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Exposure to sodium valproate during pregnancy: Facial features and signs of autism

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Abstract

BACKGROUND—Valproic acid (VPA) is the most teratogenic anticonvulsant drug in clinical use today. Children exposed prenatally to VPA have previously been shown to have dysmorphic craniofacial features, identified subjectively but not by anthropometric methods. Exposure to VPA has also been associated with an increased frequency of Autism Spectrum Disorder (ASD). An increased cephalic index (the ratio of the cranial lateral width to the cranial anterior-posterior length) has been observed in children with ASD.

METHODS—Forty-seven children exposed to VPA during the first trimester of pregnancy were evaluated for dysmorphic facial features, identified subjectively and by measurements. Each VPA-exposed child was evaluated for ASD using the SCQ (Social Communication Questionnaire), ADI-R (Autism Diagnostic Interview-Revised) and ADOS (Autism Diagnostic Observation Schedule). The same physical examination was carried out on an unexposed comparison group of 126 children. The unexposed children also had testing for cognitive performance by WISC-III (the Wechsler Intelligence Scale for Children).

RESULTS—Several dysmorphic craniofacial features, including telecanthus, wide philtrum and increased length of the upper lip were identified subjectively. Anthropometric measurements

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documented additional findings including an increased cephalic index, decreased head circumference/height index, and increased intercanthal distance. There were no differences between the craniofacial features of VPA-exposed children with and without ASD.

CONCLUSIONS—An increased frequency of dysmorphic craniofacial features was identified in children exposed to VPA during the first trimester of pregnancy. The most consistent finding was a larger cephalic index, which indicates a disproportion of increased width of the skull relative to the shortened anterior-posterior length.

Keywords

valproic acid; “valproate embryopathy”; cephalic index; Autism Spectrum Disorder

INTRODUCTION

The teratogenicity of valproic acid (VPA), when used by pregnant women as an anticonvulsant drug, was first established in a population-based malformations surveillance program in northeastern France in 1982 (Robert and Guiband, 1982). The investigators reported a 20-fold increase in the frequency of myelomeningocele in infants exposed to VPA in the first trimester of pregnancy. Subsequent case series showed that VPA-exposed infants demonstrated an increased frequency of several other malformations, including heart defects, oral clefts, hypospadias and craniosynostosis (Ardinger et al, 1988; Jentink et al, 2010; Mawhinney et al, 2012). Systematic studies, which included an unexposed comparison group, have shown that the rates of malformations were 6.7% and 9.3% in infants exposed in utero to valproate compared to 2.5% and 1.1% respectively in unexposed infants (Diav-Citrin et al, 2008; Hernandez-Diaz et al, 2012).

Dysmorphic facial features have also been described in many children who had been exposed to valproate during pregnancy (DiLiberti et al, 1984; Jäger-Roman et al, 1986; Ardinger et al, 1988; Kini et al, 2006). The dysmorphic facial features described have been: epicanthic folds, broad nasal bridge, small nose with anteverted nostrils, long and flat philtrum, and thin vermilion border of the upper lip. These descriptions have been based on subjective judgments by the examining physicians. More objective measurements of facial features, using calipers and rulers (Holmes et al, 2001) and laser light scans (Orup et al, 2014), have been used in the evaluation of children exposed to other anticonvulsant drugs during pregnancy, such as phenytoin and phenobarbital, but not, to our knowledge, in the evaluation of children exposed to VPA during pregnancy.

With any craniofacial measurements, there can be variation in the precision of the measurements. For example, L.G. Farkas used very precise instruments and techniques by direct anthropometry in which measurements are taken using anthropometric precise metallic and plexiglass instruments (Farkas and Munro, 1987; Farkas, 1994; Farkas and Deutsch, 1996). Farkas used these precise techniques to develop his normative craniofacial data. Clinical studies of other non-VPA anti-epileptics typically have used less precise plastic rulers and calipers in the examination of infants (Holmes et al, 2001).

