6 months vs 30 months_GDS1280_aging:3	197/405	0.003882	0.037008

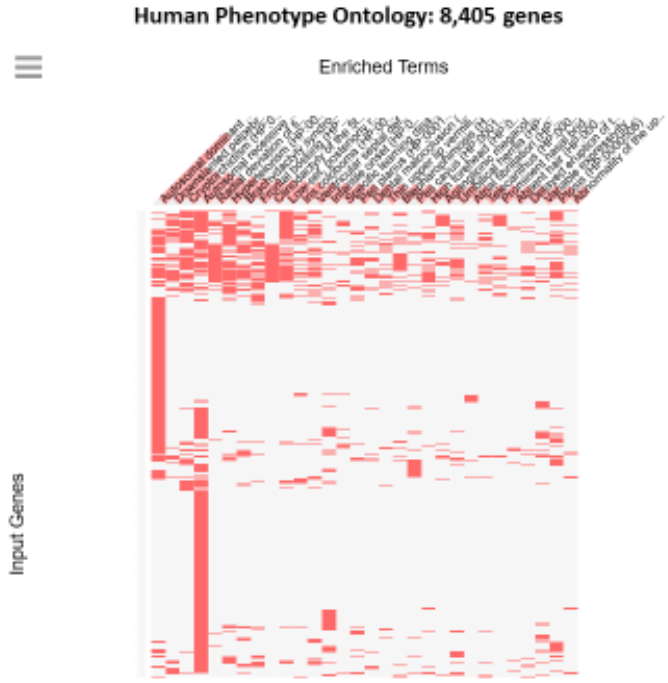


Aging Perturbations from GEO down: 8,405 genes
Top 30 significant records

Term	Overlap	P-value	Adjusted P-value
Rat_hippocampus_7 months vs 21 months_GDS4019_aging:98	196/314	1.85E-13	5.28E-11
Human_a_0 es vs 0 es_GDS5077_aging:106	175/281	4.78E-12	6.83E-10
Human_meniscus_40 years vs 55 years_GSE45233_aging:209	189/320	4.76E-10	4.54E-08
Rat_frontal cortex_4 months vs 22 months_GDS3939_aging:99	192/327	6.86E-10	4.9E-08
Mouse_skeletal muscle precursor_12 months vs 24 months_GDS4892_aging:9	137/224	5.29E-09	3.02E-07
Mouse_brain_25 weeks vs 100 weeks_GSE41018_aging:400	178/311	3.83E-08	1.68E-06
Mouse_striatum_6 months vs 21 months_GDS4153_aging:78	164/283	4.12E-08	1.68E-06
Human_meniscus_28 years vs 47 years_GSE45233_aging:208	165/286	5.7E-08	2.04E-06
Mouse_brain_8 weeks vs 104 weeks_GSE20411_aging:311	224/408	8.36E-08	2.66E-06
Human_brain (frontal cortex)_28 years vs 100 years_GSE53890_aging:228	197/356	2.32E-07	6.64E-06
Human_CD4+Tlyphocytes_61 years vs 81 years_GSE62373_aging:185	193/349	3.26E-07	8.48E-06
Human_CD4+lyphocytes_40 years vs 72 years_GSE62373_aging:163	203/373	7.65E-07	1.82E-05
Mouse_hippocampus_4 months vs 9 months_GSE48911_aging:387	230/430	8.6E-07	1.89E-05
Rat_hippocampus_7 months vs 21 months_GDS4019_aging:97	156/277	9.99E-07	2.04E-05
Mouse_cochlea_15 weeks vs 45 weeks_GSE35234_aging:164	142/250	1.58E-06	3.01E-05
Human_brain (frontal cortex)_55 years vs 82 years_GSE53890_aging:229	159/287	2.97E-06	5.31E-05
Human_retinalmacula_18 years vs 74 years_GSE32614_aging:150	182/336	4.14E-06	6.96E-05
Mouse_hippocampus_4 months vs 9 months_GSE48911_aging:381	226/429	4.52E-06	7.18E-05
Human_CD4+lyphocytes_40 years vs 61 years_GSE62373_aging:160	192/358	5.34E-06	7.77E-05
Human_CD4+Tlyphocytes_29 years vs 81 years_GSE62373_aging:176	201/377	5.44E-06	7.77E-05
Human_CD4+lyphocytes_29 years vs 61 years_GSE62373_aging:154	184/342	6.43E-06	8.75E-05
Human_retinalperiphery_32 years vs 74 years_GSE32614_aging:151	160/293	8.42E-06	0.000109
Human_brain (frontal cortex)_28 years vs 82 years_GSE53890_aging:227	154/281	9.47E-06	0.000118
Rat_hippocampus CA3 region_6 months vs 25 months_GSE14724_aging:347	141/255	1.2E-05	0.000143
Mouse_cochlea_4 weeks vs 45 weeks_GSE35234_aging:161	145/268	4.04E-05	0.000463
Rat_hippocampus dentate gyrus_18 months vs 28 months_GSE21681_aging:357	124/225	4.78E-05	0.000522
Rat_hippocampus dentate gyrus_18 months vs 28 months_GSE21681_aging:358	147/273	4.93E-05	0.000522
Human_CD4+lyphocytes_40 years vs 81 years_GSE62373_aging:165	197/380	6.24E-05	0.000638
Human_CD4+lyphocytes_29 years vs 81 years_GSE62373_aging:156	215/419	6.73E-05	0.000664
Rat_hippocampus CA1_18 months vs 28 months_GSE21681_aging:353	144/270	0.000107	0.001004

Supplemental Figure S2. Identification and characterization of genes expression of which is altered during aging of humans, rats, and mice.

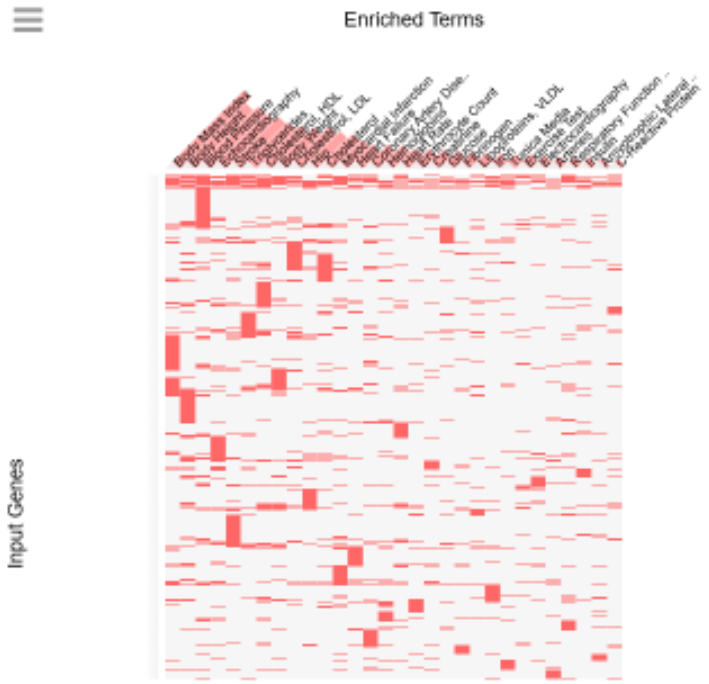




Human Phenotype Ontology (8,405 genes): Top 35 of 298 significant records

Term	Overlap	P-value	Adjusted P-value
Autosomal dominant inheritance (HP:0000006)	645/1134	2.85E-25	5.07E-22
Downslanted palpebral fissures (HP:0000494)	127/188	1.17E-12	1.04E-09
Cryptorchidism (HP:0000028)	222/379	4.46E-11	2.64E-08
Autosomal recessive inheritance (HP:0000007)	840/1722	1.14E-10	5.07E-08
Radial deviation of finger (HP:0009466)	140/229	3.75E-09	1.11E-06
Hypertelorism (HP:0000316)	190/328	3.76E-09	1.11E-06
Brachydactyly syndrome (HP:0001156)	119/192	1.77E-08	4.5E-06
Frontal bossing (HP:0002007)	129/213	3.38E-08	7.51E-06
Clinodactyly of the 5th finger (HP:0004209)	118/192	4.03E-08	7.96E-06
Low-set, posteriorly rotated ears (HP:0000368)	158/276	2.14E-07	3.8E-05
Iris coloboma (HP:0000612)	72/110	5.77E-07	9.34E-05
Ventricular septal defect (HP:0001629)	106/176	7.99E-07	0.000113
Infantile onset (HP:0003593)	145/254	8.28E-07	0.000113
Specific learning disability (HP:0001328)	36/47	1.49E-06	0.000189
Pes planus (HP:0001763)	68/105	2.09E-06	0.000246
Dental malocclusion (HP:0000689)	55/81	2.21E-06	0.000246
Thin upper lip vermilion (HP:0000219)	38/51	2.5E-06	0.000261
Blepharophimosis (HP:0000581)	67/104	3.26E-06	0.000323
Pes cavus (HP:0001761)	80/129	3.55E-06	0.000323
High forehead (HP:0000348)	69/108	3.63E-06	0.000323
Aganglionic megacolon (HP:0002251)	63/97	4.26E-06	0.000361
Umbilical hernia (HP:0001537)	91/151	4.5E-06	0.000364
Atrial fibrillation (HP:0005110)	26/32	6.52E-06	0.000505
Telecanthus (HP:0000506)	55/83	6.96E-06	0.000516
Prominent nasal bridge (HP:0000426)	64/100	7.43E-06	0.000529
Absent hair (HP:0002298)	18/20	1.14E-05	0.000781
Delayed eruption of teeth (HP:0000684)	56/86	1.28E-05	0.000841
Variable expressivity (HP:0003828)	86/144	1.34E-05	0.00085
Ptosis (HP:0000508)	180/338	1.79E-05	0.001074
Abnormality of the upper arm (HP:0001454)	29/38	1.81E-05	0.001074
Progressive disorder (HP:0003676)	86/145	1.94E-05	0.001103
Depressed nasal bridge (HP:0005280)	127/228	1.99E-05	0.001103
Sporadic (HP:0003745)	42/61	2.05E-05	0.001103
Left ventricular hypertrophy (HP:0001712)	31/42	2.93E-05	0.0015
Postaxial hand polydactyly (HP:0001162)	53/82	2.95E-05	0.0015

