RESEARCH ARTICLE

Transcription Factor Binding and Individual Genetic Risk of Valproate Teratogenicity

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Abstract

Background and Objectives

Valproate (VPA) use during pregnancy is associated with a wide range of structural birth defects, but not all exposed children are affected and there is evidence for a genetic predisposition. The development of a pharmacogenomic biomarker test that can be used for preconception counseling, allowing access to women unnecessarily denied VPA treatment because of concern about teratogenic risks if they were to become pregnant, is challenged by a poor understanding of how variation in maternal DNA could modify the risk. We hypothesized that genomic variants that affect the binding affinity of transcription factors (TFs), key regulators of gene expression, are integral to VPA-associated teratogenicity and a plausible explanation for both variance in interindividual risk and the wide range of birth defect types.

Methods

We interrogated genomic variants within maternal exomes from women recruited through international epilepsy pregnancy registries and genomics consortia. We applied a network-based approach that contextualized the variant spectra to genes associated with diverse birth defect types, gene burden tests, and evidence from multiple modalities to identify variant-sensitive TFs.

Results

Sixty-six pregnancies were exposed to VPA as monotherapy or polytherapy, leading to 28 cases with birth defects, and 184 were exposed to other antiseizure medications (ASMs), leading to 20 cases with birth defects. The variant burden within genes associated with 32 different birth defect types was higher for those exposed to VPA compared with those exposed to other ASMs (OR 1.73 [95% CI 1.39 to 2.13], $p < 0.0001$). Variants in a network comprising significant genes from VPA-exposed mothers were predicted to modify the binding affinity of 359 TFs. These variant-sensitive TFs formed a highly connected protein-protein interaction network, among which the acetyltransferase *EP300* connected to 41% (147/359) of all proteins. Profiling of coexpression between *EP300* and other TFs in an embryonic stem cell (hESC) model showed that VPA exposure alters *EP300*-TF interactions.

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Glossary

ASMs = antiseizure medications; **eQTL** = expression quantitative trait loci; **hESC** = human embryonic stem cell; **TF** = transcription factor; **VPA** = valproic acid.

Discussion

These findings suggest that VPA-induced disruption of *EP300*-related gene regulation is a teratogenic mechanism that is common to heterogeneous birth defect types and sensitive to genetic variation. This has implications for the development of pharmacogenomic risk biomarkers and safer drugs for women of childbearing potential.

Introduction

Valproic acid (VPA) is a first-line antiseizure medication (ASM) for the control of seizures in people with epilepsy. It is the most effective treatment in those with generalized epilepsies, including several syndromes such as juvenile myoclonic epilepsy, juvenile absence epilepsy, epilepsy with generalized tonic-clonic seizure alone, and epilepsy with eyelid myoclonia.[1-3] It is also widely used for the treatment of neuropathic pain, bipolar disorder, schizophrenia, and migraine[4] and is emerging as a potential cancer therapy.[5] Babies born to mothers who take VPA during pregnancy are at high risk of birth defects, lower intelligence quotient (IQ), developmental delay, attention-deficit hyperactivity disorder, autism spectrum disorder, and other neurodevelopmental disorders.[4] Globally, measures have been introduced to raise awareness and restrict VPA prescribing. The UK Medicines and Healthcare products Regulatory Agency, for instance, stipulates that valproate must not be started in new patients younger than 55 years, unless 2 specialists independently consider and document that there is no other alternative effective or tolerated treatment, or that there are compelling reasons the reproductive risks do not apply.[6] Yet, not all children exposed in utero to VPA have adverse consequences, and particularly for patients with genetic generalized epilepsies, alternatives to this potentially life-saving medication are often considerably less effective.[1,2] The teratogenic effects have been well established through large prospective registries[7,8]; administrative health care databases[9-11]; and prospective, smaller-scale, observational studies.[12,13] There remains a pressing need to elucidate the mechanism of teratogenesis and to understand risk at the molecular and individual level. A genetic susceptibility to VPA teratogenicity is suggested by studies that found increased risk of recurrent ASM-associated birth defects in women who have had an affected pregnancy,[14] those with a family history of birth defects,[15] and animal models.[16]

The nature of VPA teratogenicity is highly variable, including nonspecific structural birth defects affecting multiple different organ systems, as well as neurodevelopmental deficits.[17] During organogenesis and embryonic development, the expression of genes is tightly orchestrated in time and space by DNA-binding proteins collectively referred to as transcription factors (TFs).[18] The binding of TFs to DNA is dependent on both specific amino acid residues in the TF protein and specific bases in the target DNA sequence. Genetic variants within TF binding sites can (a) modify binding affinity strength, (b) prevent one or more TFs from binding, or (3) introduce a motif that permits aberrant TF binding.[19]

Genome-wide association studies show that sequence variation in TF binding sites comprise only 8% of the genome yet represent 31% of all trait-associated sequence variations.[20] These variants are mostly located within intronic (~50%) and intergenic (~30%) DNA regions but are also found in promoters, coding regions, and untranslated gene regions.[19] It has been shown that enhancers (regulatory DNA regions) with tissue-specific activity are enriched in intronic regions.[21] High-quality genotypes within intronic and intergenic variants are captured by the approximately 40%–60% of exome-sequencing reads that are off-target.[22] In this study, we used exome data to explore the hypothesis that variant-induced changes in TF binding might modify VPA-induced dysregulation of gene expression and thus explain both differences in interindividual risk and the heterogeneity of VPA-associated birth defects. We analyzed and compared pregnancies exposed to VPA only and non-VPA ASMs, ascertained from international epilepsy pregnancy registries, and applied a network-based approach that contextualized the variant spectra to genes associated with birth defects and integrated evidence from multiple modalities, to test our proposed mechanism of teratogenesis.

Methods

Samples

Women were recruited through an international collaboration involving pregnancy registries and genomics consortia in Australia, Europe, and North America: The Raoul Wallenberg Australian Pregnancy Register of Antiepileptic Drugs (RWAPR),[23] The UK and Ireland Epilepsy and Pregnancy Register (UKIEPR), Epilepsy Pharmacogenomics Consortium (EpiPGX), and an international epilepsy genetics research consortium, EPIGEN (details in eMethods).[24] Exome sequencing was performed at either the Broad Institute, the Institute for Genomic Medicine, Columbia University in New

York, or deCODE genetics. The raw reads were aligned to human genome build GRCh38, and standard quality filtering and imputation methods were applied (eMethods). To ensure that variant detection had not been biased by differences in the exome capture kits used, we compared (Wilcoxon signed-rank test) the median depth at the loci of high-confidence variants with 100 base-pair padding either side across capture kits.

Association Tests

The MalaCards human disease database was used to identify genes associated with "congenital structural birth defects" or "congenital genitourinary birth defects," which covered all birth defects of affected children reported in our study cohort. Gene burden association tests were undertaken using the SNP-Set (Sequence) Kernel Association Tests available in the "SKAT" R package (version 2.2.4). These methods aggregate individual SNP score statistics into a SNP set—in this case, each gene—and tests include Burden, SKAT, and SKAT-O, which allows testing for common only, rare only, or common and rare variant associations. The tests have different strengths that depend on the underlying biology; thus, 6 different tests were applied and genes with $p < 0.05$ in at least one test were included in subsequent analyses. Variant-level association tests were undertaken using Plink software version 2.0, with 10 principal components to control for population stratification and permutation to determine significance levels empirically. The Fisher exact test was used to compare the proportion of significant genes and unique gene-birth defect-type associations identified between sample subsets (VPA exposures or non-VPA ASM exposures).

