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Exome Sequencing in Patients With Antiepileptic Drug Exposure And Complex Phenotypes

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ABSTRACT

Introduction Fetal anticonvulsant syndrome (FACS) describes the pattern of physical and developmental problems seen in those children exposed to certain antiepileptic drugs (AEDs) in utero. The diagnosis of FACS is a clinical one and so excluding alternative diagnoses such as genetic disorders is essential.

Methods We reviewed the pathogenicity of reported variants identified on exome sequencing in the Deciphering Developmental Disorders (DDD) Study in 42 children exposed to AEDs in utero, but where a diagnosis other than FACS was suspected. In addition, we analysed chromosome microarray data from 10 patients with FACS seen in a Regional Genetics Service.

Results Seven children (17%) from the DDD Study had a copy number variant or pathogenic variant in a developmental disorder gene which was considered to explain or partially explain their phenotype. Across the AED exposure types, variants were found in 2/15 (13%) valproate exposed cases and 3/14 (21%) carbamazepine exposed cases. No pathogenic copy number variants were identified in our local sample (n=10).

Conclusions This study is the first of its kind to analyse the exomes of children with developmental disorders who were exposed to AEDs in utero. Though we acknowledge that the results are subject to bias, a significant number of children were identified with alternate diagnoses which had an impact on counselling and management. We suggest that consideration is given to performing whole exome sequencing as part of the diagnostic work-up for children exposed to AEDs in utero.

What is already known on this topic?

► Children exposed to certain antiepileptic drugs (AEDs) in utero can have physical and cognitive sequelae.
► Not all children exposed experience problems and there is a likely genetic predisposition.
► Fetal anticonvulsant syndrome (FACS) is a diagnosis of exclusion and current guidance is to perform chromosomal analysis as a baseline; however, genetic technologies have significantly advanced since these guidelines.

What this study adds?

► FACS remains a diagnosis of exclusion, and higher resolution analysis of patients' genomes may be warranted to better understand FACS susceptibility and exclude other primary genetic causes.
► Whole exome sequencing, while not being able to exclude a genetic disorder completely in an individual has facilitated the identification of pathogenic variants in several children exposed to AEDs in utero, which may contribute to their phenotype.
► The chance of identifying an underlying genetic condition is likely to be higher when children have features which are more severe than or atypical for FACS. Some of the genetic variants identified in patients diagnosed with FACS have counselling and clinical management implications, highlighting the importance of their identification.

BACKGROUND

The term fetal anticonvulsant syndrome (FACS) has been coined to describe children who have been exposed to antiepileptic drugs (AEDs) in utero and have a characteristic pattern of physical and cognitive difficulties.[1] Prior to women with epilepsy becoming pregnant, it is essential to optimise AED therapy to achieve a low risk of fetal harm by avoiding AED polytherapy, achieving seizure control at the lowest possible dose and avoiding drugs which are highly teratogenic.

Certain AEDs have been demonstrated to be associated with elevated rates of major congenital malformations. Current evidence suggests significantly increased risks of major congenital malformations for valproate (VPA), carbamazepine (CBZ), phenytoin (PHT), and topiramate. Adverse outcomes associated with human teratogens are typically wider than major structural malformations and often include functional deficits such as reduced intellectual functioning, altered growth parameters and more subtle physical malformations, occurring in a recognisable and consistent pattern.[2] To date, three anticonvulsant syndromes have been reported in the literature: fetal hydantoin syndrome,[3] fetal valproate syndrome[4] and fetal carbamazepine syndrome.[5] All three have distinct presentations and include major organ malformations, more minor or functional physical difficulties and neurodevelopmental deficits.

The diagnosis of FACS, which is a diagnosis of exclusion, depends on the ruling out of other

possible environmental and/or genetic causes of the presenting symptoms. In the past, the diagnosis has been accepted if there are normal cytogenetic studies. However, even chromosomal microarray (CMA) with a resolution of around 180 kb[6] will not detect single gene disorders which occur with significant frequency in individuals presenting with structural and developmental abnormalities and could be enriched in families where epilepsy is a feature. Whole exome sequencing (WES) is a more detailed genetic test than CMA and involves sequencing the entire genetic code, base-by-base, for the majority of protein-coding regions of the human genome (coverage depending on platform). The use of such genetic tests is likely to enhance the accuracy of the diagnosis of FACS by identifying additional or alternative diagnoses.

