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A ten year longitudinal examination of the incidence rate and age of childhood encephalopathy diagnoses in an autism spectrum disorder diagnosed cohort

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Autism spectrum disorder (ASD) is defined by persistent deficits in social communication/interaction and stereotypic behaviors with many diagnosed persons experiencing a developmental regression at >1 year-old. It was hypothesized that progressive childhood encephalopathy is an important etiological factor in ASD pathogenesis. This hypothesis-testing study examined the relationship between diagnosed childhood encephalopathy and ASD. The Independent Healthcare Research Database is composed of de-identified linked eligibility and claim healthcare records prospectively generated from the Florida Medicaid system. A cohort of 101,736 persons eligible for Florida Medicaid from 1990-2009 and continuously eligible with ≥10 outpatient office visits during the 120 month period following birth were examined using SAS software. There were 1,397 persons (7,223 person-years) in the ASD diagnosed cohort and 100,339 persons (980,786 person-years) in the undiagnosed cohort. The incidence rate of encephalopathy was examined using Cox proportional hazards ratio models. In the ASD cohort relative to the undiagnosed cohort, a significantly increased incidence rate of diagnosed encephalopathy was observed in the unadjusted and adjusted models. The risk for an encephalopathy diagnosed at >1 year-old was greater than for an encephalopathy diagnosed at <1 year-old. This study provides important new evidence supporting the hypothesis that a significant number of children with an eventual ASD diagnosis experience a progressive childhood encephalopathy diagnosed at >1 year-old.

Key words: autistic, cohort, encephalopathy, neurodevelopmental, PDD

INTRODUCTION

Autism spectrum disorder (ASD) is defined by persistent deficits in social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). Although an ASD diagnosis is defined behaviorally by the American Psychiatric Association (APA), other features, more physical or health related, are associated with an ASD diagnosis (Geier et al., 2012; Kern et al., 2014a). Among the most common are gastrointestinal disturbances=48%, incontinence=57%, sleep problems=57%, eating disorders=94%, hyperactivity=67%, lethargy=26%, sensory processing problems=85%, anxiety/fear=74%, behavioral problems=89%, and obsessive-compulsive behaviors=92% (Geier et al., 2012).

The exact etiology of ASD remains under debate, but some investigators suggested that the etiology of ASD is genetic in origin, and that the disorder is heritable with complex inheritance and genetic heterogeneity (AlSagob et al., 2015). High resolution genetic evaluations using chromosomal microarray, chromosomal karyotype, and Fragile X DNA testing revealed that approximately 80% of children with ASD have normal genetic test results (Shen et al., 2010; Geier et al., 2016). Of the remaining 20%, approximately half were of var-

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ious polymorphisms of unknown significance, and the other half were de novo mutations with little to no commonality (<10% of persons diagnosed with an ASD). As a consequence, direct genetic causal factors for an ASD diagnosis are identifiable in only a limited number of persons diagnosed with an ASD.

By contrast, other investigators hypothesized that progressive childhood encephalopathy through a neurodegenerative pathway is an important etiological factor in ASD pathogenesis (Kern et al., 2013). In support of their hypothesis, these investigators described that on a clinical basis a significant number of children diagnosed with an ASD were observed to undergo developmental regression manifested by a loss of verbal, nonverbal, and social abilities starting at about 1 year-old. Overall, it was described by these investigators that 15% to 62% of persons diagnosed with an ASD underwent regression. These investigators suggested that developmental regression among children diagnosed with an ASD was symptomatic of childhood encephalopathy (Kern et al., 2014a; 2014b).

In order to quantitatively evaluate the aforementioned hypothesis, the present hypothesis-testing epidemiological study evaluated the incidence rate of childhood encephalopathy diagnoses among persons diagnosed with an ASD in comparison to persons not diagnosed with an ASD. In addition, the potential impact of the age on the incidence rate of encephalopathy diagnosis among persons diagnosed with an ASD as compared to persons not diagnosed with an ASD was considered.

