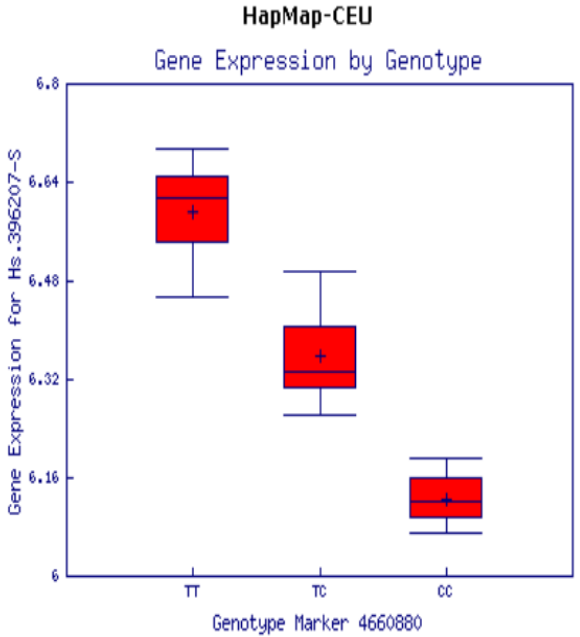
Prediction and Transcriptomics

$$T = \sum_k \omega_k X_k + \varepsilon$$



Why Predict Transcriptome Levels?

- An endo-phenotype that concentrates a substantial proportion of non-coding association signal
- A gene-based (unit-based) test with an easy to interpret direction of effect
- A natural way to iterate between human and model systems where signals often come at the level of the gene

GTEx Data on eQTLs and Transcriptome Prediction

Cis-expression is NOT polygenic; 10's not 10,000's of SNPs

Most genes have both local and distantly acting SNP predictors

Genetic regulation of a gene's transcript levels is often shared across cells/tissues

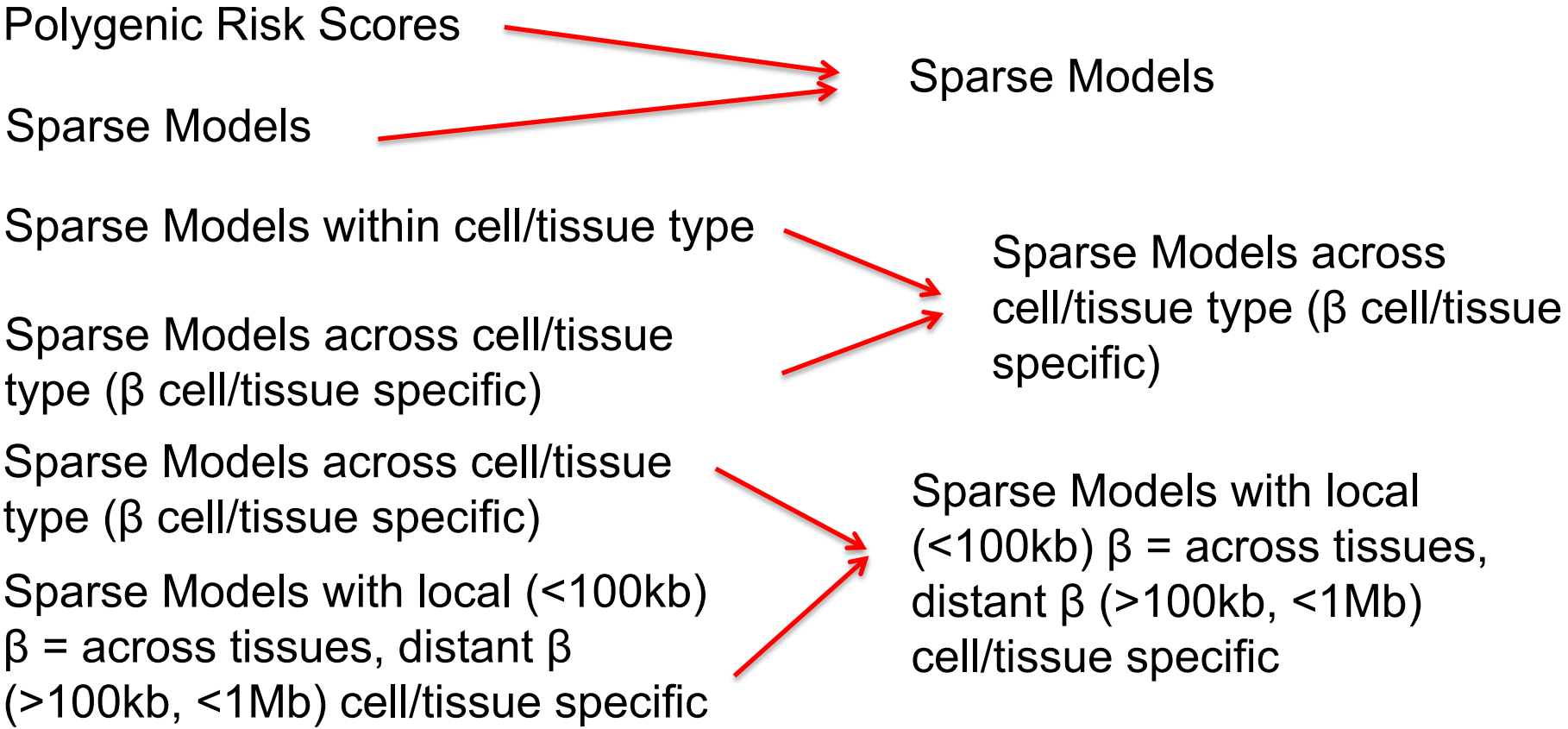
Lasso yields prediction as good or better than other models; elastic net effectively matches that prediction quality but is not as parsimonious