Children exposed during pregnancy to VPA have also been shown to have an increased frequency of developmental delay, deficits in IQ (Meador et al, 2013), autism (Rasalam et al, 2005; Christensen et al, 2013), microcephaly and postnatal growth deficiency (Ardinger et al, 1988). The presence of dysmorphic craniofacial features, such as a short nose and anteverted nostrils, in children exposed to the anticonvulsant drugs phenytoin, phenobarbital, carbamazepine and VPA have been postulated to be associated with an increased risk for cognitive dysfunction (Ornoy et al, 1996; Mawer et al, 2002; Holmes et al, 2005).

Children with autism spectrum disorder have been shown to have physical abnormalities: a large head circumference in some, but not all, affected children (Courchesne et al, 2003; Lainhart et al, 2006; Wallace and Treffert, 2004), an abnormal cephalic index, defined as the ratio of cranial lateral width to cranial anterior-posterior length (Deutsch et al, 2013) and posteriorly rotated ears (Rodier et al, 1997). Courchesne et al (2003), in a study of 48 children with ASD, reported a striking sequence of head size changes: normal at birth, an increase in head size by 2 to 5 years, and normal head size by adolescence.

We present here our findings in children who had been exposed to VPA during pregnancy, including several with autism. We evaluated whether or not they had dysmorphic facial features, identified both by subjective judgments and by measurements, and whether these features were more common in the VPA-exposed children with autism than in the VPA-exposed children without autism.

METHODS

Women who had taken VPA during pregnancy were recruited from several sources, including neurologists, the general pediatrics clinics at the MassGeneral Hospital *for* Children, the newsletter of the North American AED (antiepileptic drug) Pregnancy Registry, autism organizations and online listings of on-going research studies at the MassGeneral Hospital *for* Children. A special effort was made to recruit children who had been exposed during pregnancy to VPA who were considered to be autistic. Interested mothers called the Research Study Coordinator, who provided a description of the study and its goals. If the mother and her child were considered eligible, the mother was sent the informed consent document and the medical release form for her to sign and return by mail. Medical records were obtained from each mother's physician who had prescribed her medication during pregnancy and from the physicians who had evaluated her VPA-exposed child. The informed consent document was reviewed and approved annually by the Human Studies Committee of Partners Healthcare.

A pregnancy was considered exposed to VPA if the treatment occurred throughout the first trimester based on information recorded in the mother and/or her child's medical records. If these medical records were not available, the exposure was classified based on the mother's self-report. If the mother reported use of VPA throughout the first trimester, but her medical record stated otherwise, that child was excluded as unexposed. Children born to mothers taking another medication with known teratogenic effects in addition to VPA were excluded. VPA could have been taken for any medical condition; in this study, VPA was most often taken to prevent seizures.

There were 47 children in the final exposed group and 126 children in the final unexposed comparison group. A sensitivity power analysis was performed using GPower 3.1.5 (Faul, 2007) to determine the minimal detectable effect (for differences in measurement z-scores between the exposed and unexposed groups) which can be supported with inferential statistics with the current sample. With $\alpha = 0.05$, power = 0.80, 47 exposed, and 126 unexposed, an effect of 0.48 or greater is required to detect a difference between groups, which is considered to be a medium effect using Cohen's criteria (Cohen, 1988). There have been no pilot studies comparing the measurements of such craniofacial features between a group exposed to VPA and a control group. Similar studies do exist for other teratogenic exposures, though, such as fetal alcohol syndrome. For example, one study of multiple craniofacial measurements in children with and without fetal alcohol syndrome found effects for their significant midface findings ranging from 0.44 to 0.81 (Naidoo et al, 2006). With a minimal detectable effect of 0.48, it is expected that similarly subtle craniofacial changes would be detectable.

After the medical records were reviewed, the mother was asked, by telephone, the series of questions comprising the Social Communication Questionnaire (SCQ) (Rutter, Bailey and Lord, 2003) if the child was 4 years old or older. For the two children less than 4, the modified checklist for autism (M-CHAT) was used. These two screening tools for signs of the autism spectrum disorder (ASD) are based on an interview with child's caregiver. Each child who scored above the screening cut-off on either the SCQ or the M-CHAT was tested by experienced research examiners who were qualified as research reliable/validated for administering to each child the Autism Diagnostic Interview-Revised (ADI-R) (Rutter, Le Couteur & Lord, 2003) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al, 2012). In addition, each child tested with the ADI-R and ADOS was evaluated by a child neurologist (M.L.B.) who has specialized in the evaluation of children considered to have autism. No systematic testing of cognitive function was carried out.