To date, there has been no systematic review of the comorbid presence of genetic variants in children exposed to AEDs. This information may therefore be useful to outline its role in facilitating diagnosis and optimal clinical management and it has implications, also, for studies which aim to determine teratogenic status following in utero exposure to medications.

The Deciphering Developmental Disorders (DDD) Study used WES in over 13 000 children, of whom ~8000 also had exon resolution CMA, with a range of developmental disorders which were unexplained after clinical evaluation and baseline genetic tests including CMA in the majority of cases. Copy number variants (CNVs) were sought by analysis of the exome sequence in those not undergoing separate CMA analysis. After 1133 cases had been analysed, the study had identified a causative variant in 40% of cases.[7] As a significant number of patients recruited to the study had been exposed to AEDs in utero, this data set presented an opportunity through which the comorbid presence of effects of AED exposure and presence of potential pathogenic variants could be investigated.

METHODS

The study participants were a retrospective cohort of patients with developmental disorders exposed to AEDs in utero who have undergone WES as part of the DDD Study. A detailed overview of the DDD Study has been described previously,[8] but in brief, clinicians from UK genetic centres were invited to recruit patients with significant structural malformations and/or intellectual disability who remained undiagnosed after routine paediatric and genetic assessment to the study. Saliva samples were collected from the child and both parents and a stored DNA sample from the child and a trio of exomes sequenced. Patients were phenotyped by a Consultant Clinical Geneticist using Human Phenotype Ontology terms and comprehensive data were supplied for each patient, including details of medical history, maternal illness, and drug exposure during pregnancy and the main clinical features observed along with growth parameters and developmental milestones. Bioinformatic algorithms were used to identify variants within all genes known to cause developmental disorders at the time of the analysis. Re-analysis is undertaken periodically throughout the study as new bioinformatic algorithms and information about newly discovered genes became available. Variants considered to have contributed to the phenotype were fed back to the recruiting clinical geneticist and where appropriate were discussed with patients and validated in local NHS diagnostic laboratories. Clinicians from the regional centres were invited to study subsets of the data as Complementary Analysis Projects. The DDD Study group in Cambridge approved a Complementary Analysis Study Request for this study in February 2016.
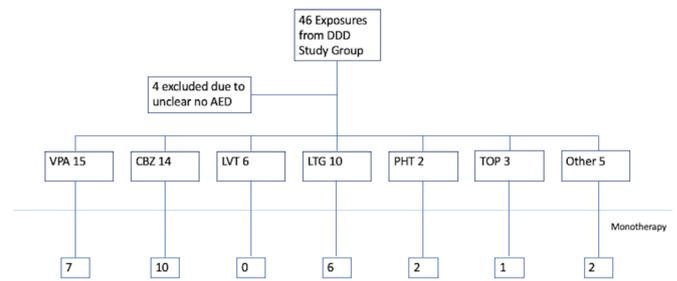

Figure 1 Breakdown of AED exposures returned from the DDD Study Group. Other 5 are diazepam, ethosuximide, clonazepam, clobazam and trihexyphenidyl. AED, antiepileptic drug; CBZ, carbamazepine; DDD, Deciphering Developmental Disorders; LTG, lamotrigine; LVT, levetiracetam; PHT, phenytoin; TOP, topiramate; VPA, valproate.

A list of variants for the 42 patients exposed to AEDs in utero was provided by the DDD Study Group. These were compared with the patient's available phenotypic data to assess whether they explained the patient's phenotype. In addition, we performed further analysis of these variants by searching online databases, interrogating evolutionary conservation and performing in silico analyses. All variants were reported and analysed on the GRCh37/hg19 genome build. These methods were used to give a pathogenicity score to each variant according to the American College of Medical Genetics (ACMG) guidelines.[9] Furthermore, referring clinicians were approached for patients identified to have pathogenic variants.

In addition to the DDD cohort, and as a source of additional genetic data, parents of a series of patients with FACS who had been referred to our Regional Genetics Centre were sent information about this study and invited to participate by allowing access to their previously obtained CMA data. These results were reviewed for any pathogenic changes. The OGT ISCA v2 8×60k microarray was used in all cases.

RESULTS

The DDD Study Group had completed WES on 46 eligible children–parent trios by the data freeze of 4293 trios on 5 July 2016 when the application for data was accepted. Figure 1 shows the breakdown of exposures. After exclusion of 4 trios due to no clear documentation of AED in utero exposure, 42 trios remained for analysis. This included 26 males and 16 females with a mean age at enrolment into DDD of 6 years and 8 months.