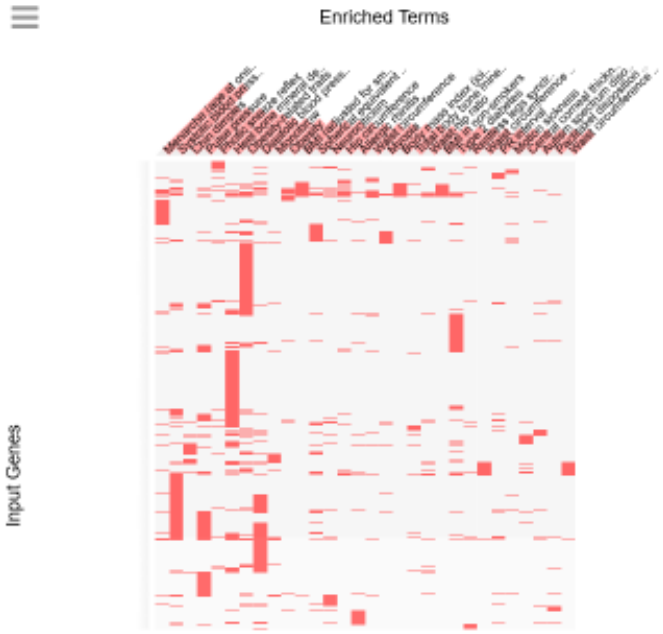
Database of Human Genotypes and Phenotypes (dbGaP): 8,405 genes



Database of Human Genotypes and Phenotypes (dbGaP): 8,405 genes
Top 20 of 136 significant records

Term	Overlap	P-value	Adjusted P-value
Body Mass Index	313/437	1.02E-36	3.46E-34
Body Height	276/385	1.32E-32	2.23E-30
Blood Pressure	310/454	3.19E-30	3.59E-28
Echocardiography	204/273	2.47E-28	2.08E-26
Stroke	211/289	6.02E-27	4.07E-25
Triglycerides	180/244	4.77E-24	2.68E-22
Cholesterol, HDL	242/357	3.57E-23	1.72E-21
Body Weight	161/216	1.91E-22	8.05E-21
Cholesterol, LDL	210/304	7.78E-22	2.92E-20
Hip	151/202	2.39E-21	8.06E-20
Cholesterol	183/268	2.28E-18	7.02E-17
Myocardial Infarction	157/229	3.32E-16	9.36E-15
Coronary Artery Disease	140/205	2.16E-14	5.31E-13
Heart Failure	130/187	2.2E-14	5.31E-13
Hemoglobins	113/157	2.38E-14	5.37E-13
Erythrocyte Count	87/115	2.09E-13	4.41E-12
Heart Rate	110/155	2.47E-13	4.86E-12
Creatinine	73/92	2.59E-13	4.86E-12
Fibrinogen	69/86	4.29E-13	7.05E-12
Lipoproteins, VLDL	69/86	4.29E-13	7.05E-12

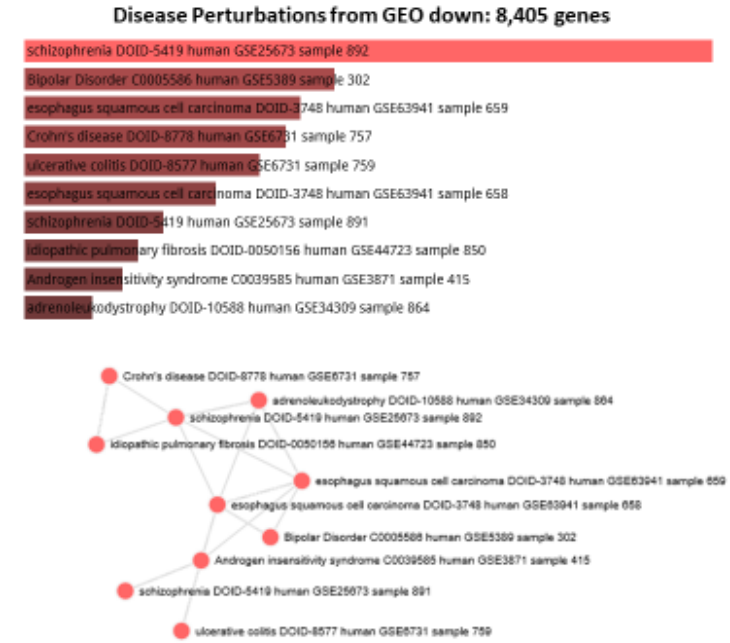
GWAS Catalog 2019: 8,405 genes

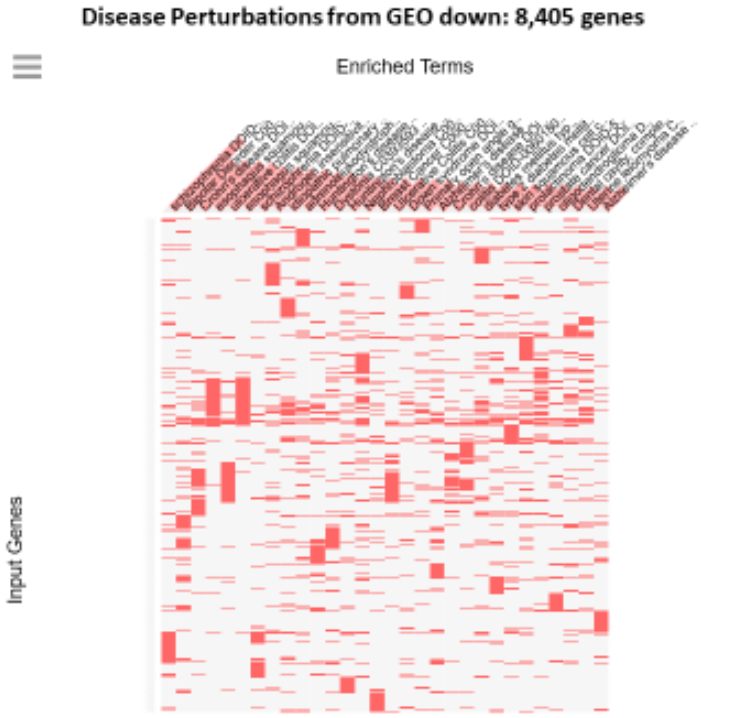


GWAS Catalog 2019 (8,405 genes): Top 40 of 241 significant records

Term	Overlap	P-value	Adjusted P-value
Menarche (age at onset)	154/212	1.12E-19	1.4E-16
Systolic blood pressure	389/657	1.62E-19	1.4E-16
Chin dimples	62/74	1.51E-13	8.68E-11
Pulse pressure	321/567	9.66E-13	4.16E-10
Photic sneeze reflex	55/65	1.6E-12	5.51E-10
Heel bone mineral density	478/898	3.09E-12	8.88E-10
Obesity-related traits	427/804	6.98E-11	1.72E-08
Diastolic blood pressure	348/646	4.87E-10	1.05E-07
Monobrow	58/76	1.19E-09	2.28E-07
Obesity	45/55	1.61E-09	2.78E-07
Height	288/527	2.32E-09	3.63E-07
Atrial fibrillation	146/240	2.83E-09	3.78E-07
BMI (adjusted for smoking behaviour)	58/77	2.85E-09	3.78E-07
Spherical equivalent or myopia (age of diagnosis)	120/191	4.87E-09	5.99E-07
Neuroticism	69/97	5.87E-09	6.74E-07
Hip circumference	52/69	1.83E-08	1.97E-06
Diverticular disease	100/156	2.04E-08	2.06E-06
Allergic rhinitis	77/114	3.13E-08	3E-06
Waist circumference	55/75	5.67E-08	3.33E-06
Myopia	52/70	4.2E-08	3.62E-06
Body mass index (joint analysis main effects and smoking interaction)	56/77	4.5E-08	3.69E-06
Hand grip strength	99/156	5.05E-08	3.96E-06
Total body bone mineral density	31/36	5.77E-08	4.32E-06
Waist-hip ratio	44/58	1.65E-07	1.14E-05
BMI in non-smokers	44/58	1.65E-07	1.14E-05
Type 2 diabetes	217/397	2.08E-07	1.38E-05
Restless legs syndrome	32/39	3.48E-07	2.22E-05
Blond vs. brown/black hair color	98/160	6.9E-07	4.25E-05
Waist circumference adjusted for BMI (adjusted for smoking behaviour)	56/81	7.21E-07	4.29E-05
Amyotrophic lateral sclerosis (sporadic)	109/182	8.3E-07	4.77E-05
PR interval	48/67	8.6E-07	4.78E-05
Motion sickness	27/32	1.01E-06	5.45E-05
Male-pattern baldness	143/251	1.15E-06	6E-05
Central corneal thickness	47/66	1.48E-06	7.14E-05
Paditaxel disposition in epithelial ovarian cancer	36/47	1.49E-06	7.14E-05
Autism spectrum disorder, ADHD, bipolar disorder, MDD, and schizophrenia (combined)	36/47	1.49E-06	7.14E-05
Rosacea symptom severity	87/141	1.84E-06	8.57E-05
Waist circumference adjusted for BMI (joint analysis main effects and smoking interaction)	54/79	1.98E-06	8.99E-05
Waist circumference adjusted for body mass index	80/128	2.29E-06	0.000101
Glaucoma (primary open-angle)	52/76	2.92E-06	0.000124

Supplemental Figure S3. Identification of genes implicated in development and manifestations of hundreds physiological and pathological phenotypes and autosomal inheritance in Modern Humans.