Network Construction and Knowledge Integration

The STRING database App within Cytoscape software (version v3.10.2) was used to generate protein-protein interaction networks from proteins encoded by significant genes, hereinafter referred to as the birth defect hub (BD-hub) for VPA exposures and non-VPA ASM exposures, and a network of TFs (TF-hub) that were predicted to have their binding affinity modified by one or more variants evidenced in the VPA exposure BD-hub. To infer the functional consequence of variants, we integrated evidence from (1) the Ensembl Variant Effect Predictor Tool, (2) expression quantitative trait loci (eQTL) from the GTEX compendium, and (3) evidence of potential disruption of TF binding sites using the DeepBind software tool (eMethods, eFigure 1). To gain insight into the susceptibility of genes to VPA, we integrated findings from differential expression analyses as reported in a rodent model[25] and a human cortical organoid model[26] of VPA teratogenesis, and a human embryonic stem cell–based neurodevelopmental toxicity assay.[27] In addition, we (1) used data from the neurodevelopmental toxicity assay to identify BD-hub genes that were differentially expressed by carbamazepine (CBZ), which can also have teratogenic effects, and (2) assessed how many BD-hub genes overlapped with a teratogenic 'gene signature' derived from a human induced pluripotent stem cell (hiPSC)–based assay for the classification of developmental toxicants.[28] We generated 1,000 comparison random gene sets to establish the specificity of signal (eMethods).

Variant Prioritization

Individual variants in genes within the BD-hub derived from VPA-exposed pregnancies were filtered based on evidence of functional consequence and considered to have a 'high level' of evidence if all of the following criteria were met: (1) the variant was nonimputed, (2) the variant was independently associated with an adverse pregnancy outcome (Plink association test, empirical $p \leq 0.05$), (3) the eQTL effect for the variant included the gene that the variant lies within, (4) DeepBind scores indicate that the variant affects the binding of at least one TF, and (5) there was evidence from at least one exposure study that VPA modified the expression of the gene harboring the variant, or of one or more of the TFs predicted to be affected by the variant. Variant filtering was undertaken using R version R/4.2.0. Circos plots were constructed using the R circlize library.

To gain insight into how *EP300* interacts with other TFs, we constructed a reference map using coexpression analyses and publicly available bulk and single-cell gene expression data. The bulk data represented postmortem brain tissue, covering the full course of human brain development, including both prenatal and postnatal periods, and the single-cell data represented fetal cerebellum and cerebrum tissue obtained from the Gene Expression Omnibus Repository (accession number GSE156793). The coexpression between *EP300* and all other TFs was determined using both the Pearson and Spearman methods (details in eMethods).

Cell Culture

H9 human embryonic stem cells (hESCs) (WiCell, WA09) were differentiated into neurons using the dual SMAD inhibition protocol[29] (eMethods). The cells were exposed to VPA throughout differentiation from days 0 to 28 and divided into 3 exposure groups of 300 μM, 700 μM, and a control with no VPA exposure. These doses represent the upper and lower bounds of the typical therapeutic range for the treatment of epilepsy, which is 50–100 mg L^{-1} (which corresponds to ~350–700 μM). Differential expression and differential coexpression analyses were undertaken (eMethods).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Melbourne Health Human Research and Ethics Committee (Reference Number: HREC/11/MH/282), and written informed consent was obtained from all participants. For the EpiPGX consortium, all participants provided written informed consent for appropriately coded use of their clinical data. Ethical approval for this study was obtained by each participating centre; for the lead organisation, approval was obtained form the National Research Ethics Service Committee London - Camden

and Islington Research Ethics Committee (reference number: 11/LO/2016).

Data Availability

The anonymized exome patient data are not being publicly shared in accordance with the ethics requirements of the Raoul Wallenberg Australian Pregnancy Register of Antiepileptic Drugs (reference number: HREC/11/MH/282). The RNA-seq data from our hESC assay are available from Gene Expression Omnibus (GEO) (accession number GSE290300).

Results

The cohort comprised 250 mothers, 48 of whom had one or more affected pregnancies (cases). Sixty-six pregnancies were exposed to VPA as monotherapy or polytherapy, with 28 cases, and 184 were exposed to other ASMs, with 20 cases (Table 1, eTable 1). The distribution of defect types was similar between children exposed to VPA and those exposed to other ASMs (eMethods, eTable 2). After postquality control filtering of the imputed data, 1,997,655 variants were analyzed (eTable 3).

Variant Burden in Genes Associated With Birth Defects

The MalaCards database identified 1,525 autosomal genes associated with one or more of 32 birth defect types (eTable 4). The number of genes with a variant burden significantly different between cases and controls was higher in pregnancies exposed to VPA (either as monotherapy or polytherapy) compared with those exposed to other ASMs (eTable 5, OR 1.73 [95% CI 1.39–2.13], $p < 0.0001$), and less than 2% (27/1,525) of genes had a variant burden that was significantly different between cases and controls in both groups. The number of unique gene-birth defect-type associations was higher for the VPA group than for the other ASM group (OR 1.56 [95% CI 1.32–1.84], $p < 0.0001$), and all 32 birth defect types were implicated in the former, but only 29 in the latter. Comparison of birth defect–related gene sets with 100 randomly sampled gene sets showed that the number of significant genes was significantly different for 3 tests (SKAT Binary [95% 1.59–2.17], $p < 0.0001$, SKAT-O [95% 1.42–1.93], $p \leq 0.0001$, and CommonRare common only [95% 1.04–1.41], $p = 0.01$). For the remaining 2 tests, none or less than 1% of the significant genes captured had a p value smaller than the smallest observed across birth defect gene sets (0% for the SKAT CommonRare test, and 0.05% for the CommonRare Rare only test).

VPA-Exposed Birth Defect Hub (BD-Hub)

When viewed as a protein-protein interaction network, 227 of encoded proteins formed a highly connected hub (BD-hub), representing genes associated with 31 different birth defect types (Figure 1B, eMethods, eTable 6). The difference in burden between cases and controls could be attributed to "common only" variants for 36 genes and "rare only" variants for 44 (Figure 1A). The number of genes associated with specific birth defect types ranged from 1 to 88 (median = 7), with microcephaly being the most frequently represented. The number of birth defect types each gene was associated with ranged from 1 to 10, with 9 genes contributing to 5 or more types (Figure 2).

Most variants within the VPA-exposed BD-hub genes lay within intronic (82%), downstream (6.92%), or upstream (4.59%) gene regions (eTable 7). This is in line with a comparison of SNP detection by 2 commercial exome capture platforms (Agilent and NimbleGen), which found that intronic variants were most represented among the roughly 25,000 to 40,000 SNPs identified by both platforms.[30] Integration of eQTL data showed that most BD-hub genes (94%, 214/227) harbor variants that modify gene expression (n = 14,658, range per gene = 1 to 846, median = 32 [IQR 10.25–82]). A single variant can affect the expression of multiple genes, including (but not always) the gene they fall within. The eQTL data indicated that the expression of 81% (183/227) of the BD-hub genes was dependent on variant(s) that they or other genes within the hub harbored, inferring that the functional impact has relevance for birth defect biology. Sixty-two percent (9,154/14,658) of the eQTL variants were predicted to affect the binding affinity of one or more TFs. The number of variants with both sources of functional evidence was lower in the BD-hub derived from non–VPA-exposed pregnancies (n = 6,584) and across 100 randomly generated gene sets (maximum 4,633, median 3,172 [IQR 2,795–3,618], eTable 8).

Integration of Published Differential Expression Data

The integration of differential expression data from multiple sources showed that 79% (180/227) of BD-hub genes had evidence of being dysregulated by VPA, including 85% (45/53) of those with the highest degree (number of direct neighbors >10) within the hub (eFigure 2). The percentage of genes with evidence of VPA-induced dysregulation was lower (68%, 90/132) in the BD-hub generated using data from non–VPA-exposed samples while the percentage of CBZ-induced dysregulated genes was slightly higher in the non–VPA-exposed BD-hub (15%, 20/132) than in the VPA-exposed BD-hub (12%, 28/227).