The physical examination by a dysmorphologist (L.B.H.) used a protocol to maintain consistency in defining the more qualitative or subjectively defined physical features looked for and in the measurements made. For the qualitative features, the examiner noted that a feature was present, absent, or unable to be examined. For the measurements, the units of measure were listed in millimeters or centimeters. Definitions of the physical features looked for and the measurements made have been published (Holmes, 2012). The tools used for measurements were a plastic ruler, a tape measure subdivided by millimeters, and calipers (Manostat) to measure the width of the philtrum and mouth, interpupillary distance, and intercanthal distance. The anterior-posterior skull length and bitemporal distance were measured using spreading calipers (GPM, Swiss Made). The examiner knew that each child being examined had been exposed during pregnancy to VPA, as all of the exposed children were examined in the same part of the study. The examiner did not know the exposure status of any of the children in the control group, as those examinations occurred in the context of a different study investigating the craniofacial features associated with various non-VPA antiepileptics. Nose length and mouth width measurements for one subject and finger measurements for another were omitted because the examiner felt that these children were not able to cooperate well enough to obtain accurate measurements of these features.

The presence of a malformation was based on the medical history provided by the mother and the child's medical records. The definition used for a malformation was a structural abnormality with surgical, medical or cosmetic importance. The criteria for inclusion and exclusion of any physical feature as a malformation have been described previously (Holmes and Westgate, 2011). Examples of physical features excluded were minor anomalies, such as short nose and thin vermillion, birth marks, and small atrial septal defects less than 3 millimeters in diameter.

The decision to obtain photographs was made after the study was underway. Photographs were taken of 40 children. The views recorded were a frontal and lateral view of the face, the dorsal and ventral aspects of each hand and the dorsum of each foot. These photographs were obtained to provide documentation of physical features for further reference.

The comparison population

The unexposed comparison population was composed of 126 children, ranging from 6 to 16 years old, who had been enrolled as the comparison group in a separate study of the teratogenicity of three drugs taken as monotherapy: phenytoin, phenobarbital and carbamazepine (Janulewicz et al, 2005). While this comparison group was not explicitly matched with the VPA group, all participants in both groups were Caucasian and of similar ages. The exposed group had a mean age of 8.3 years with a standard deviation of 4.9 years, and the unexposed group had a mean age of 9.3 years with a standard deviation of 2.8 years.

These children were recruited as the comparison group for that study from neurologists, pediatricians, and referrals from other participants. The exclusion criteria were that the enrolled children must not have been exposed to maternal insulin-dependent diabetes, could not have a history of meningitis or varicella, could not be from a multiple gestation pregnancy, could not have a hearing loss of 20 decibels or more in both ears on a screening test (AudioScope, Welch Allyn), and could not have a language other than English as her/his first language. These children were examined by the same investigator (L.B.H.) using the same equipment, the same definitions of minor physical features, and the same measurements that were used in the evaluation of the VPA-exposed group in this study. The examination protocols were the same, except that the VPA-exposed children had an additional neurological assessment for cranial nerve function, muscle tone and strength, hand-eye coordination and balance. In addition, the VPA-exposed children were screened for signs of ASD with the SCQ or M-CHAT, but the children in the comparison group were not screened systematically for signs of ASD. However, their cognitive function was tested with the WISC-III (Wechsler Intelligence Scale for Children) and an interview with an examiner with experience working with children with ASD and cognitive deficits. No signs of ASD were detected in the testing of their cognitive function by this examiner (Adams: Personal Communication, 2016).