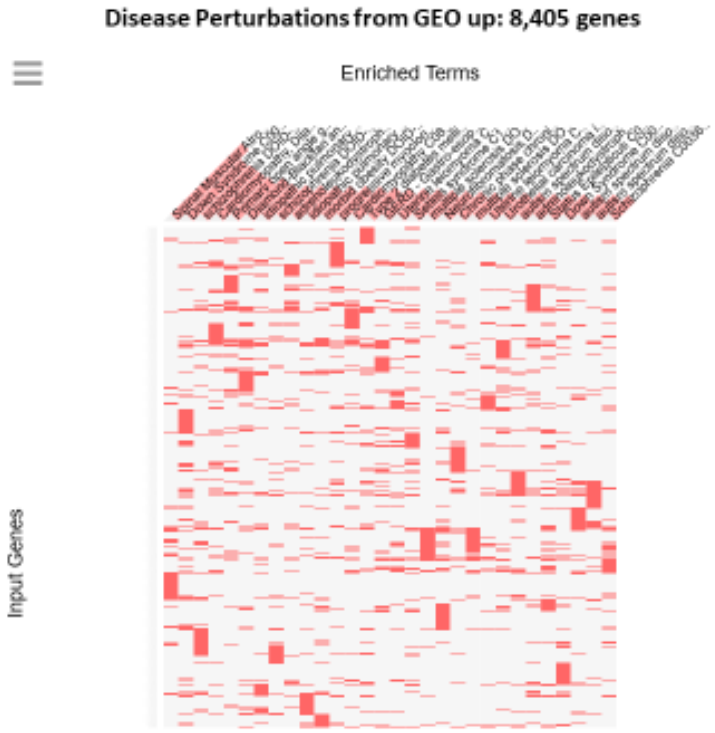




Disease Perturbations from GEO down (8,405 genes): Top 30 of 240 significant records

Term	Overlap	P-value	Adjusted P-value
schizophrenia DOID-5419 human GSE25673 sample 892	242/337	6.49E-29	5.44E-26
Bipolar Disorder C0005586 human GSE5389 sample 302	256/395	2.78E-20	1.17E-17
esophagus squamous cell carcinoma DOID-3748 human GSE63941 sample 659	252/391	1.65E-19	4.6E-17
Crohn's disease DOID-8778 human GSE6731 sample 757	240/370	3.64E-19	7.63E-17
ulcerative colitis DOID-8577 human GSE6731 sample 759	246/384	1.4E-18	2.34E-16
esophagus squamous cell carcinoma DOID-3748 human GSE63941 sample 658	256/408	1.43E-17	2E-15
schizophrenia DOID-5419 human GSE25673 sample 891	182/274	2.18E-16	2.61E-14
idiopathic pulmonary fibrosis DOID-0050156 human GSE44723 sample 850	201/312	8.28E-16	8.68E-14
Androgen insensitivity syndrome C0039585 human GSE3871 sample 415	208/327	1.94E-15	1.81E-13
adrenoleukodystrophy DOID-10588 human GSE34309 sample 864	212/338	9.23E-15	7.75E-13
Huntington's disease DOID-12858 mouse GSE3621 sample 704	219/356	6.55E-14	5E-12
Dystonia C0393593 human GSE3064 sample 329	198/317	1.27E-13	8.89E-12
Nephroblastoma C0027708 human GSE2712 sample 418	248/419	6.95E-13	4.48E-11
Huntington's disease DOID-12858 mouse GSE3583 sample 929	183/293	1.08E-12	6.44E-11
Ulcerative Colitis C0009324 human GSE6731 sample 249	213/354	3.26E-12	1.82E-10
Breast Cancer C0006142 human GSE1378 sample 52	184/299	6.23E-12	3.27E-10
Down syndrome DOID-14250 human GSE42956 sample 1060	156/247	1.41E-11	6.95E-10
Primary open angle glaucoma C0339573 human GSE2705 sample 257	156/249	3.5E-11	1.63E-09
colitis DOID-0060180 human GSE6731 sample 761	211/359	8.78E-11	3.71E-09
Alzheimer's disease DOID-10652 human GSE4757 sample 592	216/369	8.85E-11	3.71E-09
Crohn's disease DOID-8778 human GSE6731 sample 758	162/263	1.01E-10	4.01E-09
prolactinoma DOID-5394 human GSE36314 sample 636	251/440	1.05E-10	4.01E-09
diabetes mellitus type 2 DOID-9352 human GSE12643 sample 766	204/346	1.22E-10	4.34E-09
type 2 diabetes mellitus DOID-9352 human GSE13760 sample 882	221/380	1.24E-10	4.34E-09
skin squamous cell carcinoma DOID-3151 human GSE45164 sample 657	177/295	2.98E-10	1E-08
prostate cancer DOID-10283 human GSE3868 sample 638	201/344	4.9E-10	1.58E-08
Dental cavity, complex C0399396 human GSE1629 sample 175	228/401	1.12E-09	3.49E-08
Alzheimer's disease DOID-10652 human GSE36980 sample 520	190/325	1.37E-09	4.1E-08
oligodendroglioma DOID-3181 human GSE15824 sample 858	183/313	2.71E-09	7.83E-08
breast cancer DOID-1612 human GSE3744 sample 978	247/443	2.84E-09	7.95E-08

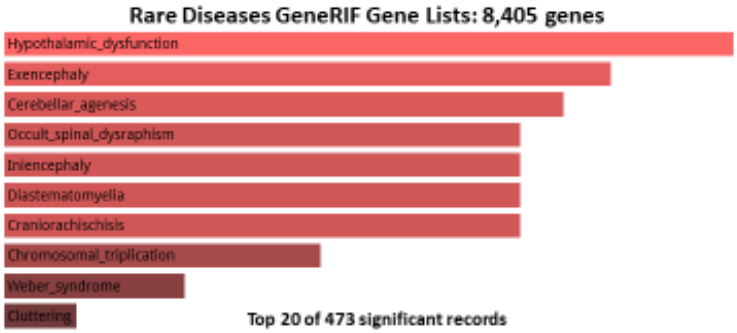
Disease Perturbations from GEO up: 8,405 genes



Disease Perturbations from GEO up (8,405 genes): Top 30 of 204 significant records

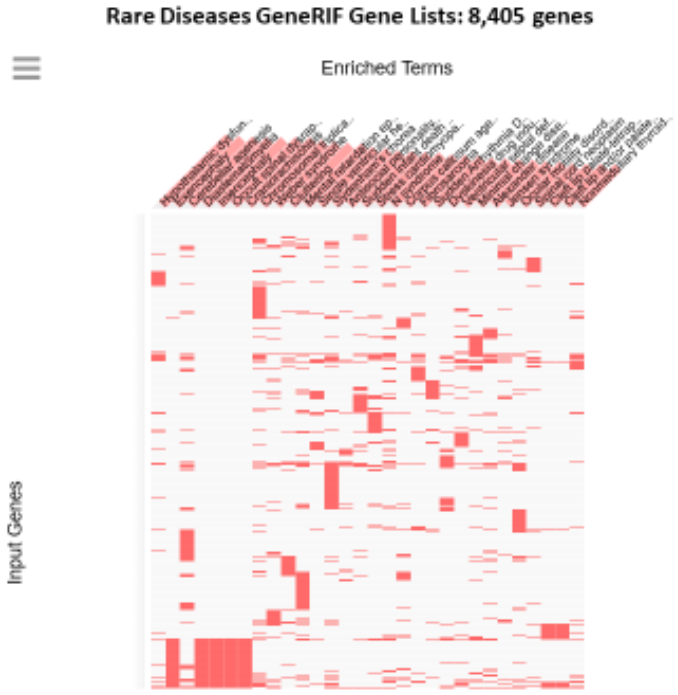
Term	Overlap	P-value	Adjusted P-value
Spinal Muscular Atrophy C0026847 mouse GSE10599 sample 235	254/368	4.69E-26	3.93E-23
Down Syndrome C0013080 human GSE5390 sample 277	288/460	2.08E-19	8.72E-17
schizophrenia DOID-5419 human GSE25673 sample 891	215/326	1.48E-18	4.14E-16
Cardiomyopathy, Dilated C0007193 human GSE3585 sample 198	209/326	4.82E-16	1.01E-13
Primary open angle glaucoma C0339573 human GSE2705 sample 257	216/351	9.23E-14	1.55E-11
Diamond-Blackfan anaemia DOID-1339 human GSE14335 sample 472	231/382	1.88E-13	2.64E-11
idiopathic pulmonary fibrosis DOID-0050156 human GSE44723 sample 851	185/300	4.2E-12	5.03E-10
schizophrenia DOID-5419 human GSE25673 sample 892	165/263	8.13E-12	8.53E-10
adrenoleukodystrophy DOID-10588 human GSE34309 sample 864	164/262	1.23E-11	1.14E-09
idiopathic pulmonary fibrosis DOID-0050156 human GSE44723 sample 850	177/288	1.83E-11	1.5E-09
morbid obesity DOID-11981 human GSE48964 sample 583	180/294	1.96E-11	1.5E-09
Cardiomyopathy C0878544 human GSE1869 sample 79	197/330	5.63E-11	3.87E-09
Type 2 diabetes mellitus C0011860 human GSE12643 sample 274	205/346	5.99E-11	3.87E-09
GERD - Gastro-esophageal reflux disease C0017168 human GSE2144 sample 27	203/346	2.46E-10	1.48E-08
Uterine leiomyoma C0042133 human GSE2725 sample 399	158/258	3.19E-10	1.78E-08
multiple sclerosis DOID-2377 human GSE38010 sample 737	183/310	9.47E-10	4.96E-08
Chronic phase chronic myelogenous leukemia DOID-8552 human GSE5550 sample 456	192/330	1.92E-09	9.47E-08
autism spectrum disorder DOID-0060041 human GSE28521 sample 1041	185/317	2.71E-09	1.26E-07
Setleis syndrome C1744559 human GSE16524 sample 285	222/393	4E-09	1.77E-07
Neurofibromatosis DOID-8712 mouse GSE1482 sample 667	210/369	4.54E-09	1.9E-07
multiple sclerosis DOID-2377 human GSE38010 sample 738	180/309	5.36E-09	2.14E-07
Uterine leiomyoma C0042133 human GSE593 sample 16	160/270	7.23E-09	2.76E-07
autism spectrum disorder DOID-0060041 human GSE28521 sample 1040	194/341	1.8E-08	6.56E-07
Urothelial carcinoma in situ C0334267 human GSE3167 sample 229	213/380	1.94E-08	6.77E-07
Status Epilepticus C0038220 rat GSE4236 sample 391	191/336	2.51E-08	8.11E-07
autism spectrum disorder DOID-0060041 human GSE28521 sample 1039	191/336	2.51E-08	8.11E-07
adrenoleukodystrophy DOID-10588 human GSE34308 sample 709	205/365	2.88E-08	8.95E-07
Down Syndrome C0013080 human GSE10758 sample 310	210/376	3.57E-08	1.07E-06
chronic lymphocytic leukemia DOID-1040 human GSE6691 sample 786	180/315	3.77E-08	1.09E-06
Oligodendroglioma C0028945 human GSE2223 sample 116	167/289	4.08E-08	1.14E-06

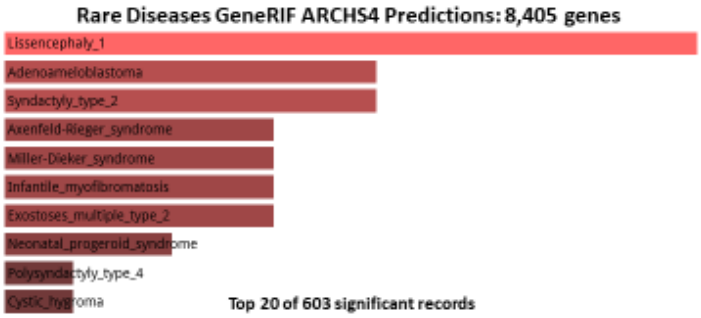
Supplemental Figure S4. Identification of genes expression of which is altered in several hundred common human disorders.