Variant Impact on TF-Binding

Variants within genes in the BD-hub were predicted to modify the binding affinities of 359 TFs. The encoded proteins formed a densely connected network: the TF-hub. Highly connected genes within the TF-hub have master regulatory roles in stem cell pluripotency (*SOX2, POU5F1, NANOG*, and *MYC*) and embryonic development (for example, members of the GATA family (GATA1-4) that play a prominent role in regulating cardiac development and *PAX6* that is involved in the regulation of forebrain development).[31] The most connected TF, linking to 41% (147/359) of all other

Table 1 Affected Pregnancies

Mother	Birth defects	In utero ASMs (mg/d)
AM21	Cryptorchidism	VPA (800)
AM11	Right renal hydronephrosis	VPA (600), LTG (100)
AM22	Extra thumb	VPA (600)
AM2	No bone in left thumb, tight fingers in both hands, and cardiac defects (VSD and ASD)	VPA (1,000)
AM10	Ventricular septal defects (bulbus cordis and cardiac septal closure)	VPA (1,000), LTG (200)
AM12	Dysmorphia	VPA (500)
AM13	Two pregnancies: retrognathia and hypertolism and hypospadias and induced TOP due to spina bifida and hydrocephalus	VPA (2000), LTG (150)
AM14	Spina bifida and ureteric obstruction	VPA (1,000)
AM15	TOP due to anencephaly	VPA (UNK), CBZ (UNK), CLN (UNK)
AM16	TOP due to anencephaly	VPA (400)
AM4	Hypospadias	VPA (800), CLN (0.25)
AM17	Bilateral deafness: left ear auditory nerve missing, right ear malformed cochlear	VPA (400)
AM3	Club foot	VPA (1,000), LEV (1,500)
AM18	TOP due to spina bifida	VPA (3,000), TPM (75)
AM19	TOP due to spina bifida	VPA (3,000)
AM20	Two pregnancies: patent foramen ovale, and cardiac defect and cleft lip and palate	VPA (500)
AM1	Arnold-Chiari malformation, clinodactyly, cardiac defects (VSD and ASD significant, PDA small)	VPA (1,500)
IM8	Cleft palate	VPA (3,000), TPM (500), PHT (350)
IM9	Neural tube defect	VPA (1,000)
IM10	Club foot	VPA (500), LTG (25)
IM11	Periauricular skin tag and brachycephalic skull	VPA (1,500)
IM12	Dysmorphic	VPA (1,000)
IM13	Limb defect (shortened arm)	VPA (1,000), LTG (25), PHT (300), OXC (1800), CLB (10), TGB (30)
IM14	Cleft palate	VPA (1,500)
IM1	Spinal deformity	VPA (1,600)
IM2	Arnold-Chiari malformation, syringomyelia, hydrocephalus, developmental delay	VPA (1,500), LTG (200)
IM3	One child with hypospadias (2 additional pregnancies with developmental delay/autism, VPA 2000 and 1200)	VPA (1,500)
IM6	Gastroschisis	VPA (800)
IM4	Cardiac (heart murmur), umbilical hernia	LTG (500), CBZ (600)
IM5	Skeletal (extra digits)	LTG (150), LEV (1,500)
IM7	Neural tube defect	CBZ (1,200), LEV (3,000)
AM23	Oral (tongue tie) and hydronephrosis	LTG (200)
AM24	Cryptorchidism	CBZ (600), LTG (100)
AM25	Hypospadias	LEV (500)
AM26	TOP due to defects (details unknown)	CBZ (800)
AM27	Twin 1 VSD and hypospadias, twin 2 VSD	CBZ (800)
AM28	TOP due to bladder obstruction	LTG (200) LEV (500)

Continued

Table 1 Affected Pregnancies *(continued)*

Mother	Birth defects	In utero ASMs (mg/d)
AM29	Bilateral club feet	CBZ (1,600)
AM30	Hypospadias	LTG (100)
AM31	VSD	LEV (1,000)
AM32	Tetralogy of Fallot	LEV (1,500)
AM5	Macrocephaly, dysmorphia, and ankyloglossia	CBZ (1,200)
AM8	Cryptorchidism	PGB (450), TPM (300)
AM33	Missing toe and syndactyly	LEV (1,000)
Am34	Bilateral club feet	LTG (200)
AM6	Hypospadias	CBZ (500)
AM35	Bicuspid aortic valve	LTG (200)
AM36	ASD	OXC (1,500), CLB (20)

Abbreviations: ASD = atrial septal defect, ASM = antiseizure medication, CLB = clobazam, CBZ = carbamazepine, CLN = clonazepam, LEV = levetiracetam, LTG = lamotrigine, OXC = oxcarbazepine, PHT = phenytoin, TGB = tiagabine, TOP = termination of pregnancy, TPM = topiramate, UNK = unknown, VPA = valproic acid, VSD = ventricular septal defect.
All births were live births unless TOP is specified.

TFs, was the acetyltransferase and transcriptional coactivator *EP300*.

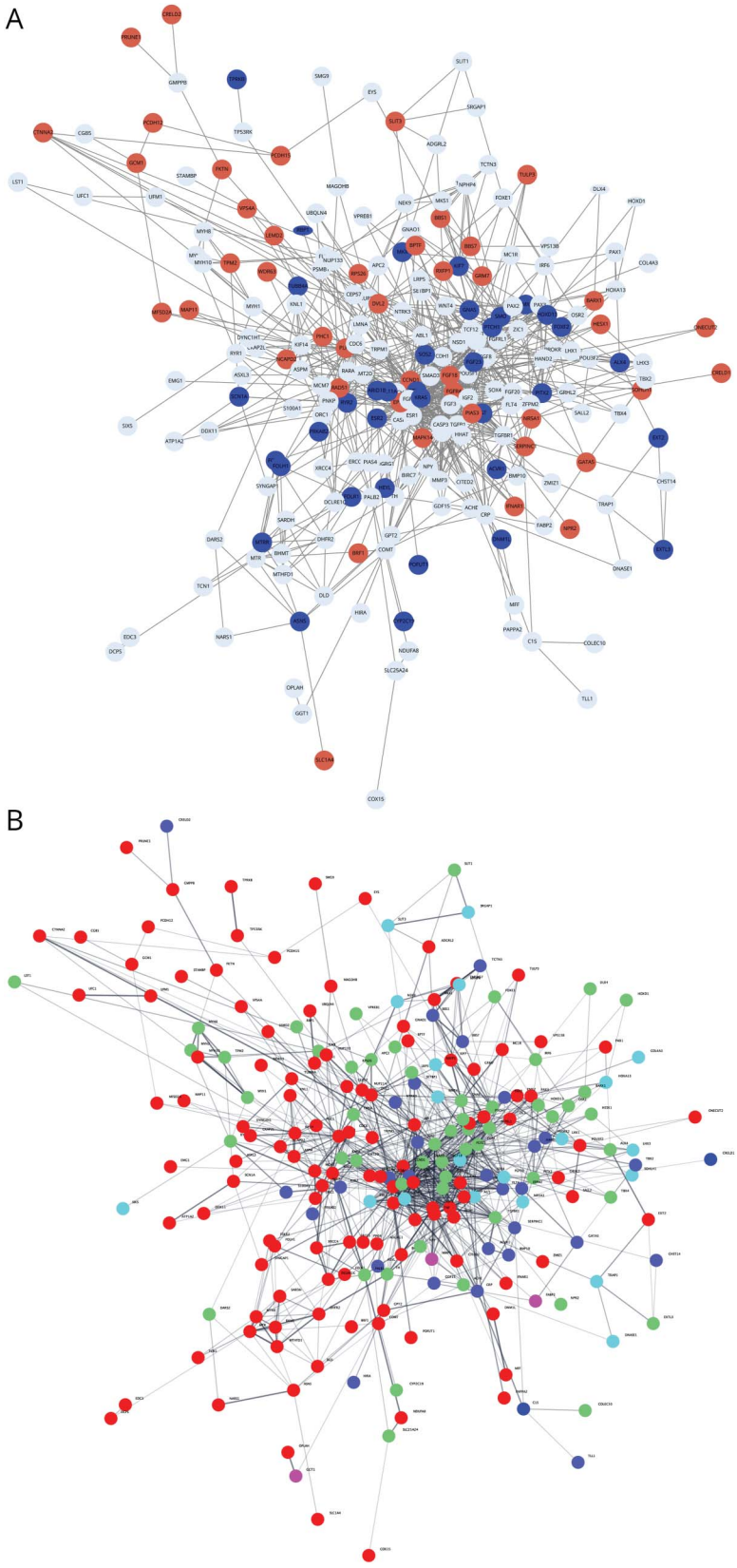
The impact of genetic variation, measured by the number of variants predicted to affect the binding affinity of any of the 50 most connected TFs, was higher for gene variants in the VPA-exposed BD-hub than in the BD-hub derived from mothers exposed to other ASMs (Wilcoxon signed-rank test [95% CI 63.50 to 106.50], p = 1.1E-09) and variants within randomly generated sets of genes (all one-sample t test/Wilcoxon signed-rank tests, $p < 0.001$, eTable 9, Figure 3). In the TF-hub, 70% (249/359) of TFs were dysregulated by VPA exposure, including 18 of 22 with a degree >70 (eFigure 3), and 12.5% (45/359) had evidence of CBZ dysregulation, with 38 being dysregulated by both drugs. There was no statistical difference across the gene hubs regarding overlap with a transcriptomic signature used to classify teratogenic compounds[28] (53/227 of VPA-exposed, 34/132 of non–VPA-exposed, and 89/359 of the TF-hub genes, χ^2 test of homogeneity: $x^2(2) = 0.29$, $p = 0.864$), and little overlap in the genes implicated across the hubs.