In parallel, 20 patients with a clinical diagnosis of FVS were identified from our Regional Genetics Centre and were invited to participate in the study. A positive response was received in 10 cases (50%). These 10 cases included 4 males and 6 female cases, all of which had CMA analysis but not WES.

COPY NUMBER VARIANTS

There was one patient with a reported CNV (DECIPHER ID 263311) from the DDD cohort, which was felt to be contributory to the child's phenotype. This was a 7 Mb gain at the 1q21.1 locus (chr1:142540048–149765695)×3 encompassing the recurrent 1q21.1 microdeletion region and including at least 128 genes. This CNV was maternally inherited although little phenotypic data were available for the mother. The mother in this case was taking 1200 mg VPA and 400 mg CBZ for post-traumatic seizures suggesting she was not affected by a genetic seizure disorder. The child's features included undescended testes, hypospadias repair, overlapping toes, thin upper lip and downturned mouth. He did not have a typical facial phenotype for fetal valproate syndrome.

The 1q21.1 recurrent microduplication syndrome (Online Inheritance in Man (OMIM) 612475) is well described in the literature and predisposes to learning disability, Autistic Spectrum Disorder (ASD), macrocephaly, hypospadias, hypertelorism and schizophrenia but is rarely associated with seizures.[10] The patient had global developmental delay, ventriculomegaly, plagiocephaly, long palpebral fissures, hypospadias and thoracic scoliosis. The opinion from the referring clinician was that this child's developmental delay was severe and a further explanation was sought from the 100,000 Genomes Project, although both the 1q21.1 microduplication and VPA exposure were thought to be contributory.

Analysis of the microarray data of 10 patients (online supplementary table 1) with a FACS diagnosis known to our centre did not find any known pathogenic variants. All patients were exposed to VPA with seven exposures in monotherapy and three polytherapy (VPA+levetiracetam (LVT), VPA+vigabatrin and VPA+lamotrigine). There was one patient exposed to both VPA and LVT who had a 327kb gain at 15q21.1 which does not contain any genes and is of uncertain significance (chr15: 46,334,812–46,661,995)x3. This patient had moderate to severe learning difficulties, ventricular septal defect, hypermobility, left hip dysplasia, mild low frequency hearing loss and repetitive autistic traits, which could all be attributed to VPA exposure and the phenotype was therefore considered to be consistent with the original fetal valproate syndrome diagnosis.

Single nucleotide variants (SNVs)

There were 19/42 (45%) patients with reported SNVs from the DDD Study WES data. Six of these variants occurring in the 42 patients (14%) were classified as pathogenic or likely pathogenic as per ACMG guidelines and thought to explain the participant's phenotype either in full or partially, alongside the AED exposure (table 1). Across the AED exposure types, variants were found in 2/15 (13%) VPA exposure cases and 3/14 (21%) CBZ exposed cases. Two further variants were initially thought to be pathogenic on bioinformatic grounds however did not appear to be contributory to the patients' phenotypes.

CLINICAL EVALUATION OF IDENTIFIED VARIANTS

A summary of the patients in whom variants were identified, their clinical and genetic findings and the details of their AED medication are summarised in table 1. Further details of ACMG pathogenicity scores can be found in online supplementary table 2.

Patient 1 NF1

This child had clinical appearances consistent with a diagnosis of Soto's syndrome, although NSD1 testing was negative and bone age was normal. His occipitofrontal circumference (OFC) was on the 97th centile and he had a long chin with a thin upper lip. He did not have any other features suggestive of FACS and consensus opinion was a Sotos-like phenotype. He, and his mother, had a few faint cafe-au-lait spots but would not fulfil criteria for neurofibromatosis. This variant was considered contributory to phenotype.

This variant was considered contributory, but the phenotype was less severe than expected. Neither of these siblings had typical FVS facial features.

Patient 4 NEXMIF

This patient had previously been investigated for Angelman syndrome due to severe developmental delay, hand flapping and repetitive mannerisms. The phenotype was felt to be consistent with other patients previously described to have NEXMIF variants. He had a tented upper lip and short upturned nose but no facial features of FACS. He had autistic features and an alternative diagnosis was considered early on. His behavioural phenotype was thought to be in keeping with others with NEXMIF variants; however, a contribution of VPA to his learning difficulties cannot be excluded.