Top 20 of 473 significant records

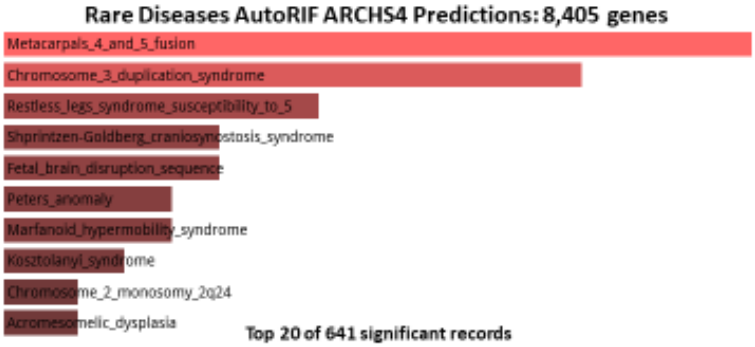
Term	Overlap	P-value	Adjusted P-value
Hypothalamic_dysfunction	90/128	8.12E-11	6.55E-08
Exencephaly	158/256	1.38E-10	6.55E-08
Cerebellar_agenesis	198/335	1.7E-10	6.55E-08
Occult_spinal_dysraphism	157/255	2.05E-10	6.55E-08
Diastematomyelia	157/255	2.05E-10	6.55E-08
Craniorachischisis	157/255	2.05E-10	6.55E-08
Inlencephaly	157/255	2.05E-10	6.55E-08
Chromosomal_triplication	201/344	4.9E-10	1.37E-07
Weber_syndrome	94/139	8.85E-10	2.2E-07
Cluttering	106/162	1.41E-09	3.16E-07
Mental_retardation_epilepsy	190/326	1.92E-09	3.9E-07
Single_ventricular_heart	73/104	5.40E-09	1.02E-06
Sydenham's_chorea	209/369	8.42E-09	1.34E-06
Chorea_minor	209/369	8.42E-09	1.34E-06
Antisocial_personality_disorder	46/59	1.93E-08	2.87E-06
Sudden_infant_death_syndrome	103/162	2.37E-08	3.31E-06
Stress_cardiomyopathy	110/176	3.16E-08	4.16E-06
N_syndrome	222/401	3.89E-08	4.83E-06
Basilar_migraine	127/210	4.85E-08	5.7E-06
Corpus_callosum_agenesis	80/138	7.92E-08	8.85E-06





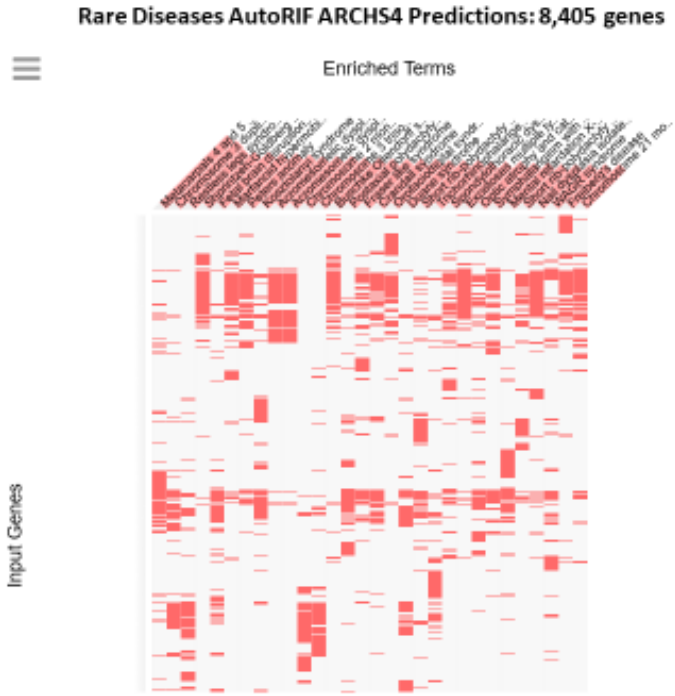
Top 20 of 603 significant records

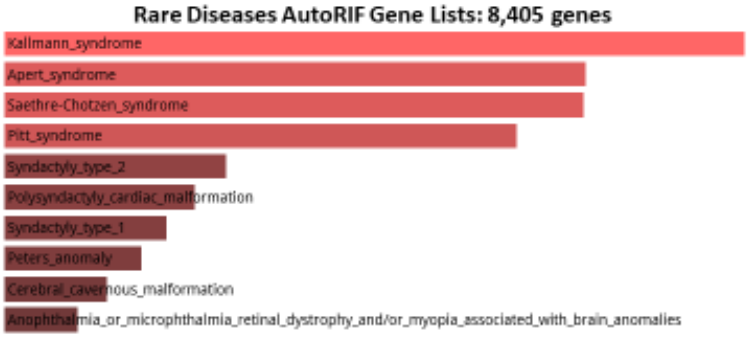
Term	Overlap	P-value	Adjusted P-value
Lissencephaly_1	154/200	4.79E-24	1.08E-20
Adenoameloblastoma	151/200	4.35E-22	3.26E-19
Syndactyly_type_2	151/200	4.35E-22	3.26E-19
Axenfeld-Rieger_syndrome	150/200	1.85E-21	5.94E-19
Miller-Dieker_syndrome	150/200	1.85E-21	5.94E-19
Exostoses_multiple_type_2	150/200	1.85E-21	5.94E-19
Infantile_myofibromatosis	150/200	1.85E-21	5.94E-19
Neonatal_progeroid_syndrome	149/200	7.69E-21	2.16E-18
Polysyndactyly_type_4	148/200	3.11E-20	6.97E-18
Cystic_hygroma	148/200	3.11E-20	6.97E-18
Acromesomelic_dysplasia_Hunter_Thompson_type	147/200	1.22E-19	2.11E-17
Acromesomelic_dysplasia	147/200	1.22E-19	2.11E-17
Exostoses_multiple_type_1	147/200	1.22E-19	2.11E-17
Scholte_syndrome	146/200	4.69E-19	6.58E-17
Congenital_diaphragmatic_hernia	146/200	4.69E-19	6.58E-17
Hereditary_multiple_osteochondromas	146/200	4.69E-19	6.58E-17
Aortopulmonary_window	145/200	1.76E-18	1.88E-16
Corpus_callosum_agenesis	145/200	1.76E-18	1.88E-16
Apert_syndrome	145/200	1.76E-18	1.88E-16
Subependymoma	145/200	1.76E-18	1.88E-16



Top 20 of 641 significant records

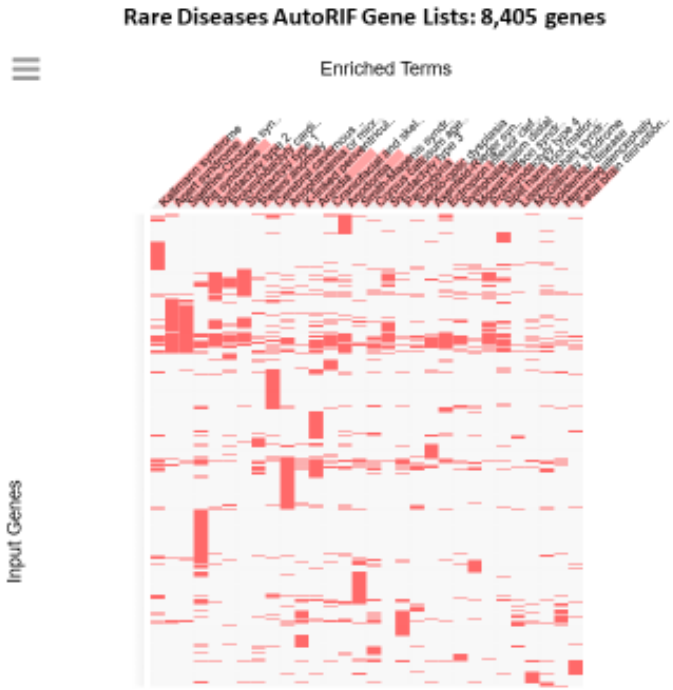
Term	Overlap	P-value	Adjusted P-value
Metacarpals_4_and_5_fusion	163/200	1.33E-30	4.97E-27
Chromosome_3_duplication_syndrome	160/200	2.69E-28	5.01E-25
Restless_legs_syndrome_susceptibility_to_5	155/200	1.01E-24	1.25E-21
Shprintzen-Goldberg_craniosynostosis_syndrome	153/200	2.21E-23	1.65E-20
Fetal_brain_disruption_sequence	153/200	2.21E-23	1.65E-20
Peters_anomaly	152/200	9.95E-23	5.3E-20
Marfanoid_hypermobility_syndrome	152/200	9.95E-23	5.3E-20
Kosztolanyi_syndrome	151/200	4.35E-22	2.03E-19
Chromosome_2_monosomy_2q24	150/200	1.85E-21	6.28E-19
Acromesomelic_dysplasia_Hunter_Thompson_type	150/200	1.85E-21	6.28E-19
Acromesomelic_dysplasia	150/200	1.85E-21	6.28E-19
Chromosome_3_trisomy_3p	149/200	7.69E-21	2.2E-18
Buschke_Ollendorff_syndrome	149/200	7.69E-21	2.2E-18
Fraser_like_syndrome	148/200	3.11E-20	6.81E-18
Crandall_syndrome	148/200	3.11E-20	6.81E-18
Orofaciodigital_syndrome_11	148/200	3.11E-20	6.81E-18
Postaxial_polydactyly_mental_retardation	148/200	3.11E-20	6.81E-18
Short_rib-polydactyly_syndrome_type_4	147/200	1.22E-19	2.28E-17
Trichorhinophalangeal_syndrome_type_3	147/200	1.22E-19	2.28E-17
Duane_syndrome	147/200	1.22E-19	2.28E-17