Most eQTLs are within 50 kb of transcription start sites

The SNP with the largest effect on most genes' transcript levels is sufficiently distant to be in no LD with variation in the gene

Local SNPs effects are more likely to be shared across cells/tissues; SNPs in distantly acting enhancers are often cell/tissue specific

A Brief History of Transcriptome Prediction



A Brief History of Transcriptome Prediction

- Improvement is greatest for cells/tissues/genes in which we had the least power in original investigations
- Genes formerly characterized as not having eQTLs often have substantial numbers of eQTLs when we borrow strength for prediction across tissues

Can we use PheRS in conjunction with genetically predicted expression of genes to identify candidate genes for Mendelian diseases?

RESEARCH ARTICLE

HUMAN GENOMICS

Phenotype risk scores identify patients with unrecognized Mendelian disease patterns

Lisa Bastarache,[1] Jacob J. Hughey,[1] Scott Hebbring,[2] Joy Marlo,[1] Wanke Zhao,[3] Wanting T. Ho,[3] Sara L. Van Driest,[4,5] Tracy L. McGregor,[5] Jonathan D. Mosley,[4] Quinn S. Wells,[4,6] Michael Temple,[1] Andrea H. Ramirez,[4] Robert Carroll,[1] Travis Osterman,[1,4] Todd Edwards,[4] Douglas Ruderfer,[4] Digna R. Velez Edwards,[7] Rizwan Hamid,[5] Joy Cogan,[5] Andrew Glazer,[4] Wei-Qi Wei,[1] QiPing Feng,[6] Murray Brilliant,[2] Zhizhuang J. Zhao,[3] Nancy J. Cox,[4] Dan M. Roden,[1,4,6] Joshua C. Denny[1,4]*

Bastarache *et al.*, *Science* **359**, 1233–1239 (2018) 16 March 2018

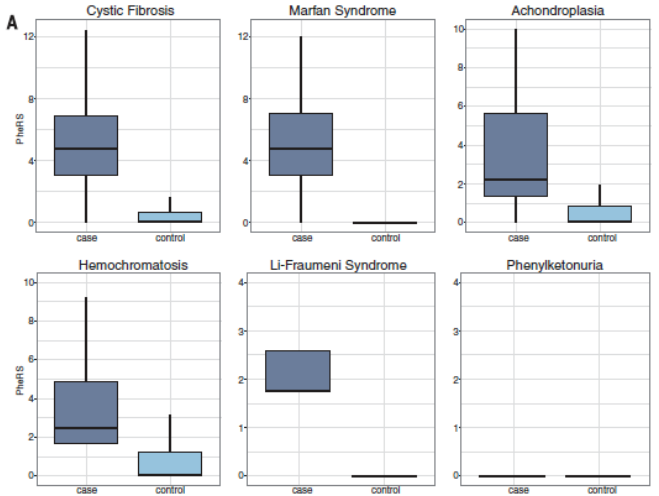
$$PheRS_i = \sum_{p=1}^{m} w_p \, x_{i,p}$$

$$where\ x_{i,p} = \begin{cases} 1\ if\ individual_i\ has\ phenotype_p \\ 0\ otherwise \end{cases}$$