METHODS

Independent Healthcare Research Database (IHRD)

The Independent Healthcare Research Database (IHRD) is composed of de-identified healthcare records generated from the Florida Medicaid system. The data contained within the IHRD were obtained from the Agency for Health Care Administration (AHCA) of the state of Florida and included eligibility and claim files. It is possible to link a person's eligibility and claim records by a unique recipient identifier code. The eligibility records included detailed information for each person regarding their month and year of enrollment, gender, date of birth, and county level residency. The claims records included detailed information for each person regarding their diagnosis status using the International Code for Disease, 9th revision (ICD-9) codes, healthcare procedure codes (medical, dental, etc.), and administered drugs using National Drug Codes (NDC). The data in the IHRD were assembled and accessed under approval by the Liberty Institutional Review Board (IRB) (Deland, FL). The SAS system for Windows, version 9.4 (Cary, NC, USA) running on a 64-bit based PC with dual core Intel® (Santa Clara, CA, USA) Xeon® CPU x5680 at 3.33 GHz, 6 cores, and 12 logical processors, with 44.0 GB of RAM, and utilizing Microsoft (Redmond, WA, USA) Windows 7 Ultimate operating system was used to examine the IHRD.

Study Participants

Fig. 1 presents a schematic flowchart of the IHRD data examined in the present study. A cohort of 8,440,941 persons of all ages with no changes or missing genders or dates of birth and eligible at specific times for Florida Medicaid from July 1990 through June 2009 was initially evaluated in this study. Among this cohort, a total of 1,871,728 persons were eligible for Florida Medicaid from their date of birth, and among those persons a total of 193,453 persons were continuously eligible for Florida Medicaid for 120 months following birth. Finally, among the cohort of 193,453 persons continuously eligible for Florida Medicaid for 120 months following birth, a sub-cohort of 101,736 persons with ≥10 outpatient office visits during the 120 month period following birth was identified.

Exposures

The exposure variable examined in this study was identified from the ICD-9 codes filed on claims for each cohort member examined. The ICD-9 codes examined included: autistic disorder, current or active state (299.00) or other specified pervasive developmental disorders, current or active state (299.80). Only persons diagnosed with at least one of the aforementioned outcomes were members of the ASD diagnosed cohort, and persons not diagnosed with either of the aforementioned outcomes were members of the undiagnosed cohort (not diagnosed with an ASD). Among those diagnosed with an ASD, the date of service for the first claim in chronological order with an ICD-9 code specifying an ASD was assumed to be the date of onset of the disorder. Overall, it was observed that 1,397 persons were in the ASD diagnosed cohort and 100,339 persons were in the undiagnosed cohort.

Outcomes

The outcome variable examined in this study was identified from the ICD-9 codes filed on claims for each

cohort member examined. All encephalopathy-related diagnoses (code: 348.3x) were examined, including: encephalopathy not elsewhere classified (348.3), encephalopathy unspecified (348.30), metabolic encephalopathy (348.31), and other encephalopathy (348.39). Persons were considered to have an encephalopathy if they had any of the encephalopathy-related diagnoses, and persons were not considered to have encephalopathy if they did not have any of the encephalopathy-related diagnoses during the study period examined. Among those with an encephalopathy diagnosis, the date of service for the first claim in chronological order with an encephalopathy diagnosis was assumed to be the date of onset of an encephalopathy. Overall, it was observed that 3,512 persons were diagnosed with encephalopathy in the undiagnosed cohort. In the ASD diagnosis cohort, a total of 554 persons were diagnosed with encephalopathy, but only 313 were diagnosed with an encephalopathy before their ASD diagnosis. Only those persons diagnosed with an encephalopathy before their ASD diagnosis were counted as having an encephalopathy diagnosis in this study.

Statistical analyses

In all statistical analyses, the statistical package in SAS was utilized, and a two-sided p-value<0.05 was considered statistically significant. The null hypothesis was that the incidence rate of encephalopathy diagnosis would have no relationship with an ASD diagnosis.