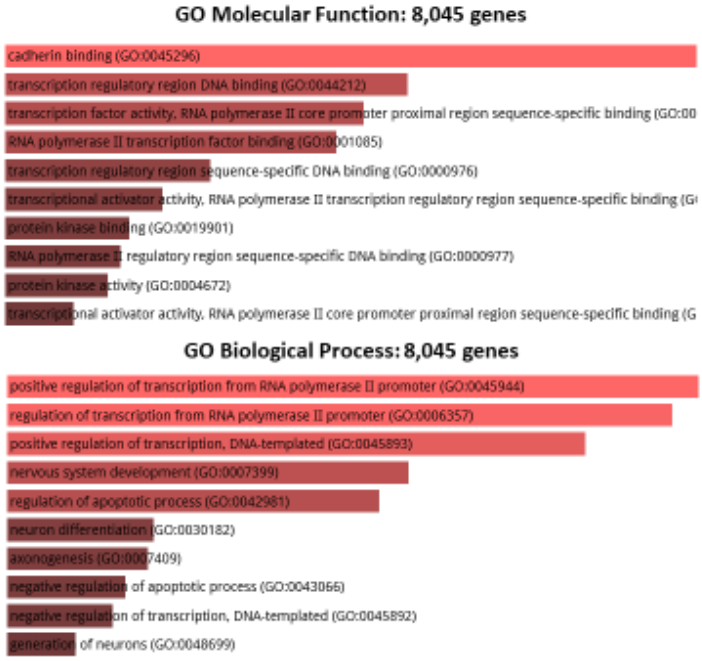


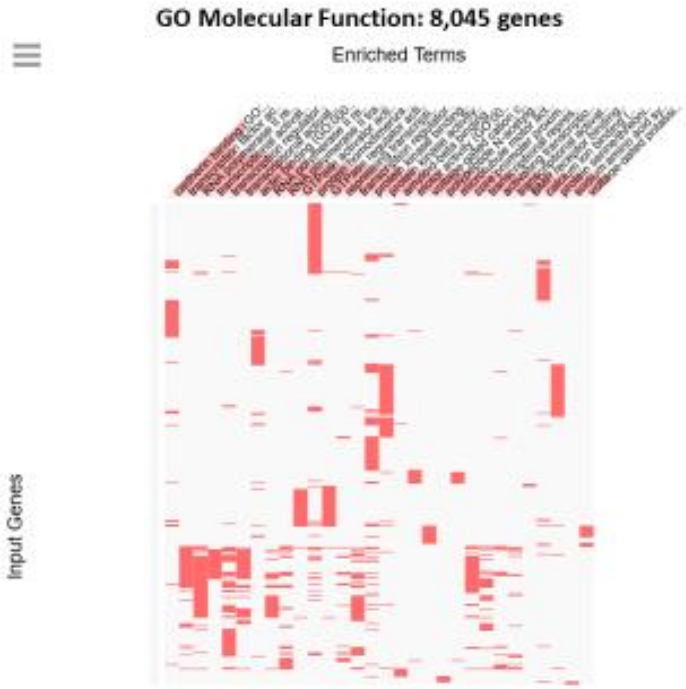
Top 20 of 1,116 significant records

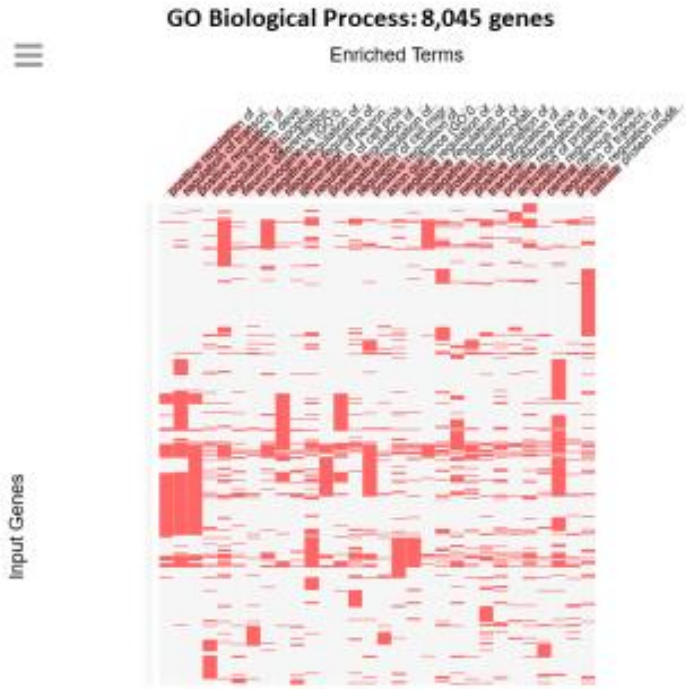
Term	Overlap	P-value	Adjusted P-value
Kallmann_syndrome	159/247	9.25E-13	5.25E-09
Apert_syndrome	143/219	2.58E-12	5.25E-09
Saethre-Chotzen_syndrome	136/206	2.62E-12	3.25E-09
Pitt_syndrome	241/410	4.01E-12	3.73E-09
Syndactyly_type_2	124/188	2.62E-11	1.95E-08
Polysyndactyly_cardiac_malformation	77/104	3.2E-11	1.98E-08
Syndactyly_type_1	122/185	3.85E-11	2.04E-08
Peters_anomaly	75/101	4.53E-11	2.1E-08
Cerebral_cavernous_malformation	157/252	5.65E-11	2.33E-08
Anophthalmia_or_microphthalmia_retinal_dystrophy_and/or_myopia_associated_with_brain_anomalies	182/301	6.82E-11	2.36E-08
X-linked_periventricular_heterotopia	89/126	6.98E-11	2.36E-08
Aniridia	171/282	1.85E-10	5.73E-08
Dominant_cleft_palate	163/267	2.37E-10	6.76E-08
Craniofacial_and_skeletal_defects	94/137	2.64E-10	7.01E-08
Anodontia	140/223	2.84E-10	7.03E-08
Glaucoma_congenital	202/345	5.48E-10	8.08E-08
Childhood-Onset_Schizophrenia	168/278	3.87E-10	8.46E-08
Hennekam_syndrome	139/223	6.68E-10	1.58E-07
Kurczynski_Casperson_syndrome	114/176	8.57E-10	1.68E-07
Osteochondroma	177/299	1.31E-09	2.44E-07



Supplemental Figure S5. Identification of genes implicated in more than 1,000 records classified as human rare diseases.





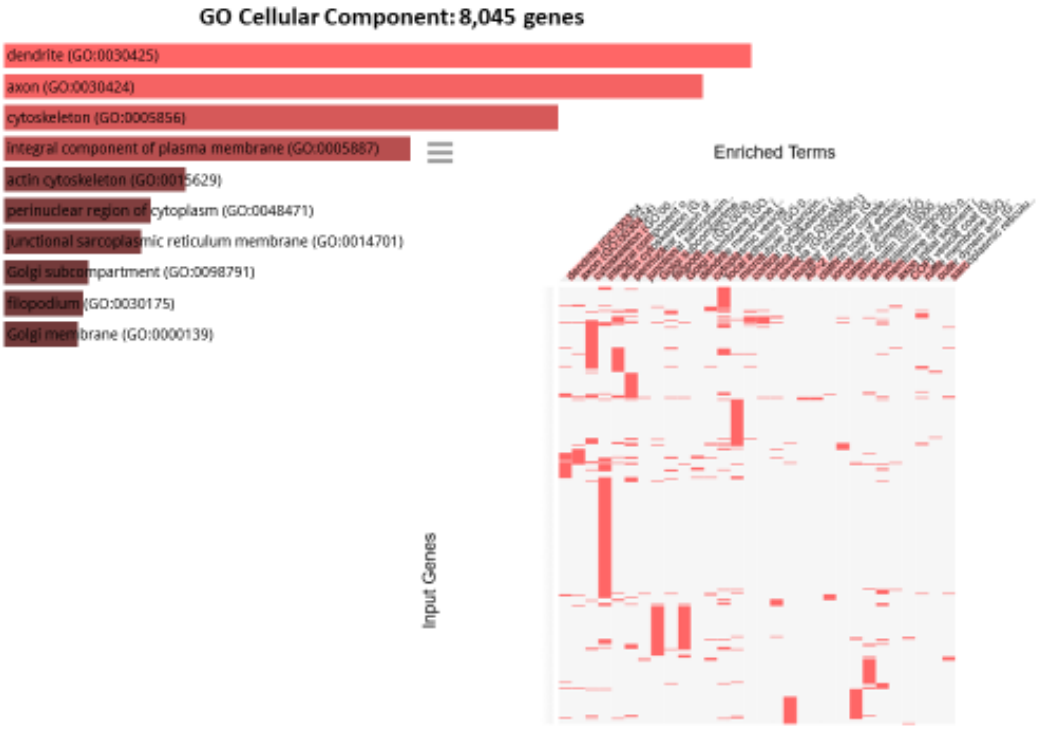


GO Biological Process (8,045 genes): Top 35 of 308 significant records

Term	Overlap	P-value	Adjusted P-value
positive regulation of transcription from RNA polymerase II promoter (GO:0045944)	485/849	1.12E-19	5.67E-16
regulation of transcription from RNA polymerase II promoter (GO:0006357)	786/1479	2.51E-19	6.37E-16
positive regulation of transcription, DNA-templated (GO:0045893)	611/1121	3.46E-18	5.85E-15
nervous system development (GO:0007399)	276/456	7.07E-16	8.98E-13
regulation of apoptotic process (GO:0042981)	453/816	1.74E-15	1.77E-12
neuron differentiation (GO:0030182)	100/140	1.57E-12	1.33E-09
axonogenesis (GO:0007409)	146/224	1.88E-12	1.37E-09
negative regulation of apoptotic process (GO:0043066)	279/486	3.63E-12	2.31E-09
negative regulation of transcription, DNA-templated (GO:0045892)	437/814	5.44E-12	3.07E-09
generation of neurons (GO:0048699)	93/131	1.66E-11	7.69E-09
regulation of cell proliferation (GO:0042127)	400/741	1.67E-11	7.69E-09
positive regulation of nucleic acid-templated transcription (GO:1903508)	284/503	2.98E-11	1.26E-08
negative regulation of transcription from RNA polymerase II promoter (GO:0000122)	314/566	4.51E-11	1.76E-08
regulation of cell migration (GO:0030334)	190/317	7.61E-11	2.76E-08
positive regulation of gene expression (GO:0010628)	410/772	1.69E-10	5.73E-08
axon guidance (GO:0007411)	106/159	2.78E-10	8.82E-08
negative regulation of cellular process (GO:0048523)	295/535	4.21E-10	1.26E-07
negative regulation of cell proliferation (GO:0008285)	210/364	9.35E-10	2.64E-07
negative regulation of programmed cell death (GO:0043069)	232/409	1.08E-09	2.88E-07
protein phosphorylation (GO:0006468)	261/471	2.26E-09	5.62E-07
negative regulation of gene expression (GO:0010629)	332/619	2.32E-09	5.62E-07
positive regulation of macromolecule metabolic process (GO:0010604)	165/277	2.5E-09	5.78E-07
transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169)	224/397	3.91E-09	8.63E-07
positive regulation of cell differentiation (GO:0045597)	122/195	5.25E-09	1.11E-06
activation of protein kinase activity (GO:0032147)	142/234	5.85E-09	1.19E-06
positive regulation of protein phosphorylation (GO:0001934)	231/413	6.36E-09	1.24E-06
central nervous system development (GO:0007417)	133/218	1.1E-08	2.06E-06
negative regulation of cellular macromolecule biosynthetic process (GO:2000113)	278/513	1.29E-08	2.34E-06
regulation of transcription, DNA-templated (GO:0006355)	776/1590	2.65E-08	4.64E-06
positive regulation of epithelial cell migration (GO:0010634)	55/75	3.67E-08	6.13E-06
cellular protein modification process (GO:0006464)	504/1002	3.74E-08	6.13E-06
positive regulation of cell migration (GO:0030335)	133/222	5.27E-08	8.35E-06
positive regulation of cell proliferation (GO:0008284)	233/425	5.42E-08	8.35E-06
neuron projection morphogenesis (GO:0048812)	103/164	5.98E-08	8.94E-06
positive regulation of multicellular organismal process (GO:0051240)	123/203	6.72E-08	9.66E-06