The normal standards

Farkas' normative data for anthropometric proportions (2004) was used for head length, head circumference, bitemporal distance, intercanthal distance, nose length, upper lip length, philtrum width, mouth width, ear length, and body height. Data from Farkas and Munro

(1987) was used for cephalic index (the ratio of cranial width to cranial anterior-posterior length), philtrum-mouth index (the ratio of the cutaneous upper lip width to the mouth width between the cheilions), and head-size body height index (the proportion of head circumference to body height). The Handbook of Physical Measurements (2007) by Hall, Froster-Iskenius and Allanson was used for hand length, third finger length and interpupillary distance. CDC Clinical Growth Charts were used for calculating BMI (Kuczmarski et al, 2000).

The findings among the VPA-exposed children are presented: a) in comparison to the unexposed control group, and b) as a comparison between the two VPA-exposed subgroups: those with autism and those without autism. The results of these comparisons were tabulated for qualitative measures (the presence versus absence of physical features) for the face and hands (Table 1), for quantitative measurements of facial features, head size, and body size scaled on population standards by age and sex (Table 2), and measures of features subgrouped by percentile cut-off points when population standards were not available (Table 3).

Statistical analysis

Utilizing craniofacial normative databases (Farkas, 1994; Farkas & Munro, 1987) (Table 2), standardized (Z-) scores, conditioned on demographic characteristics, were calculated for each child (Deutsch et al, 2003). These scores represent the number of standard deviations from the mean of the normative data. Standardized Z-scores computed were averaged for each group: those exposed to VPA, unexposed to VPA, ASD cases exposed to VPA, and cases exposed to VPA who did not exhibit ASD. Then, one-way ANOVA was used to compare: 1) the entire exposed group with the unexposed group, and 2) the VPA-exposed group with ASD with the VPA-exposed group without ASD. Group F-statistics were calculated, and p-values are reported. When population means and standard deviations were not available, proportions of children with the presence or absence of features were contrasted with a two-tailed Z-test. The Benjamini-Hochberg False Discovery Rate procedure was used to correct for alpha-inflation due to multiple comparisons (Benjamini, 1995). This procedure controls for the False Discovery Rate (FDR), i.e. the expected fraction of null hypotheses rejected mistakenly (Benjamini, 1995). Unlike many other methods, the FDR operates on actual significance levels achieved, providing a practical compromise to more conservative methods, such as the Bonferroni (Sedgwick, 2014).

For measurements that were taken twice by the same examiner and then averaged (head circumference, bitemporal distance, intercanthal distance, nose length, upper lip length, and philtrum width), the Intraclass Correlation Coefficient (ICC) (Shrout et al, 1979) was calculated to assess test-retest reliability.

RESULTS

Sixty-seven women with 91 children were interviewed about participation in this study. The mothers of 66 children returned the signed informed consent document and medical record release forms. All were invited to be interviewed using the Social Communication Questionnaire and to have a physical examination by one of the investigators (L.B.H.), either

at the hospital or in the home. Fifty-eight children and their parents participated initially, but eleven were excluded: three because their medical records suggested that their mothers did not take VPA during pregnancy, two because their mothers did not take VPA during the first trimester of their pregnancies, one because the child did not receive the ADI-R or ADOS testing despite screening positive on the SCQ, and 5 because their mothers took another teratogenic drug during pregnancy in addition to VPA (phenytoin [2], carbamazepine [2], and lithium [1]).

Forty-two of the final 47 child participants had a report of exposure indicated in their or their mother's medical record, and five had records that did not clearly indicate exposure but had a positive maternal self-report and records that did not contradict their self-report. The dose of VPA prescribed was obtained from the medical record for 37 children, self-report of the mothers for 9 children, and one mother reported having used valproate through the pregnancy, but did not recall the dose and no documentation was available. The doses often changed throughout pregnancy and the medical records were sometimes inconsistent with the mother's self-report. Because we did not find the dose information to be adequately reliable, we did not analyze the dose in relation to the child's physical features.

Of the 47 VPA-exposed children, 20 scored above the screening cut-off on the SCQ and were subsequently screened for ASD using the ADI-R and ADOS. One of the children who scored above the screening cut-off for the SCQ was unable to adequately cooperate for the ADI-R and ADOS, and was excluded. Thirteen of the children who scored above the screening cut-off for the SCQ also scored above the screening cut-off for both the ADI-R and ADOS and thus were considered to have Autism Spectrum Disorder. Six of the children who scored above the screening cut-off for the SCQ went on to score above the screening cut-off for the ADI-R but not the ADOS. These children were not considered to have ASD for the purpose of this study. No children scored above the screening cut-off for the ADOS but not the ADI-R. Ten of the 13 children considered to have ASD for the study had a prior diagnosis of ASD. Three children not considered to have ASD for the study had a prior diagnosis of ASD. For the purpose of this study, only screening scores and not prior diagnoses were considered when grouping children into the ASD and non-ASD groups.