A regression analysis of encephalopathy diagnosis based on the Cox proportional hazards model was used to evaluate the incidence rate of encephalopathy diagnoses among those persons in the ASD diagnosed cohort as compared to the undiagnosed cohort. Ties in the failure times were handled using the exact method. In the undiagnosed cohort, person-years of follow-up began on the date of birth and continued until the end of eligibility (a maximum of 120 months after birth) or until the date of the first encephalopathy diagnosis. In the ASD diagnosed cohort, person-years of follow-up began on the date of birth and continued until an ASD diagnosis was made (a maximum of 120 months after birth) or until the date of the first encephalopathy diagnosis. In addition, the potential impact of the age of

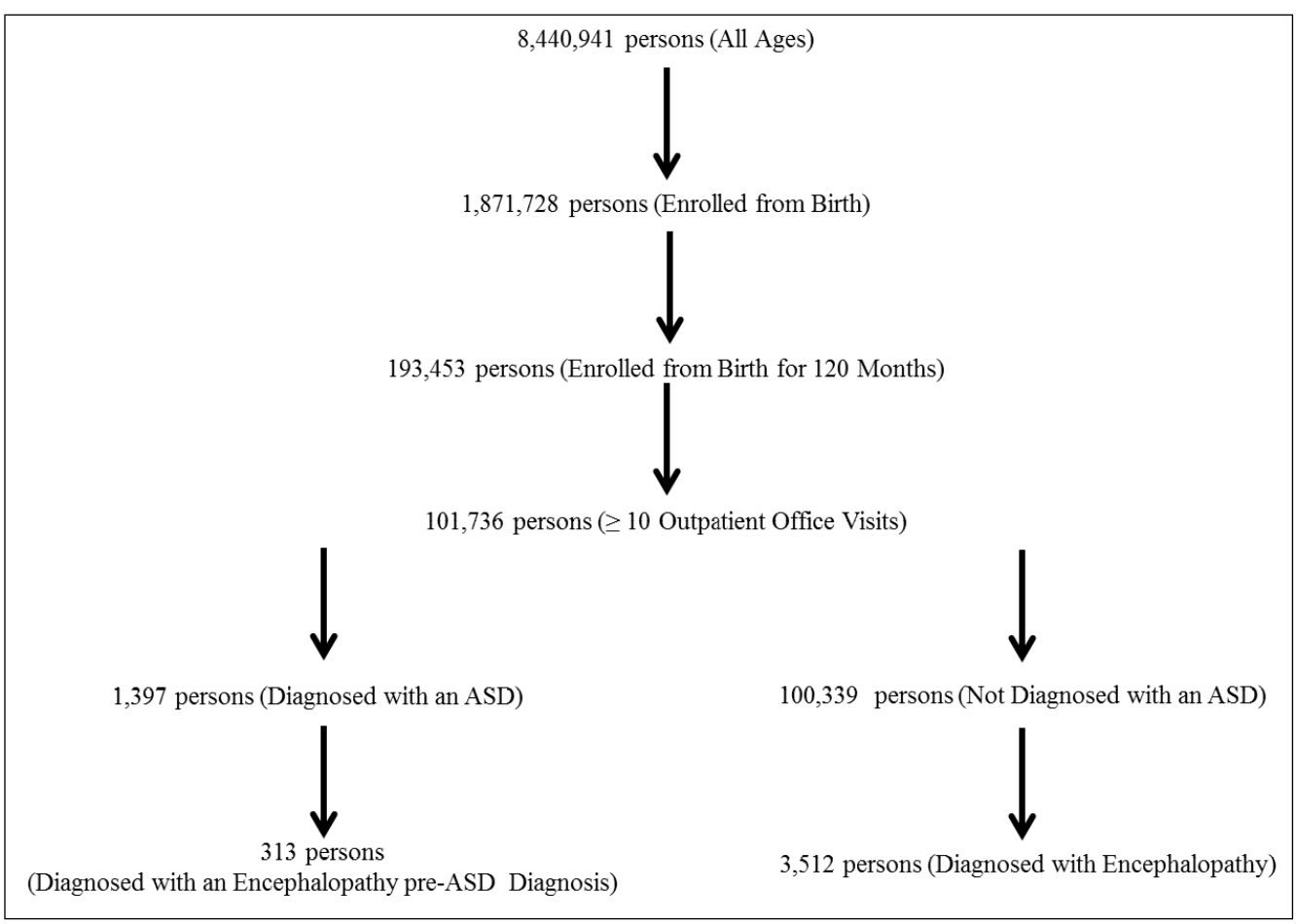

Fig. 1. A schematic flowchart of the data examined in the present study.