GO Molecular Function (8,045 genes): Top 30 of 81 significant records

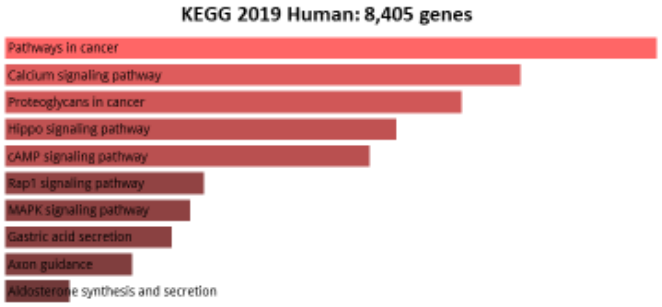
Term	Overlap	P-value	Adjusted P-value
cadherin binding (GO:0045296)	191/314	1.1E-11	1.26E-08
transcription regulatory region DNA binding (GO:0044212)	216/375	6.56E-10	3.76E-07
transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding (GO:0000982)	168/281	1.22E-09	4.66E-07
RNA polymerase II transcription factor binding (GO:0001085)	84/122	1.79E-09	5.11E-07
transcription regulatory region sequence-specific DNA binding (GO:0000976)	171/293	1.06E-08	2.43E-06
transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding (GO:0001228)	166/285	2.1E-08	4.01E-06
protein kinase binding (GO:0019901)	268/496	3.35E-08	5.4E-06
RNA polymerase II regulatory region sequence-specific DNA binding (GO:0000977)	251/461	3.78E-08	5.4E-06
protein kinase activity (GO:0004672)	276/514	4.5E-08	5.72E-06
transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding (GO:0001077)	109/176	7.35E-08	8.4E-06
GTPase regulator activity (GO:0030695)	154/276	2.44E-06	0.000254
amyloid-beta binding (GO:0001540)	37/50	4.47E-06	0.000426
protein serine/threonine kinase activity (GO:0004674)	197/369	5.99E-06	0.000518
repressing transcription factor binding (GO:0070491)	39/54	6.67E-06	0.000518
RNA polymerase II regulatory region DNA binding (GO:0001012)	116/202	6.8E-06	0.000518
GTPase activator activity (GO:0005096)	139/250	9.43E-06	0.000675
voltage-gated cation channel activity (GO:0022843)	64/101	1.2E-05	0.000807
motor activity (GO:0003774)	56/86	1.28E-05	0.000811
regulatory region DNA binding (GO:0000975)	126/225	1.52E-05	0.000916
core promoter proximal region sequence-specific DNA binding (GO:0000987)	152/279	1.65E-05	0.000943
protein homodimerization activity (GO:0042803)	332/665	1.79E-05	0.000973
actin binding (GO:0003779)	140/255	2.09E-05	0.001086
PDZ domain binding (GO:0030165)	43/63	2.29E-05	0.001141
RNA polymerase II core promoter proximal region sequence-specific DNA binding (GO:0000978)	143/263	3.29E-05	0.001569
transcriptional repressor activity, RNA polymerase II transcription regulatory region sequence-specific binding (GO:0001227)	92/160	5.43E-05	0.002452
protein tyrosine kinase activity (GO:0004713)	86/148	5.57E-05	0.002452
microtubule motor activity (GO:0003777)	41/61	6.16E-05	0.002608
tubulin binding (GO:0015631)	138/256	7.7E-05	0.003145
microtubule binding (GO:0008017)	109/196	8.13E-05	0.003208
acetylgalactosaminyltransferase activity (GO:0008376)	34/49	9.81E-05	0.003742

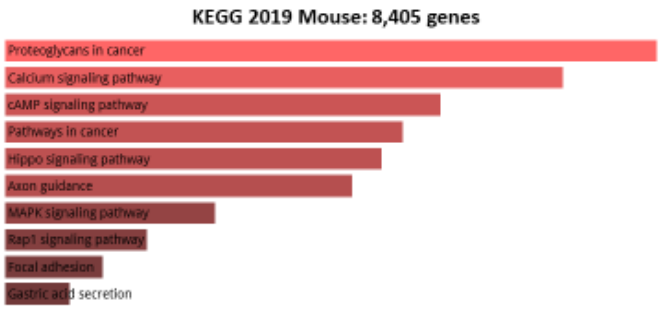


GO Cellular Component (8,045 genes): 29 significant records

Term	Overlap	P-value	Adjusted P-value
cytoskeleton (GO:0005856)	284/521	4.18E-09	1.85E-06
dendrite (GO:0030425)	131/216	2.35E-08	5.12E-06
axon (GO:0030424)	92/142	3.47E-08	5.12E-06
actin cytoskeleton (GO:0015629)	169/295	7.67E-08	8.23E-06
integral component of plasma membrane (GO:0005887)	711/1464	9.29E-08	8.23E-06
focal adhesion (GO:0005925)	194/357	1.52E-06	0.000112
Golgi subcompartment (GO:0098791)	250/480	4.46E-06	0.000282
perinuclear region of cytoplasm (GO:0048471)	198/379	3.32E-05	0.001839
cytoplasmic vesicle membrane (GO:0030659)	38/55	4.48E-05	0.002203
Golgi membrane (GO:0000139)	225/443	0.000104	0.004546
cortical cytoskeleton (GO:0030863)	36/53	0.000123	0.004546
cortical actin cytoskeleton (GO:0030864)	36/53	0.000123	0.004546
caveola (GO:0005901)	38/57	0.000148	0.00505
filopodium (GO:0030175)	40/61	0.000173	0.005461
membrane raft (GO:0045121)	70/120	0.000224	0.006622
endoplasmic reticulum lumen (GO:0005788)	142/271	0.000339	0.009382
microtubule organizing center (GO:0005815)	251/508	0.000401	0.010456
cytoplasmic vesicle (GO:0031410)	115/216	0.000546	0.013065
chromatin (GO:0000785)	153/297	0.00056	0.013065
nuclear chromatin (GO:0000790)	132/254	0.000826	0.018285
catenin complex (GO:0016342)	21/29	0.0009	0.018978
ionotropic glutamate receptor complex (GO:0008328)	27/40	0.001001	0.020165
dendrite membrane (GO:0032590)	16/21	0.001571	0.030261
cation channel complex (GO:0034703)	40/66	0.00177	0.032667
centrosome (GO:0005813)	225/462	0.00199	0.035259
main axon (GO:0044304)	23/34	0.002257	0.038461
junctional sarcoplasmic reticulum membrane (GO:0014701)	10-Sep	0.002537	0.040494
microtubule cytoskeleton (GO:0015630)	191/389	0.002642	0.040494
actin-based cell projection (GO:0098858)	42/71	0.002651	0.040494
ruffle membrane (GO:0032587)	33/54	0.003598	0.053124

Supplemental Figure S6. Gene ontology analyses of putative regulatory targets of genetic loci harboring human-specific SNCs.