Among the VPA-exposed subjects (n=47), three children with malformations were identified based on the parents' report and confirmed by a review of the medical records of each child: cleft palate (1); hypoplastic aortic arch, abnormal aortic valve and bilateral ptosis (1); coarctation of aorta and bicuspid aortic valve (1). In the unexposed comparison group (n=126), there was one infant with bilateral Grade II ureteral reflux.

In the clinical examination, several facial features were found to be more common in the VPA-exposed children than in the unexposed comparison group: telecanthus (increased width of soft tissues in the innercanthi), increased length of the upper lip and wide philtrum (Table 1). No significant differences were found in the prevalence of epicanthal folds, broad or depressed bridge of the nose, short nose, anteverted nostrils, stiff IP joints, or a number of finger features (hypoplastic fingernails, tapered fingers, stiff IP joints, or clinodactyly).

In the course of the clinical examinations, the examiner (L.B.H.) noted that 8 of the 47 VPA-exposed children had features of the typical “anticonvulsant face.” These 8 children also had, on average, a greater cephalic index than the group of VPA-exposed children as a whole. Since the examiner knew that each child had been exposed to VPA, these findings are of limited value. However, it suggests that among the 47 study subjects, a blinded examination may have identified a subset of children with more distinctive facial features, which has been characterized as “the VPA embryopathy” (DiLiberti et al, 1984; Jäger-Roman et al, 1986; Ardinger et al, 1988; Kini et al, 2006).

The measurements added surprising new findings in the VPA-exposed children compared to the unexposed children: a shorter head length ($p = 0.005$) and wider head width ($p = 0.006$), which are the characteristics of a large cephalic index ($p = 0.000$) (Table 2). The large cephalic index was present in VPA-exposed children both with and without ASD. To our knowledge, these measurements of skull width and length have not been reported previously in VPA-exposed children. However, one of us (C.K.D.) has observed the large cephalic index in an unpublished study of children with autism (Deutsch et al, 2013). The measurements also identified an increased intercanthal distance ($p = 0.000$) (Table 2). The VPA-exposed children were also taller than the children in the unexposed comparison group ($p = 0.008$) and had a decreased head circumference/height index ($p = 0.003$) (Table 3).

The Intraclass Correlation Coefficient (ICC) showed a high degree of reliability in the comparison of measurements made in duplicate. ICCs ranged from 0.92 to 0.99 for duplicate measurements taken in the VPA-exposed children, and from 0.91 to 0.99 in the VPA-unexposed group. ICCs > 0.75 are interpreted as excellent (Fleiss, 1986) suggesting strong consistency in repeated measurements.

The neurologic examination, which was performed only on the VPA-exposed children, showed abnormal deep tendon reflexes in 16 out of 42 children tested (38.1%) in the VPA-exposed group, including 9 out of 12 children tested (75.0%) in the VPA-exposed ASD subgroup. Muscle tone was decreased in 10 out of 43 children tested (23.3%) in the VPA-exposed group, including 6 out of 13 children tested (46.2%) in the ASD subgroup. Finger-to-nose accuracy was impaired in 6 out of 39 children (15.4%) in the VPA-exposed group, including 3 out of 11 (27.3%) in the ASD subgroup. These findings were assessed subjectively; no standardized methodology was used.