High-Confidence Variants

One hundred thirty-five variants met our high-confidence filtering criteria. Comparison of the mean read depths (Wilcoxon signed-rank test) at the loci of high-confidence variants in VPA-exposed samples found no significant difference between NextEra and the Broad Institute custom kit (n = 51 vs n = 12 [95% CI −5.00 to 7.00], p = 0.85) or NimbleGen (n = 3 [95% CI −11.0 to 22.0], p = 0.865) or between NimbleGen and the Broad Institute custom kit ([95% CI −9.99 to 13.99], p = 0.51, eFigure 4, A and B). These variants were ranked based on the potential to disrupt one or more "master regulators" (degree >70) identified within the TF-hub (eTable 10). The highest ranking "high-confidence" variant (chr11:49173538:T:C) lies within an intronic region of *FOLH1*, the gene encoding folate hydrolase 1, which is associated with neural tube defects[32] and downregulated by VPA in a cortical organoid model.[26] The variant was detected by all exome capture kits (eFigure 4C). *FOLH1* met the significance cutoff for only one SKAT test, which evaluated the contribution of both common and rare variants (p = 0.02), but the frequency of this single variant was higher in VPA-exposed cases compared with controls (empirical p = 0.007). Relevant to the homozygous reference genotype TT, the TC genotype has tissue-specific and cell type–specific impact: reduced expression of *FOLH1* in arterial and nerve cells and tissues of the colon, esophagus, and omentum, and increased expression of a brain-specific transcript (RP11-707M1.1), across 6 different brain regions. The DeepBind scores predict that this variant modifies the binding of 15 TFs, including 6 master regulators (*CEBPB, EP300, FOS, HDAC2, JUN*, and *STAT3*), 4 of which show evidence of VPA-induced dysregulation (*EP300, FOS*, *JUN*, and *STAT3*). Together, these observations suggested that both genetic variant and prenatal VPA exposure could modify the activity of multiprotein complexes, with their combination potentially having an additive effect.

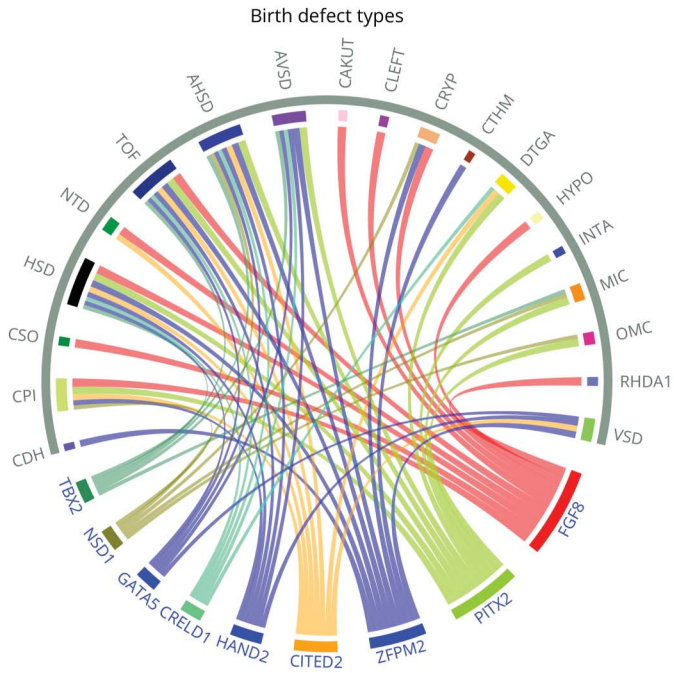
The overall spectra of high-confidence variants suggested that genetic variation across multiple genes can converge to modify a specific TF protein complex. An example is the GATA family of TFs, which comprises 6 proteins (GATA1-6) that play a critical role in the early stages of cell differentiation and organ development, involving tissues from all germ layers.[33] The dysregulation of pleiotropic activity and tissue-specific regulation of this type of multiprotein complex might

Figure 1 BD-Hub Comprising Genes With Significantly Different Variant Burdens Between Affected and Unaffected VPA-Exposed Pregnancies



(A) Variant burden: for genes colored in blue (n = 36), the difference in burden between affected and unaffected pregnancies was attributed to 'common only' variants, and those attributed to 'rare only' variants are shown in orange (44). (B) Defect types: hub genes are colored by specific birth defect types: red (n = 5)—neural tube defects, anencephaly, encephalocele, microcephaly, and microphthalmia; dark blue (n = 7)—atrial heart septal defect, atrioventricular septal defect, conotruncal heart malformations, transposition of the great arteries dextro-looped, heart septal defect, tetralogy of Fallot, and ventricular septal defect; pink (n = 3)—biliary atresia, gastroschisis, and intestinal atresia; green (n = 10)—diaphragmatic hernia, congenital, cleft palate isolated, craniosynostosis, cleft lip with or without cleft palate, clubfoot, fryns microphthalmia syndrome, microtia, omphalocele, and esophageal atresia; and light blue (n = 4)—congenital anomalies of the kidney and urinary tract, renal hypodysplasia, cryptorchidism, and hypospadias. Two conditions, atrioventricular septal defect and meningocele, are not shown because they were each associated with a single gene that was also implicated (and colored) in other conditions. VPA = valproic acid.

Figure 2 Association Between Genes Within the VPA-Exposed BD-Hub and Birth Defect Types



Abbreviation	Birth defect types
AHSD	Atrial heart septal defect
AVSD	Atrioventricular septal defect (AVSD)
CAKUT	Congenital anomalies of the kidneys and urinary tracts
CDH	Diaphragmatic hernia, congenital
CLEFT	Cleft lip with or without cleft palate
CPI	Cleft palate, isolated
CRYP	Cryptorchidism
CSO	Craniosynostosis
CTHM	Conotruncal heart malformations
DTGA	Transposition of the great arteries, dextro-looped
HSD	Heart septal defect
HYPO	Hypospadias
INTA	Intestinal atresia
MIC	Microcephaly
NTD	Neural tube defects
OMC	Omphalocele
RHDA1	Renal hypodysplasia/aplasia 1
TOF	Tetralogy of Fallot
VSD	Ventricular septal defect

The ribbons represent the association between genes (blue labels bottom of circle) and specific birth defects; abbreviations (green labels top of circle, full names in the table) based on information from the Human Disease Database MalaCards. VPA = valproic acid.

explain the wide range of VPA-associated birth defects (Figure 4).