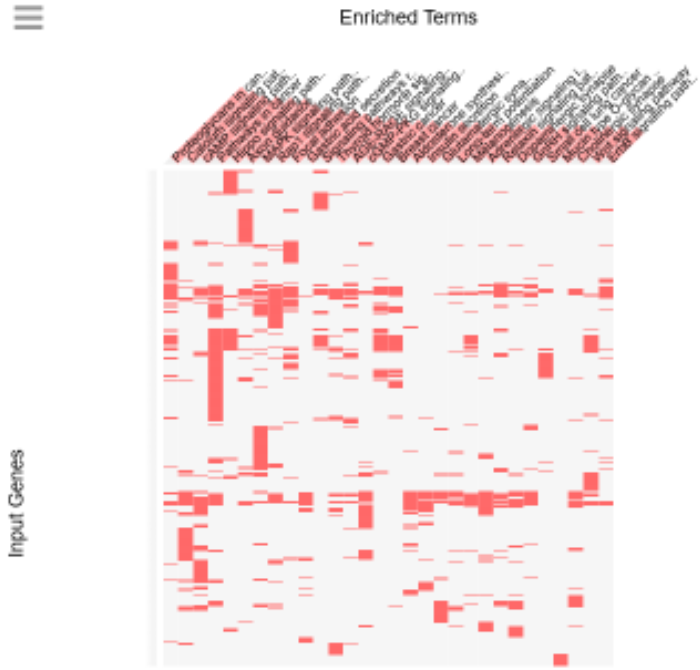




KEGG 2019 Human (8,405 genes): Top 40 of 129 significant records

Term	Overlap	P-value	Adjusted P-value
Pathways in cancer	512/530	2.04E-15	6.28E-13
Calcium signaling pathway	130/188	4.19E-14	6.45E-12
Proteoglycans in cancer	136/201	1.55E-13	1.59E-11
Hippo signaling pathway	112/160	6.61E-13	5.09E-11
cAMP signaling pathway	140/212	1.21E-12	7.48E-11
Rap1 signaling pathway	133/206	4.85E-11	2.49E-09
MAPK signaling pathway	179/295	6.48E-11	2.85E-09
Gastric acid secretion	59/75	9.75E-11	3.75E-09
Axon guidance	118/181	2.39E-10	8.17E-09
Aldosterone synthesis and secretion	71/98	9.54E-10	2.94E-08
cGMP-PKG signaling pathway	108/166	1.62E-09	4.54E-08
Focal adhesion	125/199	2.39E-09	6.14E-08
AGE-RAGE signaling pathway in diabetic complications	70/100	1.35E-08	3.15E-07
Dopaminergic synapse	87/131	1.42E-08	3.13E-07
Signaling pathways regulating pluripotency of stem cells	91/139	1.94E-08	3.98E-07
Wnt signaling pathway	101/158	2.15E-08	4.13E-07
Adrenergic signaling in cardiomyocytes	94/145	2.36E-08	4.28E-07
Gastric cancer	96/149	2.67E-08	4.56E-07
Amoebiasis	67/96	3.33E-08	5.32E-07
Thyroid hormone signaling pathway	78/116	3.45E-08	5.32E-07
PI3K-Akt signaling pathway	199/354	4.17E-08	6.11E-07
Long-term potentiation	50/67	5.95E-08	8.32E-07
Melanogenesis	69/101	8.04E-08	1.08E-06
Breast cancer	93/147	1.57E-07	2.02E-06
Insulin secretion	60/86	1.75E-07	2.16E-06
Cushing syndrome	97/155	1.84E-07	2.18E-06
Circadian entrainment	66/97	1.94E-07	2.22E-06
Glutamatergic synapse	75/114	2.48E-07	2.73E-06
Mucin type O-glycan biosynthesis	27/31	2.65E-07	2.81E-06
Relaxin signaling pathway	83/130	4.06E-07	4.17E-06
GnRH signaling pathway	63/93	4.68E-07	4.65E-06
Cholinergic synapse	73/112	6.18E-07	5.95E-06
Oxytocin signaling pathway	94/153	9.5E-07	8.87E-06
Inflammatory mediator regulation of TRP channels	66/100	1.08E-06	9.78E-06
Small cell lung cancer	62/93	1.35E-06	1.19E-05
Human papillomavirus infection	181/350	1.53E-06	1.31E-05
Neuroactive ligand-receptor interaction	184/358	2.41E-06	2.01E-05
Colorectal cancer	57/86	4.72E-06	3.82E-05
Gap junction	58/88	5.1E-06	4.03E-05
TGF-beta signaling pathway	59/90	5.49E-06	4.22E-05

KEGG 2019 Mouse: 8,405 genes



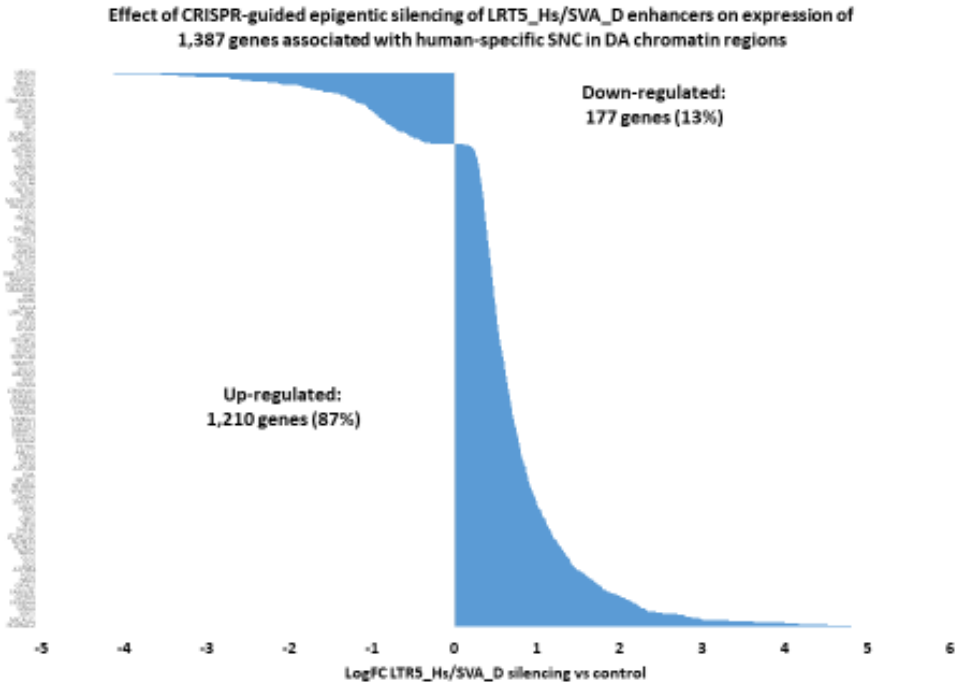
KEGG 2019 Mouse (8,405 genes): Top 35 of 106 significant records

Term	Overlap	P-value	Adjusted P-value
Proteoglycans in cancer	136/203	4.94E-13	1.5E-10
Calcium signaling pathway	127/189	2.09E-12	3.16E-10
cAMP signaling pathway	137/211	1.36E-11	1.38E-09
Pathways in cancer	300/535	2.41E-11	1.83E-09
Hippo signaling pathway	108/159	3.32E-11	2.01E-09
Axon guidance	119/180	5.22E-11	2.64E-09
MAPK signaling pathway	176/294	4.31E-10	1.86E-08
Rap1 signaling pathway	131/209	1.22E-09	4.62E-08
Focal adhesion	125/199	2.39E-09	8.05E-08
Gastric acid secretion	56/74	3.97E-09	1.2E-07
Signaling pathways regulating pluripotency of stem cells	90/137	1.8E-08	4.96E-07
Thyroid hormone signaling pathway	77/115	5.58E-08	1.41E-06
AGE-RAGE signaling pathway in diabetic complications	69/101	8.04E-08	1.88E-06
cGMP-PKG signaling pathway	106/172	1.58E-07	3.43E-06
Gastric cancer	94/150	2.59E-07	5.22E-06
Breast cancer	92/147	3.76E-07	7.12E-06
Aldosterone synthesis and secretion	68/102	4.22E-07	7.52E-06
Insulin secretion	59/86	5.53E-07	9.32E-06
Glutamatergic synapse	74/114	6.6E-07	1.05E-05
Long-term potentiation	48/67	8.6E-07	1.3E-05
Melanogenesis	66/100	1.08E-06	1.56E-05
Adrenergic signaling in cardiomyocytes	91/148	1.34E-06	1.84E-05
PI3K-Akt signaling pathway	194/357	1.52E-06	1.98E-05
Relaxin signaling pathway	82/131	1.57E-06	1.98E-05
Dopaminergic synapse	84/135	1.68E-06	2.04E-05
GnRH signaling pathway	60/90	1.99E-06	2.32E-05
Small cell lung cancer	61/92	2.17E-06	2.43E-05
Mucin type O-glycan biosynthesis	24/28	2.36E-06	2.56E-05
Cholinergic synapse	72/113	2.6E-06	2.72E-05
Wnt signaling pathway	96/160	3.27E-06	3.3E-05
ErbB signaling pathway	56/84	4.34E-06	4.24E-05
Circadian entrainment	64/99	4.53E-06	4.29E-05
Oxytocin signaling pathway	92/154	6.68E-06	6.13E-05
Cushing syndrome	94/159	9.8E-06	8.74E-05
Gap junction	56/86	1.28E-05	0.000111

Supplemental Figure S7. KEGG analyses of putative regulatory targets of genetic loci harboring human-specific SNCs.

Association with networks of human-specific regulatory sequences (HSGRS) and stem cell-associated retroviral sequences (SCARS) of 8,405 genes associated with 35,074 fixed human-specific single nucleotide changes located in differentially-accessible chromatin regions during human neurogenesis in cerebral organoids

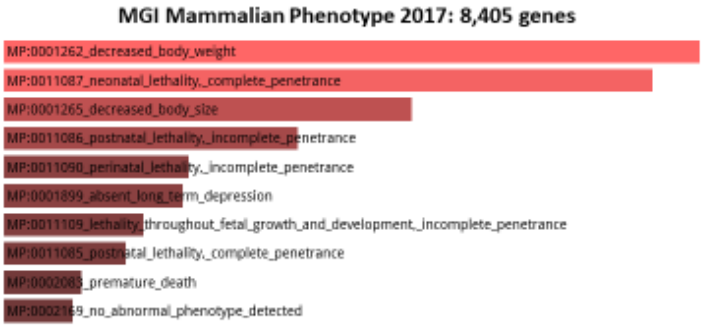
Classification category	Number of genes	Perent
Unique genes	8405	100.00
In network of human-specific genomic regulatory sequences (HSGRS)	7406	88.11
LTR5_Hs/SVA_D enhancers-regulated genes	1387	16.50
HERVH lncRNA-regulated genes	3191	37.97
LTR7Y/B enhancers-regulated genes	3306	39.33
In network of stem cell-associated retroviral sequences (SCARS)	4029	47.94
Both HSRGS & SCARS-regulated genes	3602	42.86
All HSGRS & SCARS-regulated genes	7833	93.19

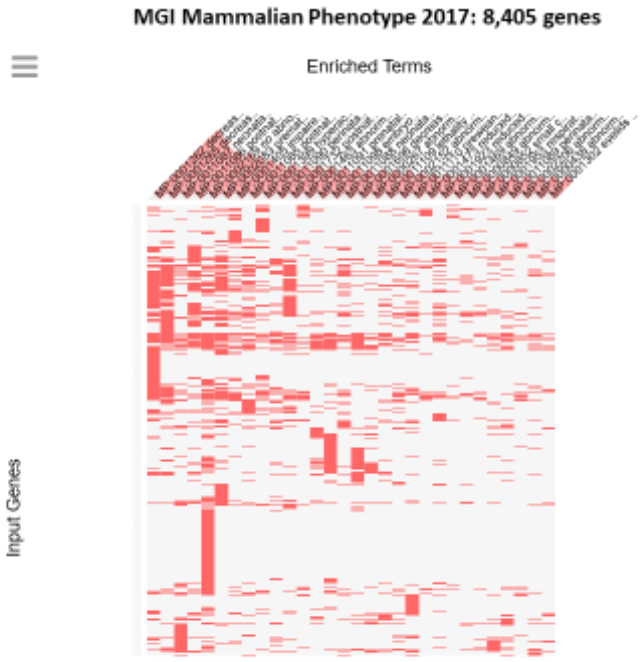


Effects of stem cell-associated regulatory sequences (SCARS) on expression of 4,029 genes associated with human-specific SNCs located in DA chromatin regions

Classification category	Number of genes	Down-regulated	Percent	Up-regulated	Percent
LTR5_Hs/SVA_D enhancers-regulated genes	1387	1210	87.24	177	12.76
HERVH lncRNA-regulated genes	3191	1733	54.31	1458	45.69
LTR7Y/B enhancers-regulated genes	3306	2494	75.44	812	24.56

Supplemental Figure S8. Structurally, functionally, and evolutionary distinct classes of HSRS share the relatively restricted elite set of common genetic targets.