DISCUSSION

Our findings of the craniofacial features of 47 valproate-exposed children (all as monotherapy) confirmed some of the impressions of several experienced clinicians (DiLiberti et al, 1984; Jäger-Roman et al, 1986; Winter et al, 1987; Ardinger et al, 1988; Kini et al, 2006). Our qualitative exam confirmed the presence of a pattern of facial features that included a wide philtrum, long upper lip and telecanthus (Table 1). Our quantitative exam confirmed the increased intercanthal distance (Table 2) in addition to introducing a new finding of the increased cephalic index. Some of the facial features of valproate-exposed children, specifically the wide philtrum and long upper lip, have also been observed in children exposed to phenytoin and phenobarbital (Orup et al, 2014). This overlap suggests

that these very different medications may have some similar effects on the craniofacial features of the exposed fetus. However, the children exposed during pregnancy to phenytoin and phenobarbital were not found to have an increased intercanthal distance, i.e. telecanthus, on laser light scans (Orup et al, 2014) nor an increased cephalic index (Holmes et al, 1995). Because of these differences, the overall gestalt of the facial features of the VPA-exposed children was different from the appearance of children exposed to phenytoin and phenobarbital.

Other new findings included an increased height and decreased head circumference/height index in the exposed group. VPA exposure has been associated with low birth weight and length (Kacirova et al, 2015) but not, to our knowledge, with increased height. A decreased head circumference/height index has also not been previously observed. Because the head circumference by itself was not significantly decreased but height was significantly increased, it is possible that this finding is due to an increased height in the exposed group rather than a truly decreased head circumference. It is also possible that the head circumference is truly decreased but was masked by the increased height. This could be elucidated with further investigation into the cranial features found in this study, especially how they change over time throughout childhood and with physical growth.

One of the strengths of this study was its systematic nature. The detailed surface examination followed a protocol, which prompted the examiner to look for the presence or absence of specific physical features. Previous studies of newborn infants (Leppig et al, 1987; Méhes et al, 1973) have demonstrated the value of using an examination protocol to improve consistency in the evaluation of features. Even so, the subjective nature of physical features has been associated with poor reproducibility by two examiners of the same child (Holmes et al, 1987). In addition, when the examiner focuses on the presence/absence of specific physical features, such as a short nose with anteverted nostrils, this introduces an observer bias (Harvey et al, 1994). With this focus, an increased rate of detection of the physical features of interest may be expected. This potential bias underscores the importance of including objective measurements as part of the examination.

It was notable that in some cases, the findings based upon measurements differed from the subjective findings. For example, upper lip length and philtrum width were found to be increased in the VPA-exposed group in the clinical examination (Table 1), but the measurements of these features were not significantly different from the measurements in control children (Table 2). This could be an example of the inherent observer bias that exists when looking for subjectively defined features, or it could be an example of the limitations of relying on measurements made with less precise instruments and/or imprecise landmarks. Méhes (Holmes, 2010) used the term “pseudoaccuracy” in referring to the difficulty of identifying end points in a rounded structure, such as the end of the nose or the heel. In addition, measurements made by two examiners using the same methodology can differ, presumably because of different interpretations of the landmarks to be used (Harvey et al, 1994). It is difficult to discern whether some of the discrepancies between the observed features and the measurements are due to inaccuracy of the observations or inaccuracy of the measurements. The findings in the measurements of the interpupillary distance (Table 3) illustrate the potential effect of less precise measurements. The portion of VPA-exposed

children with a measurement less than the third centile was significant, but the portion with a measurement above the 97th centile was not, despite both portions being increased overall. A more precise measurement might have clarified these findings.

For the measurements that were performed twice on the same subject, we calculated the Intraclass Correlation Coefficient to assess test-retest reliability. There was a high degree of reliability shown with all ICC values exceeding .90, suggesting strong re-measurement consistency. This reliability, and the fact that the same examiner measured all children in the study, exposed and unexposed, with the same protocol and equipment, assures us that we can safely draw conclusions from direct comparisons between the exposed and unexposed groups. Any error in measurement due to imprecise instruments or human error would likely be consistent in both groups.