AND

$$w_p = log\frac{N}{n_p}$$

Population of N individuals, with n_p the number with phenotype p



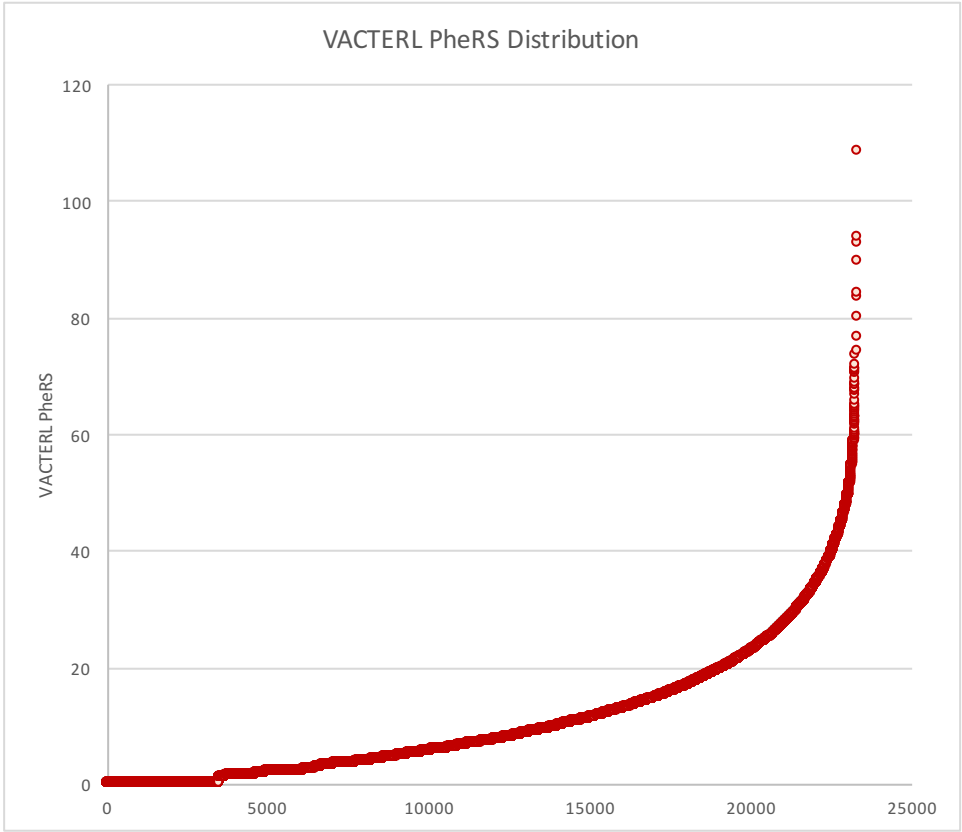
VACTERL: at least 3 of…

vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities

Calculating Phenotype Risk Score (PheRS) for VACTERL

Using SD-BioVU:

1) Identify all subjects with VACTERL diagnosis, or with “VACTERL” in physician notes
2) Read all notes to determine which patients have VACTERL as a diagnosis
3) Identify ICD codes significantly associated with true VACTERL diagnosis
4) Create PheRS with significantly associated codes
5) Deploy VACTERL PheRS in BioVU genotyped subjects



GReX Associated with VACTERL PheRS

Gene	P	Tissue
SNX25	8.39E-18	ArteryAorta
UBE2W	8.39E-18	Brain_Cerebellum
RPS17	8.39E-18	Brain_NucleusAccumbensBasalGanglia
MYBL1	8.39E-18	Liver
HLA_DQB1	8.39E-18	Vagina
PCSK4	8.39E-18	WholeBlood
HLA_DQA2	4.90E-16	Spleen
HLA_DQA1	1.27E-13	Vagina
DHODH	2.27E-11	Prostate
ACTA2	3.60E-09	SkinNOTSunExposed
POLQ	1.56E-07	ArteryAorta
VSX1	1.56E-07	Brain_Cerebellum
ZFYVE1	1.56E-07	Brain_NucleusAccumbensBasalGanglia
LILRB4	1.56E-07	WholeBlood
ACSL1	2.24E-07	Adrenal

GREX associated with VACTERL PheRS

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J Biol Chem. 2016 Feb 5;291(6):3030-42. doi: 10.1074/jbc.M115.676601. Epub 2015 Nov 24.

Loss of the Ubiquitin-conjugating Enzyme UBE2W Results in Susceptibility to Early Postnatal Lethality and Defects in Skin, Immune, and Male Reproductive Systems.