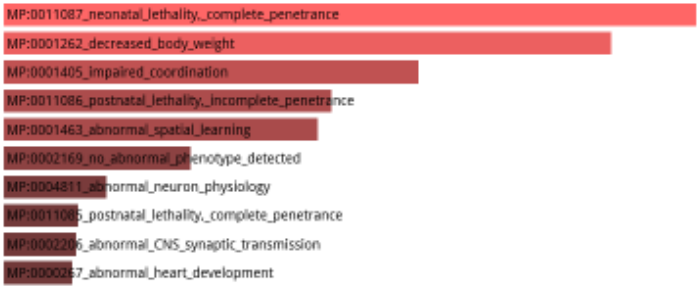


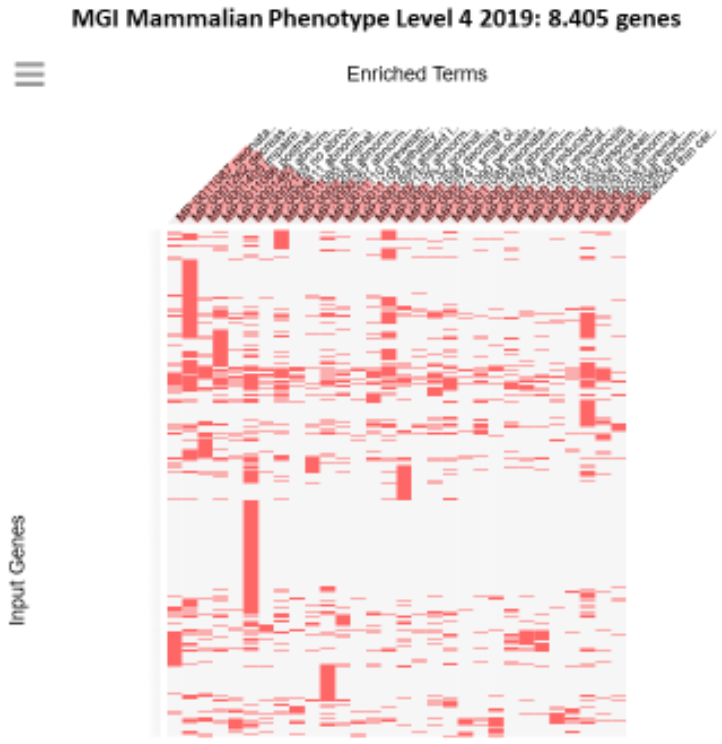


MGI Mammalian Phenotype 2017 (8,405 genes): Top 40 of 749 significant records

Term	Overlap	P-value	Adjusted P-value
MP:0001262_decreased_body_weight	692/1189	4.71E-31	2.44E-27
MP:0001265_decreased_body_size	472/774	2.25E-27	5.82E-24
MP:0011087_neonatal_lethality,_complete_penetrance	293/462	2.47E-23	4.25E-20
MP:0011086_postnatal_lethality,_incomplete_penetrance	346/563	4.06E-21	5.26E-18
MP:0002169_no_abnormal_phenotype_detected	882/1674	2.98E-20	3.08E-17
MP:0002083_premature_death	474/834	1.12E-18	9.65E-16
MP:0001405_impaired_coordination	215/332	5.43E-17	2.54E-14
MP:0011085_postnatal_lethality,_complete_penetrance	258/384	1.57E-15	1.02E-12
MP:0001399_hyperactivity	216/344	4.36E-15	2.51E-12
MP:0011090_perinatal_lethality,_incomplete_penetrance	152/226	1.28E-14	6.65E-12
MP:0001732_postnatal_growth_retardation	359/590	1.43E-14	6.75E-12
MP:0001463_abnormal_spatial_learning	115/162	6.87E-14	2.96E-11
MP:0011091_prenatal_lethality,_complete_penetrance	174/272	1.81E-13	7.22E-11
MP:0011098_embryonic_lethality_during_organogenesis,_complete_penetrance	319/559	2.9E-13	1.07E-10
MP:0011088_neonatal_lethality,_incomplete_penetrance	163/255	1.15E-12	3.97E-10
MP:0001698_decreased_embryo_size	273/472	1.96E-12	6.36E-10
MP:0000267_abnormal_heart_development	109/157	3.11E-12	9.48E-10
MP:0011109_lethality_throughout_fetal_growth_and_development,_incomplete_penetrance	116/170	5.92E-12	1.13E-09
MP:0002152_abnormal_brain_morphology	104/152	3.9E-11	1.06E-08
MP:0011110_preweaning_lethality,_incomplete_penetrance	222/381	8.85E-11	2.29E-08
MP:0001473_reduced_long_term_potentiation	77/106	1.52E-10	3.75E-08
MP:0001923_reduced_female_fertility	142/227	5.06E-10	7.2E-08
MP:0004811_abnormal_neuron_physiology	67/90	4.06E-10	8.85E-08
MP:0000788_abnormal_cerebral_cortex_morphology	98/145	4.1E-10	8.85E-08
MP:0001406_abnormal_gait	180/302	4.48E-10	9.28E-08
MP:0001469_abnormal_contextual_conditioning_behavior	45/54	4.96E-10	9.82E-08
MP:0000849_abnormal_cerebellum_morphology	68/92	5.12E-10	9.82E-08
MP:0000266_abnormal_heart_morphology	159/224	1.03E-09	1.91E-07
MP:0000852_small_cerebellum	52/66	1.15E-09	2.05E-07
MP:0001953_respiratory_failure	98/147	1.29E-09	2.23E-07
MP:0011089_perinatal_lethality,_complete_penetrance	135/217	1.41E-09	2.35E-07
MP:0002206_abnormal_CNS_synaptic_transmission	66/90	1.6E-09	2.59E-07
MP:0000438_abnormal_cranium_morphology	90/135	1.91E-09	2.93E-07
MP:0001302_eyelids_open_at_birth	43/52	1.97E-09	3E-07
MP:0000807_abnormal_hippocampus_morphology	63/86	4.04E-09	5.97E-07
MP:0006009_abnormal_neuronal_migration	57/76	5.14E-09	7.28E-07
MP:0001954_respiratory_distress	111/174	5.2E-09	7.28E-07
MP:0001899_absent_long_term_depression	25/26	5.88E-09	7.95E-07
MP:0002906_increased_susceptibility_to_pharmacologically_induced_seizures	65/90	5.99E-09	7.95E-07
MP:0003633_abnormal_nervous_system_physiology	74/106	6.53E-09	8.12E-07

MGI Mammalian Phenotype Level 4 2019: 8.405 genes





MGI Mammalian Phenotype Level 4 2019 (8,405 genes): top 40 of 407 significant records

Term	Overlap	P-value	Adjusted P-value
MP:0011087_neonatal_lethality,_complete_penetrance	315/517	1.55E-18	8.15E-15
MP:0001262_decreased_body_weight	773/1471	2.04E-17	5.36E-14
MP:0001405_impaired_coordination	247/405	6.90E-15	1.21E-11
MP:0011086_postnatal_lethality,_incomplete_penetrance	362/643	9.65E-14	1.27E-10
MP:0001463_abnormal_spatial_learning	120/172	1.46E-13	1.54E-10
MP:0002169_no_abnormal_phenotype_detected	958/1944	6.87E-12	6.02E-09
MP:0004811_abnormal_neuron_physiology	78/107	8.69E-11	6.53E-08
MP:0002206_abnormal_CNS_synaptic_transmission	75/103	2.18E-10	1.27E-07
MP:0000267_abnormal_heart_development	111/168	2.43E-10	1.28E-07
MP:0011085_postnatal_lethality,_complete_penetrance	246/432	2.04E-10	1.34E-07
MP:0011110_preweaning_lethality,_incomplete_penetrance	360/669	2.90E-10	1.39E-07
MP:0011109_lethality_throughout_fetal_growth_and_development,_incomplete_penetrance	122/191	8.21E-10	3.60E-07
MP:0001899_absent_long_term_depression	27/28	1.11E-09	4.50E-07
MP:0001732_postnatal_growth_retardation	360/677	1.85E-09	6.48E-07
MP:0001698_decreased_embryo_size	293/537	2.09E-09	6.88E-07
MP:0002152_abnormal_brain_morphology	119/187	1.84E-09	6.92E-07
MP:0011090_perinatal_lethality,_incomplete_penetrance	154/256	3.23E-09	9.44E-07
MP:0002741_small_olfactory_bulb	35/40	3.07E-09	9.51E-07
MP:0001469_abnormal_contextual_conditioning_behavior	48/61	5.45E-09	1.43E-06
MP:0001475_reduced_long_term_potentiation	84/124	6.05E-09	1.45E-06
MP:0000788_abnormal_cerebral_cortex_morphology	104/161	5.79E-09	1.45E-06
MP:0011088_neonatal_lethality,_incomplete_penetrance	172/293	5.33E-09	1.48E-06
MP:0001954_respiratory_distress	121/194	7.90E-09	1.81E-06
MP:0001575_cyanosis	129/210	1.00E-08	2.19E-06
MP:0001955_respiratory_failure	103/161	1.47E-08	3.09E-06
MP:0002906_increased_susceptibility_to_pharmacologically_induced_seizures	70/101	2.60E-08	5.25E-06
MP:0002910_abnormal_excitatory_postsynaptic_currents	59/83	7.68E-08	1.50E-05
MP:0002083_premature_death	499/997	9.80E-08	1.84E-05
MP:0002066_abnormal_motor_capabilities/coordination/movement	102/164	1.41E-07	2.55E-05
MP:0006254_thin_cerebral_cortex	53/74	2.36E-07	4.14E-05
MP:0011098_embryonic_lethality_during_organogenesis,_complete_penetrance	339/656	2.60E-07	4.28E-05
MP:0000807_abnormal_hippocampus_morphology	65/92	2.56E-07	4.34E-05
MP:0006009_abnormal_neuronal_migration	59/85	2.94E-07	4.69E-05
MP:0011108_embryonic_lethality_during_organogenesis,_incomplete_penetrance	143/247	3.20E-07	4.95E-05
MP:0000031_abnormal_cochlea_morphology	44/59	3.84E-07	5.77E-05
MP:0000852_small_cerebellum	58/84	4.91E-07	7.17E-05
MP:0002063_abnormal_learning/memory/conditioning	42/56	5.43E-07	7.52E-05
MP:0003633_abnormal_nervous_system_physiology	79/123	5.36E-07	7.62E-05
MP:0010025_decreased_total_body_fat_amount	263/498	5.94E-07	8.01E-05
MP:0009937_abnormal_neuron_differentiation	75/116	7.01E-07	9.22E-05

Supplemental Figure S9. Interrogation of MGI Mammalian Phenotype databases identifies genes associated with human-specific SNCs and implicated in premature death and embryonic, perinatal, neonatal, and postnatal lethality phenotypes.