EP300-TF Coexpression Reference Map

EP300 was significantly coexpressed with 91% (2,144/2,367) of TFs tested in bulk RNA-seq data from the BrainSpan repository, representing cortical and subcortical structures across the full course of human brain development (eFigure 5A). In scRNA-seq data, 238 coexpressed gene modules involving TFs were detected across 2 tissues and 10 cell types (eTable 11, Figure S5B). Of these, 12% (29/238) contained *EP300*. Within these modules, *EP300* was coexpressed with 86% (2,011/2,347) of TFs tested, and 83% (1,812/2,176) of coexpressed pairs were found in both bulk and single-cell settings. Overall, the number of times a unique coexpression relationship was observed ranged from 1 to 18 out of a possible 25 biological settings (median = 9), with 1,053 observed in 10 or more settings. For 71% (1,662/2,341) of TFs profiled, the direction of correlation differed across biological settings and this was observed more across tissues (55%, 1,206/2,176) compared with cell types (26%, 535/2,011).

Impact of VPA Exposure on *EP300* Coexpression

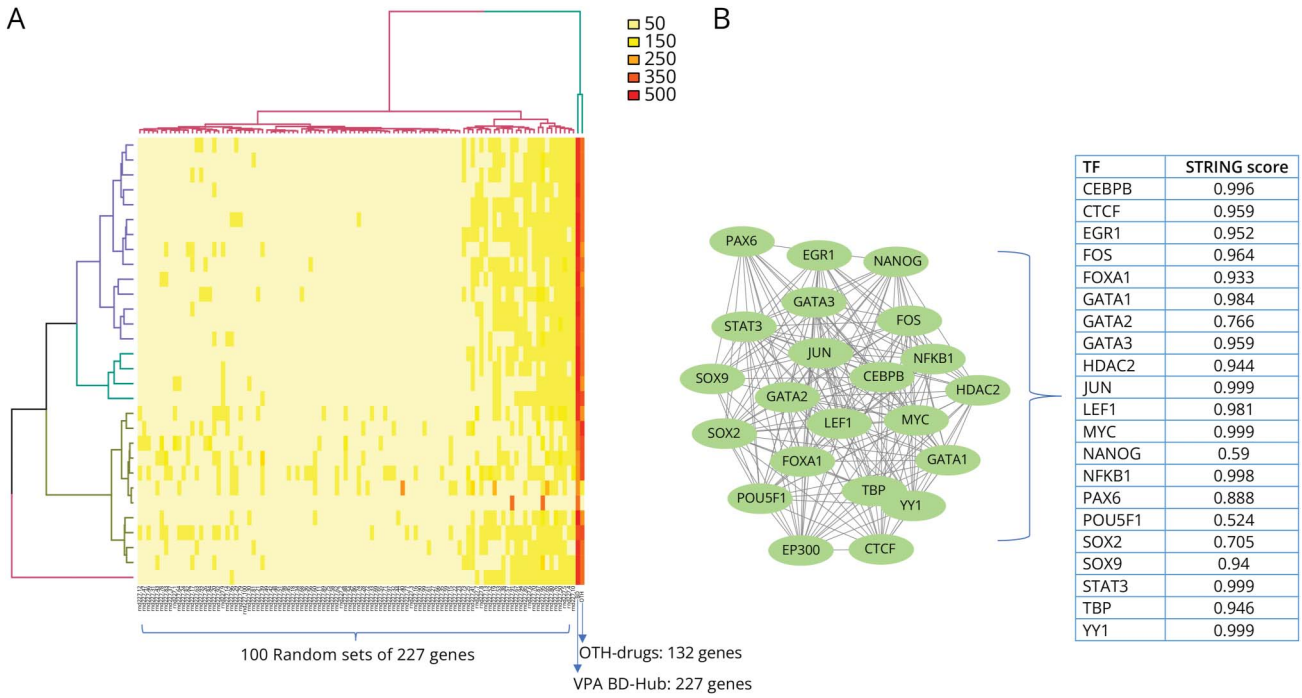
Gene expression data from unexposed hESCs (eFigure 6) showed *EP300* to be coexpressed with 400 TFs, and 99% (396/400) of these relationships were observed in one or more settings in the reference map (Figure 5A, range 1–18, median 10). Similarly, almost all coexpressed pairs observed in cells exposed to VPA were present in the reference (99% (225/228) at 300 μM and 99% (405/408) at 700 μM, Figure 5A). By contrast, only 30% (119/400) of the coexpressed pairs observed in unexposed cells were present in cells exposed to VPA (45 at 300 μM and 89 at 700 μM, with 15 observed at both doses, Figure 5B). Among the 281 TFs for which coexpression with *EP300* was lost, just over half (60%, 169/281) were also differentially expressed at one or both VPA doses. Similarly, 58% (269/467) of pairs observed only in VPA-exposed cells were also dysregulated at one or both doses. There was no significant difference in the level of *EP300* mRNA after exposure (adjusted $p = 0.239$ at 300 μM, adjusted $p = 0.395$ at 700 μM). These findings indicate that the impact of VPA on the activity of regulatory mechanisms is likely not fully exposed by studies that focus on differential expression alone.

Discussion

By contextualizing maternal exome variants to structural birth defect biology and evaluating impact on TF-driven gene regulation, we find evidence in support of a putative mechanism for VPA anatomical teratogenicity that involves the "master gene transcription regulator" *EP300*. Our findings support the notion that variant-affected TF activity could explain variation in both risk and VPA-associated birth defect types in women treated during pregnancy. These findings provide a foundation for more clinically translational studies.

As would be expected, given that TFs rarely act in isolation, we found that variants within different genes potentially have

Figure 3 (A) Variant Burden



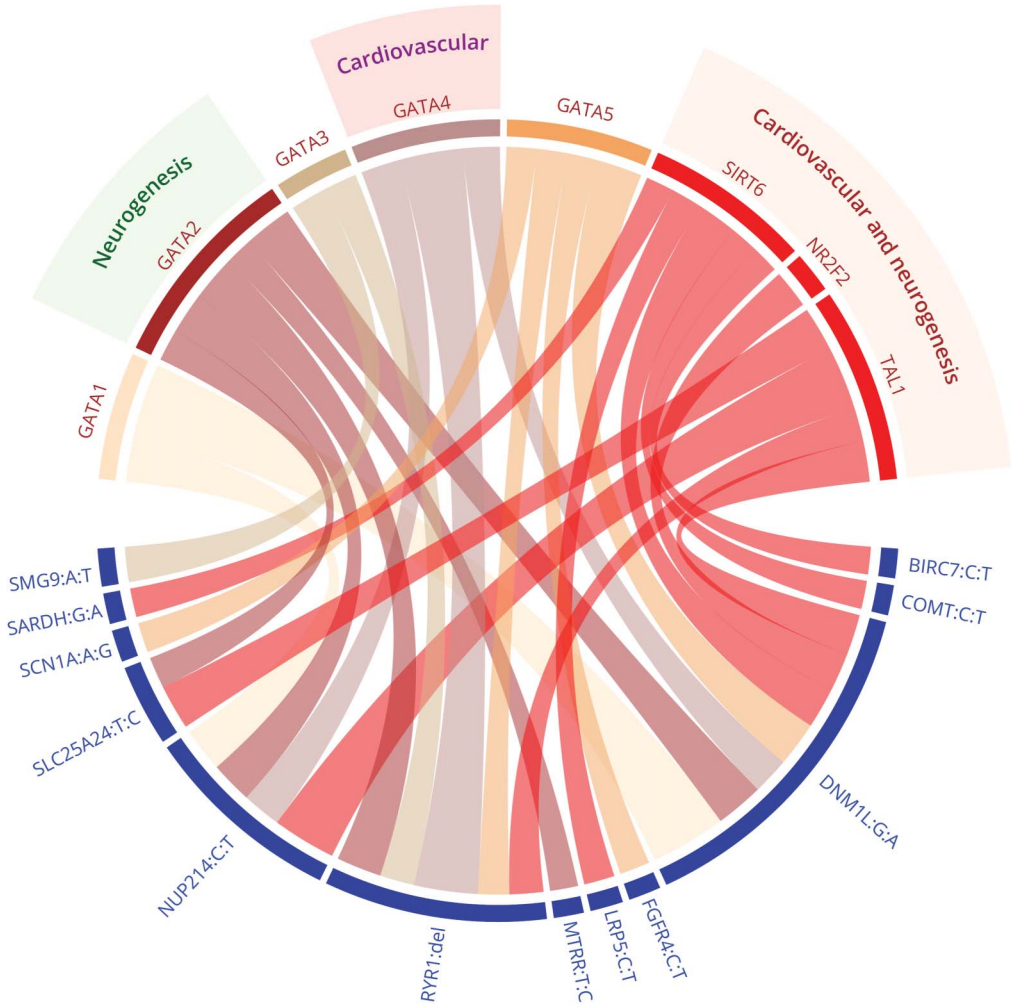
TF	STRING score
CEBPB	0.996
CTCF	0.959
EGR1	0.952
FOS	0.964
FOXA1	0.933
GATA1	0.984
GATA2	0.766
GATA3	0.959
HDAC2	0.944
JUN	0.999
LEF1	0.981
MYC	0.999
NANOG	0.59
NFKB1	0.998
PAX6	0.888
POU5F1	0.524
SOX2	0.705
SOX9	0.94
STAT3	0.999
TBP	0.946
YY1	0.999