Patient 5 DCX

It was very difficult to separate the effects of prenatal CBZ exposure from the effects of the DCX variant. The grandmother of the index case had the DCX variant but did not have epilepsy. Three of her daughters inherited the DCX variant, had epilepsy and a variable degree of developmental disorder, and were on CBZ during their pregnancies. One of her daughters did not inherit the DCX variant and had normal development and no epilepsy. Of the three children in the third generation, who had prenatal exposure to CBZ, all have speech delay, two have inherited the DCX variant and also have epilepsy, one did not and has no epilepsy (online supplementary figure). This DCX variant has previously been reported as likely pathogenic (rs587783577) causing white matter heterotopia. There was also a maternally inherited variant of unknown significance (VUS) in SRCAP (ENST00000262518.4:c.3212T>C (p.Leu1071Pro)) in the index patient. Pathogenic variants of this gene cause Floating-Harbor syndrome (FHS) (OMIM 136140). A blended phenotype is possible, but neither the patient, nor her mother, have short stature, a key feature of FHS.

Patient 6 EHMT1

This patient was felt to have a phenotype entirely consistent with the *EHMT1* variant identified. She did not have typical features of FACS and an alternative diagnosis was considered early on. In this case, CBZ was taken for the management of bipolar affective disorder. Interestingly, Kleefstra syndrome has recently been reported as a phenocopy in a patient presumed to have fetal valproate syndrome highlighting that FACS should remain a diagnosis of exclusion.[11]

Variants not contributory to phenotype
Patient 7 KIF5C

A 41bp deletion in KIF5C (ENST00000435030.1:c.2679_2719 del) identified by WES causes frameshift and hence was thought likely to be pathogenic however did not correlate with clinical findings. This patient was known to have a maternally inherited 16p12.2 deletion, which was pathogenic. This microdeletion syndrome is known to cause developmental delay, cognitive impairment and seizures, although a variable phenotype.[12] He also has a paternally inherited 20q13.33 duplication of uncertain significance. Both of these CNVs were identified by CMA prior to referral to DDD Study as neither variant was thought completely explanatory to the phenotype. WES also found a VUS in KMT2D (ENST00000301067.7:c.5980G>A (p.Asp1994Asn)), a gene associated with Kabuki syndrome. He did not have typical features of either of these conditions so the SNVs in KIF5C and KMT2D were felt unlikely to be clinically significant.

Patient 8 FOXP1

This patient's facial and behavioural phenotype was not typical for FACS. The FOXP1 variant (ENST00000491238.1:c.1372C>T (p.Gln458Ter)), although a nonsense variant, was felt not adequate to explain entire the phenotype and FACS was felt

Table 1 Pathogenic variants in patients with DDD exposed to AEDs in utero

Patient No	Exposure DECIPHER ID	Variant	Inheritance	Patient phenotype	OMIM phenotype	ACMG classification for pathogenicity
1	VPA male 263768	NF1 ENST00000358273.4:c.5488C>T (p.Arg1830Cys)	Mat.	Pectus excavatum, GDD, dysmorphism, speech articulation difficulties, abnormal aggressive, impulsive or violent behaviour, poor motor coordination	Neurofibromatosis-Noonan syndrome (601321)	Likely pathogenic
2 and 3	VPA, LVT male, siblings x2 270782 270783	EEF1A2 ENST00000217182.3:c.505G>A (p.Glu169Lys)	Mat.	Cognitive impairment, generalised seizures, IUGR	Epileptic encephalopathy (616409)	Likely pathogenic
4	CBZ male 260205	NEWXMIF ENST00000055682.6:c.2096C>A (p.Ser699Ter)	Mat.	Intellectual disability, ataxia, wide mouth	X-linked mental retardation (300912)	Pathogenic
5	CBZ female 269952	DCX ENST00000338081.3:c.910G>A (p.Gly304Arg)	Mat.	GDD, joint hypermobility, high palate, delayed speech and language development, abnormal aggressive impulsive or violent behaviour, white matter neuronal heterotopia	X-linked lissencephaly (300067)	Likely pathogenic
6	CBZ female 270822	EHMT1 ENST00000460843.1:c.3310G>A (p.Glu1104Lys)	De novo	GDD, periventricular leucomalacia, seizures, thoracolumbar scoliosis, dysmorphism	Kleefstra syndrome (610253)	Likely pathogenic
7	VPA, CBZ male 263311	(Chr1: 142540048–149765695)x3	Mat.	GDD, ventriculomegaly, plagiocephaly, long palpebral fissures, hypospadias, thoracic scoliosis	1q21.1 duplication syndrome 612475	Documented as clinically significant in multiple peer-reviewed publications*

*CNV unable to classify with ACMG criteria for sequence variants.