Wang B[1], Merillat SA[2], Vincent M[3], Huber AK[2], Basrur V[4], Mangelberger D[5], Zeng L[2], Elenitoba-Johnson K[4], Miller RA[6], Irani DN[2], Dlugosz AA[5], Schnell S[3], Scaglione KM[7], Paulson HL[8].

Author information

Abstract

UBE2W ubiquitinates N termini of proteins rather than internal lysine residues, showing a preference for substrates with intrinsically disordered N termini. The in vivo functions of this intriguing E2, however, remain unknown. We generated Ube2w germ line KO mice that proved to be susceptible to early postnatal lethality without obvious developmental abnormalities. Although the basis of early death is uncertain, several organ systems manifest changes in Ube2w KO mice. Newborn Ube2w KO mice often show altered epidermal maturation with reduced expression of differentiation markers. Mirroring higher UBE2W expression levels in testis and thymus, Ube2w KO mice showed a disproportionate decrease in weight of these two organs (~50%), suggesting a functional role for UBE2W in the immune and male reproductive systems. Indeed, Ube2w KO mice displayed sustained neutrophilia accompanied by increased G-CSF signaling and testicular vacuolation associated with decreased fertility. Proteomic analysis of a vulnerable organ, presymptomatic testis, showed a preferential accumulation of disordered proteins in the absence of UBE2W, consistent with the view that UBE2W preferentially targets disordered polypeptides. These mice further allowed us to establish that UBE2W is ubiquitously expressed as a single isoform localized to the cytoplasm and that the absence of UBE2W does not alter cell viability in response to various stressors. Our results establish that UBE2W is an important, albeit not essential, protein for early postnatal survival and normal functioning of multiple organ systems.

Andrologia. 2019 Feb;51(1):e13171. doi: 10.1111/and.13171. Epub 2018 Oct 15.

Paternal factors and embryonic development: Role in recurrent pregnancy loss.

Dhawan V[1], Kumar M[1], Deka D[2], Malhotra N[2], Singh N[2], Dadhwal V[2], Dada R[1].

Author information

Abstract

The events occurring at the maternal-foetal interface define a successful pregnancy but the current paradigm has shifted towards assessing the contribution of spermatozoa for embryogenesis. Spermatozoa with defective DNA integrity may fertilise the oocyte but affect subsequent embryonic development. The present case-control study was conducted in male partners of couples experiencing recurrent pregnancy loss (RPL) to assess the gene expression of spermatozoal FOXG1, SOX3, OGG1, PARP1, RPS6, RBM9, RPS17 and RPL29. This was correlated with reactive oxygen species (ROS) levels and DNA Fragmentation Index (DFI). Semen samples were obtained from 60 cases and 30 fertile controls. Gene expression was done by qPCR analysis, and relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method. Chemiluminescence and the sperm chromatin structure assay were used to measure the ROS and DFI levels respectively. FOXG1, OGG1, RPS6 and RBM9 were seen to be upregulated, while SOX3 and PARP1 were downregulated. Relative expression of SOX3, OGG1, RPS6 and RPS17 showed a significant difference between patients and controls ($p < 0.05$). RPL patients were seen to have high ROS (>27.8; $p = 0.001$) and DFI (>30.7; $p < 0.0001$) with respect to controls. Sperm transcript dysregulation and oxidative DNA damage can be "carried over" after implantation, thus affecting embryogenesis and health of the future progeny.

GReX Associated with VACTERL PheRS

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Mol Genet Metab. 2016 Sep;119(1-2):83-90. doi: 10.1016/j.ymgme.2016.06.008. Epub 2016 Jun 14.

Elevated plasma dihydroorotate in Miller syndrome: Biochemical, diagnostic and clinical implications, and treatment with uridine.

Duley JA[1], Henman MG[2], Carpenter KH[3], Bamshad MJ[4], Marshall GA[5], Ooi CY[6], Wilcken B[7], Pinner JR[8].

Author information

Abstract

BACKGROUND: Miller syndrome (post-axial acrofacial dysostosis) arises from gene mutations for the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH). Nonetheless, despite demonstrated loss of enzyme activity dihydroorotate (DHO) has not been shown to accumulate, but paradoxically urine orotate has been reported to be raised, confusing the metabolic diagnosis.