Heatmap representing the predicted impact of variants on the binding affinity of the top 30 most highly connected TFs in the TF-hub. The rows represent TFs, and the columns are gene sets. The first 2 columns on right-hand side of the x-axis represent the impact of variants within a gene hub derived from genes significantly different between cases and controls exposed to other ASMs and variants within the VPA-exposed BD-hub. The remaining columns represent variants within 100 randomly generated gene sets. A stronger color represents a higher number of variants predicted to modify a TF. (B) *EP300* interactions; protein-protein interactions between *EP300* and other TFs within the TF-hub. Oval shapes represent genes encoding the proteins, and the lines connecting genes are determined by STRING database scores (shown in the table), which are ranked from 0 to 1 depending on how likely STRING judges an interaction to be true. ASMs = antiseizure medications; VPA = valproic acid.

converging effects on a single regulatory mechanism, and that a single variant could disrupt the binding of multiple TFs. The potential sensitivity of *EP300*-mediated TF complexes to genetic variation was an important, novel finding in this study. Our expression correlation analyses in hESCs indicate that the activity of *EP300*-related TF complexes differs considerably between unexposed cells and VPA-exposed cells, yet correlated pairs in both exposure settings had high overlap with those observed in the reference map, suggesting that VPA activates alternative biologically valid, but context inappropriate, signaling cascades. Given that any single TF can regulate more than one gene and the same gene can be regulated by multiple TFs, additional investigative methods are required to gain insight into the biological significance of the actual dysregulated coexpression pairs.

The *EP300* gene encodes p300, a member of the cAMP response element-binding protein (CREB)-binding protein family (CBP) with histone acetyltransferase activity. p300 plays a critical role in embryogenesis. Complete loss of p300 in a mouse model resulted in embryonic lethality, and heterozygotes also manifested considerable embryonic lethality.[34] In contrast to findings from rodent studies, we found no significant difference in the level of *EP300* mRNA after exposure to VPA in our cell model. In utero exposure in a mouse model of neural tube defects found p300 mRNA, but not protein levels, to be downregulated at 1 and 3 hours after exposure to VPA in GD9 mouse embryos[35] while both mRNA and protein levels of p300 were decreased after exposure in a mouse cell line.[36] Among embryos exposed or not to VPA, the levels of p300 mRNA and protein expression in those with closed or open neural tubes were not significantly different.[35] *EP300* is regulated by mechanisms other than transcription including post-translational modifications and co-activator interactions[37] and thus potentially drives major biological transitions without a change in mRNA or protein expression. In addition, altered p300 activation may be a contributing factor in pathogenicity. The enzyme is activated by propionate and butyrate, which are short-chain fatty acids, as is VPA.[38] Beyond its canonical acetyltransferase activity, p300 has a broad functional repertoire conditioned on cellular metabolism of cognate short-chain acyl-CoA cofactors. It has been shown, for instance, that p300-catalyzed histone crotonylation directly stimulates transcription to a greater degree than histone acetylation, and it is proposed that differential acetylation vs crotonylation couples cellular metabolism to gene regulation.[39] Appropriately designed assays will be required to determine the relevance of these functions to VPA-induced teratogenicity.

Figure 4 Transcription Factors as a Link Between Genetic Variation and Birth Defects

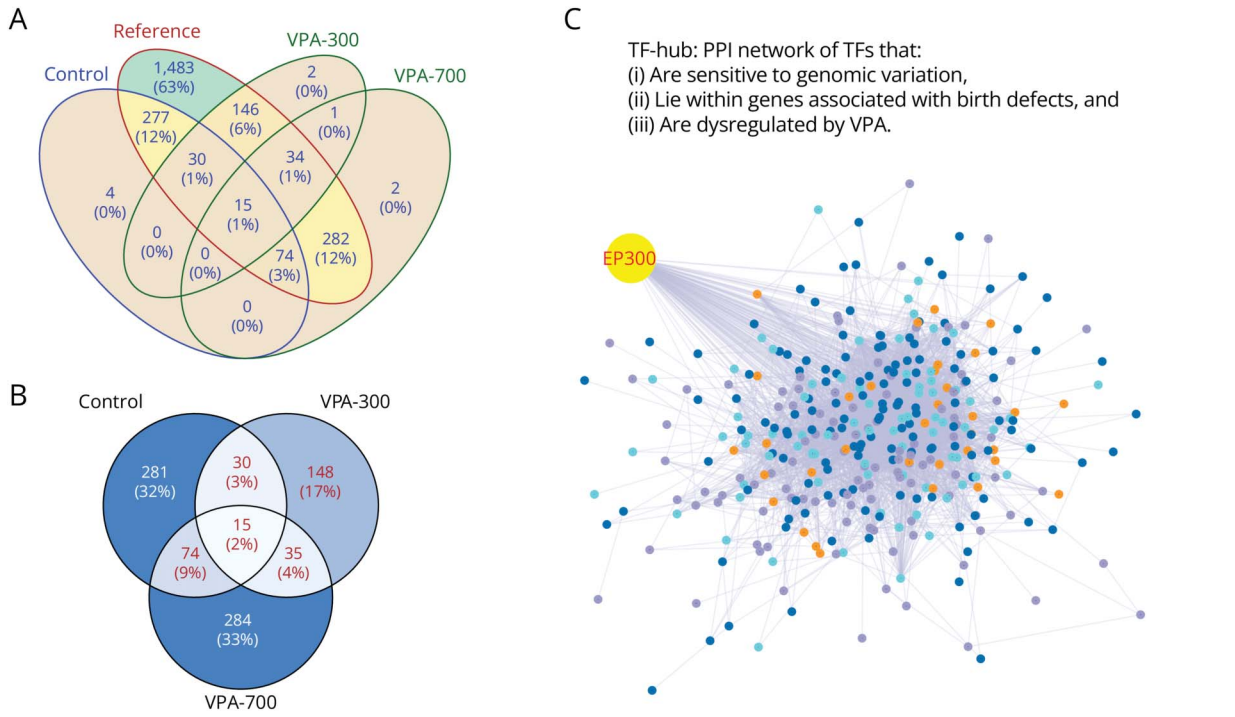


The ribbons in the Circos plot link 12 gene:variants (blue text) to TFs with tissue-specific effects (*GATA1-5*, *NR2F2*, *SIRT6*, and *TAL1*). The thickness of a connection is proportional to the score generated by DeepBind, which represents the difference in predicted binding affinity between wild-type and variant-containing alleles.