One weakness of this study was the fact that the examiner was not blind to which participants had been exposed to VPA during pregnancy. This was due to the nature of the study in that the recruitment and examination of the exposed children occurred separately from the recruitment and examination of the unexposed children (though the methods of recruitment and examination were similar in both cases and all examinations in both groups were done by the same examiner). The examiner was blind to the exposure status of the comparison group, but this group was not examined concurrently. It should also be noted that a special effort was made to recruit VPA-exposed children considered to have features of autism, which might artificially increase the observed prevalence of the associated autism spectrum disorder. In addition, there was a potential volunteer bias in that parents volunteering to participate in the study may have been more likely to have concerns about their exposed child than parents who did not volunteer. For this reason, the incidence of ASD in the exposed group is not a useful statistic. More unbiased estimates have been developed in population-based studies. For example, Christensen et al (2013) reported on the incidence of ASD in all children born in Denmark within a 10-year timeframe. They found an absolute risk of 4.4% for ASD in children exposed during pregnancy to valproate compared to an absolute risk of 1.5% in children not exposed to valproate.

Another limitation is that the valproate-exposed children diagnosed in our study with autism did not have testing for the potential molecular changes that have been identified in many individuals with autism. For example, chromosome microarray testing could have identified microdeletion/duplication at 15q13.2q13.3 (Miller et al, 2008) or 16p11.2 (Blumenthal et al, 2014). Whole-genome sequencing might have identified mutations in the gene POGZ (MIM: 614787) [Stessman et al, 2016], *SCN2A*, *KATNAL2* or *CHD8* (Sanders et al, 2012). This study was part of a project in which DNA was to be collected from VPA-exposed children with and without autism. One hypothesis being tested was whether polymorphisms of several different genes, such as *HOXA1*, *WNT2* and *RELN*, were more common in children with autism. Could the autistic child exposed to VPA during pregnancy be more likely to have these polymorphisms (Rodier, 2004)? DNA from the VPA-exposed children with and without autism is being sequenced, in a separate study underway now.

The changes identified in the head shape of the VPA-exposed children suggest that this prenatal exposure may alter brain development. To our knowledge, no CNS imaging studies

have been reported in VPA-exposed children. Alterations in the basolateral nucleus of the amygdala (Olde Loohusi et al, 2015) and the cerebellum (Kliemann et al, 2012) of rats exposed to valproate during pregnancy have been reported. The finding of an increased cephalic index in the VPA-exposed group is especially interesting given recent research demonstrating an increased cephalic index in a sample of children with autism (Deutsch et al, 2013). This raises questions about potential parallels between the effects on the developing brain of VPA and of various genetic abnormalities that could contribute to the development of ASD.

The high prevalence of neurological abnormalities in the VPA-exposed children, and even more so in the VPA-exposed children with ASD, are of interest. Although the neurologic exam was not standardized and was not performed in the control group, the very high prevalence of neurologic abnormalities may be seen as evidence for an increased prevalence of neurologic abnormalities in children exposed to VPA. This is especially interesting given that children with ASD have been shown to exhibit an increased prevalence of minor neurological dysfunction. One analysis of 122 children with ASD found that 74% of the children, compared with 6% in a reference group, exhibited minor neurological dysfunction based on the standardized Touwen neurological examination (de Jong et al, 2011). In addition, animal studies have demonstrated significantly smaller cerebella in rats exposed to VPA during neural tube closure, mirroring cerebellar changes found in some human cases of ASD (Ingram et al, 2000).

Much more information is needed as to how prenatal exposure to VPA affects the brain and the associated shape of the skull. It will be important to investigate how the valproate-associated cranial findings reported here change throughout childhood and adolescence. More information is available on the brain structure of children with autism. Serial MRIs have shown abnormal regulation of brain growth including early overgrowth in young children with autism (Courchesne et al., 2001). Such studies of children exposed to VPA during pregnancy are needed for comparison.

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Table 1

Comparison of percentage of children affected by abnormalities of the face and fingers identified subjectively in the surface examinations in the VPA-exposed (with and without ASD) children and unexposed populations