ACMG, American College of Medical Genetics; AEDs, antiepileptic drugs; CBZ, carbamazepine; CNV, copy number variant; DDD, Deciphering Developmental Disorders; GDD, global developmental delay; IUGR, intrauterine growth restriction; LTG, lamotrigine; LVT, levetiracetam; mat., maternal; PHT, phenytoin; VPA, valproate.

to possibly have contributed. Variants in this gene cause intellectual disability with language impairment (OMIM 613670); however, she did not completely demonstrate this phenotype. She had occasional bouts of aggressive behaviour and dysmorphic features not consistent with FACS nor any obvious alternative diagnosis.

DISCUSSION

This study is the first to investigate potential underlying genetic diagnoses using CMA or WES to identify a potential explanatory, or contributory causes, for the clinical phenotypes in children exposed to AEDs in utero who have developmental disorders. Clinically relevant SNVs (and one CNV) were identified in 17% (7/42) of individuals.

This high yield might be expected, given that these individuals were recruited into the DDD Study because clinicians felt that their symptoms remained unexplained by the diagnosis of FACS alone. However, this study demonstrates well that children exposed to AEDs in utero can, in some cases, have underlying genetic causes for their symptoms and therefore the prenatal AED exposure may be of no or of limited relevance. These results also emphasise that FACS should be a diagnosis of exclusion following clinical assessment and appropriate investigation, which should include WES where available.

The clinical diagnosis of children with developmental disorders is often difficult and the added complication of AED exposure in utero may make the task even more challenging. The diagnostic guidelines for FACS were published by Dean et al[1] and have yet to be updated in the light of ever more sensitive newer genetic investigations (such as CMA and WES). These criteria stress that FACS is a diagnosis of exclusion citing normal karyotype and 22q11 studies as a prerequisite to diagnosis. It is reasonable to suggest, based on our findings here that those children diagnosed with FACS some time ago based on the Dean criteria could potentially be found to have an alternate genetic diagnosis if further investigation was undertaken with CMA or WES. Consistent with our work published here, in 2012, Douzgou et al[13] reported on 80 children referred for a possible diagnosis of fetal alcohol syndrome. Using a combination of fluorescence in situ hybridisation and CMA, 9% of these children were found to have an alternative genetic diagnosis.

As well as the implications for clinical diagnosis of the FACS, these results also have implications for research aimed at delineating the risks associated with in utero exposure to medications. This has comprised case reports and series but also cohort studies or population studies. While the inclusion of a control group from the same source and excluding children with a known genetic diagnosis may assist in ensuring the results of such studies are not biased by the inclusion of children with physical and neurodevelopmental difficulties which are in fact not linked to the teratogenic exposure, the data presented here suggest that genetic testing should also be considered in future studies if completely reliable risk estimates are to be generated.

The current study is limited by a small sample size and almost certain recruitment bias as patients will probably have been entered into DDD Study due to clinical suspicion that their phenotype was not complete in keeping with FACS. Many less complex or more mildly affected FACS patients would not have been entered into study as clinicians would be confident with the FACS diagnosis. This most likely explains why some patients with DDD had complex phenotypes which did not specifically fit with current knowledge of FACS. The additional cases from the Regional Genetics Centre had more extensive phenotypic data available and were not selected on the basis of an unclear diagnosis, but rather were sequential patients attending a genetic clinic, however, WES data were not available for them.

Reverse phenotyping has been established as a useful tool in variant calling from exome data and describes clinical evaluation of a patient once again after the variant has been found.[14] We feel this technique may be useful in rare disorders such as FACS although we must consider that a 'negative' exome does not completely rule out a contributing genetic cause in our patients. Further study using genome sequencing or epigenetic techniques may play a role in elucidating the complex genetics behind a FACS diagnosis.

CONCLUSION

This study is the first of its kind to analyse the exomes of a series of individuals with developmental disorders who were exposed to AEDs in utero. Using WES in those children with severe phenotypes or who were exposed to relatively low doses of AED (below 800 mg daily of VPA for instance) would be useful in identifying alternative diagnoses. This will also be important in establishing if there are any FACS 'genocopies' suggesting that a child may have a purely genetic disorder which mimics FACS. This would have both significant implications for genetic counselling in future pregnancies and potentially for the processes currently used to determine teratogenic status of medications.

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