This study considered only structural birth defects; however, variant perturbation of the same or different TFs might also be an important modifier of the risk of neurodevelopmental disorders, which also occur more frequently in women who take VPA during pregnancy.[11,40-42] TF-driven regulatory networks that were enriched in rare and common genetic autism spectrum disorder risk variants have been identified in postmortem brains from individuals diagnosed with the condition.[43]

The *FOLH1* gene was included in the BD-hub because of its association with valproate-induced birth defects. A variant within *FOLH1*, chr11:49173538:T:C, with eQTL effects on *FOLH1* in tissues of the digestive tract and on pseudogenes expressed in brain tissues, met all high-confidence criteria. The variant is predicted to affect the binding activity of 15 TFs, including *EP300*, which might explain the eQTL effects. *FOLH1* encodes a transmembrane glycoprotein that acts as a glutamate carboxypeptidase on substrates including the nutrient folate. In a rat model of hypertension, Folh1 was expressed in the small intestine and animals with mutated Folh1 had reduced folate clearance and lowered plasma cysteine and homocysteine levels.[44] It is interesting to speculate that individuals who carry this variant may have a predisposition to folate deficiency, due to impaired folate processing, which is exacerbated by VPA treatment, or reduced capacity to benefit from folate supplementation. It is noteworthy that folate supplementation has not been found to protect against VPA-associated neural tube defects or other teratogenic birth defects in the same way it has for the general population.[45] One consideration is that the variant is silent under baseline conditions (within euchromatin) and the functional consequence is only realized after VPA-induced changes in chromatin accessibility, a scenario that would not apply to the general population.

Figure 5 Results From Coexpression Analyses



(A) Comparison between reference and hESC model: Venn diagram showing good overlap in correlated expression of *EP300* with other TFs between reference data and data obtained from our hESC exposure model. (B) Comparison between exposure groups: Venn diagram showing the extent of differences in correlated expression of *EP300* between unexposed and VPA-exposed cells. (C) Differentially expressed and coexpressed TFs in the TF-hub: overlay of results comparing unexposed and VPA-exposed cells at either dose found that 70% (251/359) of the TFs in the hub were either both differentially expressed and differentially coexpressed with *EP300* (n = 77, turquoise blue nodes), differentially coexpressed with *EP300* only (n = 34, orange nodes), or differentially expressed only (n = 140, dark blue nodes). hESC = human embryonic stem cell; TF = transcription factor; VPA = valproic acid.

The use of whole-exome, rather than whole-genome, sequence data is a limitation of this study. However, it has been shown that enhancers, with tissue-specific activity, are enriched in intronic gene regions[21] while genes that are ubiquitously expressed, such as housekeeping genes, are controlled by intergenic enhancers.[21] The relatively small sample size in this study, despite the largest published whole-exome genomic study of ASM teratogenicity, is also a limitation. We could not determine how specific our putative teratogenic mechanism is to VPA. It would be interesting to determine whether a drug's capacity to modify TF and chromatin dynamics is linearly associated with its known teratogenicity risk or whether there are TF drug–specific relationships that are sensitive to genetic variation. In addition, the sample size and grouping strategy we applied did not allow us to determine whether effects differed between polytherapy and monotherapy treatment regimes. In the VPA-exposed group, the proportion of mothers treated with monotherapy was equivalent to the proportion of affected offspring exposed to monotherapy, 59% (39/66) in the former and 60% (17/28) in the latter. Gene expression analyses comparing VPA and CBZ exposure found both common and drug unique regulation of genes (including those that encode TFs and their targets).[27] For this reason, we consider that there may be additive, antagonistic, or synergistic effects for polytherapy and that mechanistic studies using cell-based assays are required to understand this because these complex interactions may be obscured at the clinical phenotype level.

We acknowledge that the large number of TF binding sites implicated likely include spurious calls due to limitations of the DeepBind tool used to detect them. By coupling these predictions to more robust eQTL data, we helped to overcome this limitation. While we cannot yet conclude with certainty that VPA modifies the binding of TFs, or their *EP300*-dependent regulation during embryogenesis, our findings align with the broader literature and provide a proof-of-concept that this type of genetic variation could contribute to VPA teratogenicity and warrants further investigation.

In conclusion, the findings of this study advance the understanding of the genomic mechanisms underlying VPA-associated teratogenicity and bring us one step closer to the development of a biomarker test that can be used for preconception counseling. Further investigations with larger sample sizes and whole-genome sequencing coupled with chromatin assays, to determine regions of the DNA where accessibility to, and function of, TFs is modified by VPA, are required to make this a reality. This is clinically important given that concerns over teratogenicity have led to broad restrictions on the use of VPA, disadvantaging women who

might benefit therapeutically from it, particularly those with genetic generalized epilepsy and bipolar disorder.

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Author Contributions

A. Anderson: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. P. Perucca: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. E. Vianca: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. D. Sandvik: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. A. Antonic-Baker: study concept or design; analysis or interpretation of data. R. Krause: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. D. Jazayeri: major role in the acquisition of data. A. Hitchcock: major role in the acquisition of data. J. Graham: major role in the acquisition of data. M. Todaro: major role in the acquisition of data. T. Tomson: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. D. Battino: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. E. Perucca: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Martinez Ferri: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. A. Rochtus: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. L. Lagae: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M.P. Canevini: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. E. Zambrelli: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. E. Campbell: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. A. Rakitin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. B. Koeleman: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. I.E. Scheffer: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S.F. Berkovic: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. P. Kwan: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S.M. Sisodiya: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. J.J. Craig: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. F.J. Vajda: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. T.J. O'Brien: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data.

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Appendix Coinvestigators

Coinvestigators are listed at Neurology.org/N.