	VPA-exposed ASD (n=13)	VPA-exposed non-ASD (n=34)	Z-test p-value	VPA-exposed (n=47)	Unexposed (n=126)	Z-test p-value
FACE						
Epicanthal fold, left	16.7	3.2	0.12	7.0	7.2	0.97
Epicanthal fold, right	7.7	2.9	0.47	4.3	9.5	0.26
Broad bridge of nose	23.1	26.5	0.81	25.5	21.6	0.58
Telecanthus	46.1	47.1	0.95	44.7	21.4	0.002*
Depressed bridge of nose	7.7	2.9	0.46	4.3	0.8	0.12
Short nose	38.5	18.2	0.15	21.3	17.5	0.57
Anteverted nostrils	38.5	26.5	0.42	29.8	29.6	0.98
Long upper lip	38.5	44.1	0.73	42.6	22.2	0.008*
Wide philtrum	33.3	33.3	1.0	33.3	11.3	0.0008*
FINGERS						
Hypoplastic fingernails	23.1	27.3	0.77	26.1	15.8	0.12
Tapered fingers	7.7	9.1	0.88	8.7	7.9	0.87
Stiff IP joints	7.7	6.1	0.84	6.5	5.6	0.83
Clinodactyly, left	33.3	6.1	0.02	13.3	9.9	0.53
Clinodactyly, right	30.8	6.1	0.02	13.0	11.9	0.84

● Z-test: This z-test for difference of proportions gives information about whether the proportion of children affected was significantly different between the two groups. A significant p-value indicates that there is likely a true difference between the two groups in regards to the proportion of children affected by the given trait.

* Significant VPA exposed vs. unexposed contrast after Benjamini-Hochberg correction for multiple comparison ($q^*=0.11$ significance level)

* Significant VPA exposed ASD vs. VPA exposed non-ASD contrast after Benjamini-Hochberg correction for multiple comparison ($q^*=0.0036$ significance level)

Table 2

Comparison of anthropometric measurement mean z-scores in VPA-exposed (with and without ASD) and unexposed populations.

	Difference in z-scores [▲] (exposed z-score – unexposed z-score)	F-test [●] p-value
Head circum.	–0.254	0.219
A-P dist.	–0.623	0.005 *
Bitemporal width	0.8	0.006 *
Cephalic index	1.012	0.000 *
Intercanthal dist.	1.304	0.000 *
Nose length	–0.254	0.312
Upper lip	0.285	0.232
Philtrum width	0.432	0.09
Mouth width	–0.194	0.391
Philtrum-mouth width index	0.256	0.383
Height	0.755	0.008 *
Head circ/height	–0.623	0.003 *

● F-test: The F-test gives information about whether the mean values for a specific trait differ between the two groups. A significant p-value indicates that there is likely a true difference between the two groups in regard to the measurement in question.

▲ Z-score: The average z-score for the group is the average number of standard deviations that that group's average measurement is above or below the mean population measurement for a given trait.

A-P distance = head length

Bitemporal distance = head width

Cephalic index = [(head width * 10)/(head length)]

Intercanthal distance = distance between inner canthi, or most medial portions of the eyelids

* Significant after Benjamini-Hochberg correction for multiple comparison ($q^*=0.033$ significance level)

Note that there were no significant differences between the VPA-exposed children with autism spectrum disorders (ASD) and those without ASD for any of these measurements.

Table 3

Comparison of proportions of children with given trait in VPA-exposed (with and without ASD) and unexposed populations

	VPA-exposed (n=47)	Unexposed (n=126)	Z-test ●
	%	%	p-value
Decreased interpupillary distance (< 3 rd percentile)	8.9	0.0	0.0007 *
Increased interpupillary distance (> 97 th percentile)	20.0	7.0	0.014
Small hand (< 3 rd percentile)	2.1	2.0	0.97
Large hand (> 97 th percentile)	8.5	6.0	0.55
Short 3 rd finger (< 3 rd percentile)	6.4	0.8	0.03
Long 3 rd finger (> 97 th percentile)	4.3	5.0	0.85
Low BMI (< 3 rd percentile)	0.0	3.0	0.23
High BMI (> 97 th percentile)	8.9	7.0	0.67

● Z-test: This z-test for difference of proportions gives information about whether the proportion of children affected was significantly different between the two groups. A significant p-value indicates that there is likely a true difference between the two groups in regards to the proportion of children affected by the given trait.

* Significant after Benjamini-Hochberg correction for multiple comparison (q*=.013 significance level)

Note that there were no significant differences between exposed with ASD and exposed without ASD groups for any of these features.