METHODS: We analysed plasma and urine from a 4-year-old male Miller syndrome patient. DHODH mutations were determined by PCR and Sanger sequencing. Analysis of DHO and orotic acid (OA) in urine, plasma and blood-spot cards was performed using liquid chromatography-tandem mass spectrometry. In vitro stability of DHO in distilled water and control urine was assessed for up to 60h. The patient received a 3-month trial of oral uridine for behavioural problems.

RESULTS: The patient had early liver complications that are atypical of Miller syndrome. DHODH genotyping demonstrated compound-heterozygosity for frameshift and missense mutations. DHO was grossly raised in urine and plasma, and was detectable in dried spots of blood and plasma. OA was raised in urine but undetectable in plasma. DHO did not spontaneously degrade to OA. Uridine therapy did not appear to resolve behavioural problems during treatment, but it lowered plasma DHO.

CONCLUSION: This case with grossly raised plasma DHO represents the first biochemical confirmation of functional DHODH deficiency. DHO was also easily detectable in dried plasma and blood spots. We concluded that DHO oxidation to OA must occur enzymatically

solved the biochemical conundrum in previous reports of Miller syndrome patients, and opened the ening.

G X E

Front Immunol. 2017 Aug 24;8:1013. doi: 10.3389/fimmu.2017.01013. eCollection 2017.

Decidual Macrophage Functional Polarization during Abnormal Pregnancy due to *Toxoplasma gondii*: Role for LILRB4.

Li Z[1], Zhao M[2], Li T[1], Zheng J[3], Liu X[1], Jiang Y[1], Zhang H[1], Hu X[1].

Author information

Abstract

During gestation, *Toxoplasma gondii* infection produces a series of complications including stillbirths, abortions, and congenital malformations. The inhibitory receptor, LILRB4, which is mainly expressed by professional antigen-presenting cells (especially macrophages and dendritic cells) may play an important immune-regulatory role at the maternal-fetal interface. To assess the role of LILRB4 during *T. gondii* infection, LILRB4$^{-/-}$ and *T. gondii* infected pregnant mouse models were established. Further, human primary-decidual macrophages were treated with anti-LILRB4 neutralizing antibody and then infected with *T. gondii*. These *in vivo* and *in vitro* models were used to explore the role of LILRB4 in *T. gondii*-mediated abnormal pregnancy outcomes. The results showed that abnormal pregnancy outcomes were more prevalent in LILRB4$^{-/-}$ infected pregnant mice than in wild-type infected pregnant mice. In subsequent experiments, expression levels of LILRB4, M1, and M2 membrane-functional molecules, arginine metabolic enzymes, and related cytokines were assessed in uninfected, infected, LILRB4-neutralized infected, and LILRB4$^{-/-}$ infected models. The results demonstrated *T. gondii* infection to downregulate LILRB4 on decidual macrophages, which strengthened M1 activation functions and weakened M2 tolerance functions by changing M1 and M2 membrane molecule expression, synthesis of arginine metabolic enzymes, and cytokine secretion profiles. These changes contributed to abnormal pregnancy outcomes. The results of this study provide not only a deeper understanding of the immune mechanisms operational during abnormal pregnancy, induced by *T. gondii* infection, but also identify potential avenues for therapeutic and preventive treatment of congenital toxoplasmosis.

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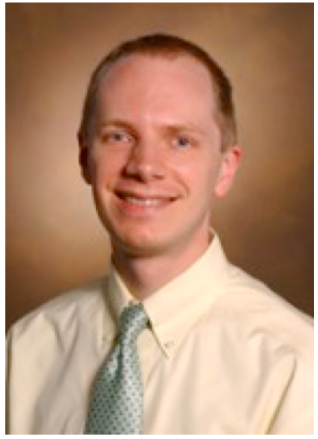
Phenome!

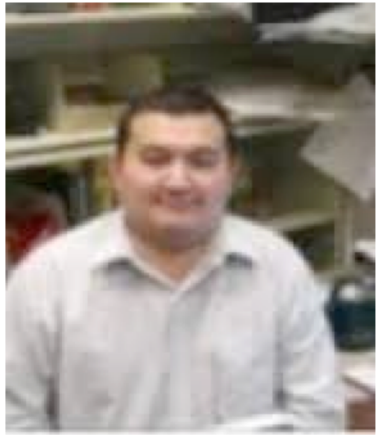
Eric Gamazon

Dan Roden

Lisa Bastarache



Josh Denny



Anuar Konkashbaev

Jessica Dennis

Tyne Miller

Megan Shuey

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NHGRI, FNIH, NIMH, NIMHD, AHA