References

1. Marson A, Burnside G, Appleton R, et al. The SANAD II study of the effectiveness and cost-effectiveness of valproate versus levetiracetam for newly diagnosed generalised and unclassifiable epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial. *Lancet*. 2021;397(10282):1375-1386. doi:10.1016/S0140-6736(21)00246-4
2. Marson AG, Al-Kharusi AM, Alwaidh M, et al. The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet*. 2007;369(9566):1016-1026. doi:10.1016/S0140-6736(07)60461-9
3. Hirsch E, French J, Scheffer IE, et al. ILAE definition of the idiopathic generalized epilepsy syndromes: position statement by the ILAE task force on nosology and definitions. *Epilepsia*. 2022;63(6):1475-1499. doi:10.1111/epi.17236
4. Gotlib D, Ramaswamy R, Kurlander JE, DeRiggi A, Riba M. Valproic acid in women and girls of childbearing age. *Curr Psychiatry. Rep*. 2017;19(9):58-7. doi:10.1007/s11920-017-0809-3
5. Natale G, Fini E, Calabrò PF, Carli M, Scarselli M, Bocci G. Valproate and lithium: old drugs for new pharmacological approaches in brain tumors? *Cancer Lett*. 2023;560:216125. doi:10.1016/j.canlet.2023.216125
6. GOV.UK. *Valproate (Belvo, Convulex, Depakote, Dyzantil, Epilim, Epilim Chrono or Chronosphere, Episenta, Epival, and Syonell): New Safety and Educational Materials to Support Regulatory Measures in Men and Women Under 55 Years of Age*. Agency MaHpR; 2024.
7. Tomson T, Battino D, Bonizzoni E, et al. Dose-dependent teratogenicity of valproate in mono- and polytherapy an observational study. *Neurology*. 2015;85(10):866-872. doi:10.1212/WNL.0000000000001772
8. Vajda FJE, Hitchcock A, Graham J, et al. Foetal malformations and seizure control: 52 months data of the Australian Pregnancy Registry. *Eur J Neurol*. 2006;13(6):645-654. doi:10.1111/j.1468-1331.2006.01359.x
9. Cohen JM, Alvestad S, Cesta CE, et al. Comparative safety of antiseizure medication monotherapy for major malformations. *Ann Neurol*. 2023;93(3):551-562. doi:10.1002/ana.26561
10. Christensen J, Grønborg TK, Sørensen MJ, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA*. 2013;309(16):1696-1703. doi:10.1001/jama.2013.2270
11. Hernández-Díaz S, Straub L, Bateman BT, et al. Risk of autism after prenatal topiramate, valproate, or lamotrigine exposure. *N Engl J Med*. 2024;390(12):1069-1079. doi:10.1056/nejmoa2309359
12. Meador KJ, Baker GA, Browning N, et al. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. *N Engl J Med*. 2009;360(16):1597-1605. doi:10.1056/NEJMoa0803531
13. Meador KJ, Baker GA, Browning N, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. *Lancet Neurol*. 2013;12(3):244-252. doi:10.1016/S1474-4422(12)70323-X
14. Vajda FJE, O'Brien TJ, Lander CM, Graham J, Roten A, Eadie MJ. Teratogenesis in repeated pregnancies in antiepileptic drug-treated women. *Epilepsia*. 2013;54(1):181-186. doi:10.1111/j.1528-1167.2012.03625.x
15. Tomson T, Battino D, Bonizzoni E, et al. Dose-dependent risk of malformations with antiepileptic drugs: an analysis of data from the EURAP epilepsy and pregnancy registry. *Lancet Neurol*. 2011;10(7):609-617. doi:10.1016/S1474-4422(11)70107-7
16. Lundberg YW, Cabrera RM, Greer KA, Zhao J, Garg R, Finnell RH. Mapping a chromosomal locus for valproic acid-inducedexencephaly in mice. *Mamm Genome*. 2004;15(5):361-369. doi:10.1007/s00335-004-2345-9
17. Vajda F, O'Brien T, Graham J, et al. Specific fetal malformations following intrauterine exposure to antiseizure medication. *Epilepsy Behav*. 2023;142:109219. doi:10.1016/j.yebeh.2023.109219
18. Andrey G, Mundlos S. The three-dimensional genome: regulating gene expression during pluripotency and development. *Development*. 2017;144(20):3646-3658. doi:10.1242/dev.148304
19. Tseng C-C, Wong MC, Liao WT, et al. Genetic variants in transcription factor binding sites in humans: triggered by natural selection and triggers of diseases. *Int J Mol Sci*. 2021;22(8):4187. doi:10.3390/ijms22084187
20. Nishizaki SS, Ng N, Dong S, et al. Predicting the effects of SNPs on transcription factor binding affinity. *Bioinformatics*. 2020;36(2):364-372. doi:10.1093/bioinformatics/btz612
21. Borsari B, Villegas-Mirón P, Pérez-Lluch S, et al. Enhancers with tissue-specific activity are enriched in intronic regions. *Genome Res*. 2021;31(8):1325-1336. doi:10.1101/gr.270371.120
22. Guo Y, Long J, He J, et al. Exome sequencing generates high quality data in non-target regions. *BMC Genomics*. 2012;13(1):194-10. doi:10.1186/1471-2164-13-194
23. Vajda F, Lander C, O'brien T, et al. Australian pregnancy registry of women taking antiepileptic drugs. *Epilepsia*. 2004;45(11):1466. doi:10.1111/j.0013-9580.2004.451103.x
24. Perucca P, Anderson A, Jazayeri D, et al. Antiepileptic drug teratogenicity and de novo genetic variation load. *Ann Neurol*. 2020;87(6):897-906. doi:10.1002/ana.25724
25. Feleke R, Jazayeri D, Abouzeid M, et al. Integrative genomics reveals pathogenic mediator of valproate-induced neurodevelopmental disability. *Brain*. 2022;145(11):3832-3842. doi:10.1093/brain/awac296
26. Cui K, Wang Y, Zhu Y, et al. Neurodevelopmental impairment induced by prenatal valproic acid exposure shown with the human cortical organoid-on-a-chip model. *Microsyst Nanoeng*. 2020;6(1):49. doi:10.1038/s41378-020-0165-z
27. Schulpen SHW, Pennings JLA, Piersma AH. Gene expression regulation and pathway analysis after valproic acid and carbamazepine exposure in a human embryonic stem cell-based neurodevelopmental toxicity assay. *Toxicol Sci*. 2015;146(2):311-320. doi:10.1093/toxsci/kfv094
28. Cherianidou A, Seidel F, Kappenberg F, et al. Classification of developmental toxicants in a human iPSC transcriptomics-based test. *Chem Res Toxicol*. 2022;35(5):760-773. doi:10.1021/acs.chemrestox.1c00392
29. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009;27(3):275-280. doi:10.1038/nbt.1529
30. Asan n, Xu Y, Jiang H, et al. Comprehensive comparison of three commercial human whole-exome capture platforms. *Genome Biol*. 2011;12(9):R95-12. doi:10.1186/gb-2011-12-9-r95
31. Georgala PA, Carr CB, Price DJ. The role of Pax6 in forebrain development. *Dev Neurobiol*. 2011;71(8):690-709. doi:10.1002/dneu.20895
32. Molloy AM, Pangilinan F, Brody LC. Genetic risk factors for folate-responsive neural tube defects. *Annu Rev Nutr*. 2017;37:269-291. doi:10.1146/annurev-nutr-071714-034235
33. Tremblay M, Sanchez-Ferras O, Bouchard M. GATA transcription factors in development and disease. *Development*. 2018;145(20):dev164384. doi:10.1242/dev.164384

34. Yao T-P, Oh SP, Fuchs M, et al. Gene dosage–dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell*. 1998;93(3):361-372. doi:10.1016/s0092-8674(00)81165-4
35. Shafique S, Winn LM. Role of Cbp, p300 and Akt in valproic acid induced neural tube defects in CD-1 mouse embryos. *Reprod Toxicol*. 2020;95:86-94. doi:10.1016/j.reprotox.2020.05.008
36. Lamparter CL, Winn LM. Valproic acid exposure decreases Cbp/p300 protein expression and histone acetyltransferase activity in P19 cells. *Toxicol Appl Pharmacol*. 2016;306:69-78. doi:10.1016/j.taap.2016.07.001
37. Shahib AK, Rastegar M, van Wijnen AJ, Davie JR. Neurodevelopmental functions and activities of the KAT3 class of lysine acetyltransferases. *Biochem Cell Biol*. 2024;102(6):430-447. doi:10.1139/bcb-2024-0156
38. Thomas SP, Denu JM. Short-chain fatty acids activate acetyltransferase p300. *Elife*. 2021;10:e72171. doi:10.7554/eLife.72171
39. Sabari BR, Tang Z, Huang H, et al. Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Mol Cell*. 2015;58(2):203-215. doi:10.1016/j.molcel.2015.02.029
40. Honybun E, Cockle E, Malpas CB, et al. Neurodevelopmental and functional outcomes following in utero exposure to antiseizure medication: a systematic review. *Neurology*. 2024;102(8):e209175. doi:10.1212/WNL.0000000000209175
41. Meador K, Reynolds MW, Crean S, Fahrbach K, Probst C. Pregnancy outcomes in women with epilepsy: a systematic review and meta-analysis of published pregnancy registries and cohorts. *Epilepsy Res*. 2008;81(1):1-13. doi:10.1016/j.eplepsyres.2008.04.022
42. Wood AG, Nadebaum C, Anderson V, et al. Prospective assessment of autism traits in children exposed to antiepileptic drugs during pregnancy. *Epilepsia*. 2015;56(7):1047-1055. doi:10.1111/epi.13007
43. Wamsley B, Bicks L, Cheng Y, et al. Molecular cascades and cell type–specific signatures in ASD revealed by single-cell genomics. *Science*. 2024;384(6698):eadh2602. doi:10.1126/science.adh2602
44. Šilhavý J, Krijt J, Sokolová J, et al. Dissecting the role of Folr1 and Folh1 genes in the pathogenesis of metabolic syndrome in spontaneously hypertensive rats. *Physiol Res*. 2018;67(4):657-662. doi:10.33549/physiolres.933932
45. Vajda FJ, O'Brien TJ, Graham JE, et al. Folic acid dose, valproate, and fetal malformations. *Epilepsy Behav*. 2021;114(Pt A):107569. doi:10.1016/j.yebeh.2020.107569