encephalopathy diagnosis was examined. Specifically, modeling was constructed to evaluate the impact of age of an encephalopathy by examining those persons diagnosed with an encephalopathy at <1 year of age and >1 year of age. All models were constructed without adjustment for covariates (Model I) and with adjustment for the covariates of gender, date of birth, and county of residence (Model II).

RESULTS

Table I displays the demographic characteristics of the persons evaluated in this study. Overall, there were a total 1,397 persons in the ASD diagnosed, cohort contributing a total of 7,223 person-years and 100,339 persons in the undiagnosed, cohort contributing a total of 980,786 person-years. The gender distribution in the ASD diagnosed cohort (male/female ratio=3.2) was greater than the undiagnosed cohort (male/female ratio=1.12). The mean date of birth was similar for the ASD diagnosed and undiagnosed cohorts. The incidence rate of diagnosed encephalopathy was significantly higher at 22.4% (95% confidence interval=20.25 to 24.61) in the ASD diagnosed cohort as compared to 3.50% (95% confidence interval=3.39 to 3.62) in the undiagnosed cohort.

Table II shows the demographic characteristics of the 3,825 persons diagnosed with an encephalopathy. Slightly more males than females were diagnosed with an encephalopathy (male/female ratio=1.5) with the ratio being higher than those observed in the undiagnosed cohort but lower than in the ASD diagnosed cohort. The mean date of birth for persons diagnosed with an encephalopathy (1995) was similar in the ASD diagnosed and undiagnosed cohorts.

Table III reveals the Cox proportional hazards model results examining the relationship between the diagnosed incidence rates of encephalopathy among those persons with an ASD diagnosis in comparison to those persons not diagnosed with an ASD. It was observed when comparing the ASD diagnosed cohort relative to the undiagnosed cohort that the hazard ratio for the incidence rate of encephalopathy diagnosis was significantly increased in both the unadjusted (hazard ratio=9.65) and adjusted (hazard ratio=8.98) models. It was also observed in the adjusted model that gender and county of residence were significant covariates.

Fig. 2 is a Cox proportional hazards survival plot evaluating the incidence rate of diagnosed encephalopathy cases over the age in years for persons were examined in the ASD and undiagnosed cohorts. The plot reveals that in the initial period (< 3 months) there was a similar incidence rate of diagnosed encephalopathy among the ASD and undiagnosed cohorts (adjusted hazard ratio=1.79, p=0.085). The incidence rate of diagnosed encephalopathy cases subsequently steadily increased in the ASD diagnosed cohort relative to the undiagnosed cohort, so that by the end of the follow-up period at 10 years, there was more than a 10-fold increase in the incidence rate of diagnosed encephalopathy cases in the ASD diagnosed cohort relative to the undiagnosed cohort.

Table IV reveals the impact of age on the incidence rate of encephalopathy diagnosis in the ASD diagnosed and undiagnosed cohorts. It was observed when comparing the ASD diagnosed cohort to the undiagnosed cohort that the incidence rate of diagnosed encephalopathy at >1 year-old (unadjusted hazard ratio=13.22 or adjusted hazard ratio=12.30) relative to the incidence rate of diagnosed encephalopathy at <1 year-old (unadjusted hazard ratio=3.91 or adjusted hazard ratio=3.65), the risk was significantly increased.

Table I. Demographic characteristics of the cohorts examined in this study[1].

Parameter Examined	ASD Diagnosed Cohort[2] (n=1,397)	Undiagnosed Cohort[3] (n=100,339)
Person-Years	7,223	980,786
Gender (%)		
Male	1,060 (75.88%)	53,094 (52.91%)
Female	337 (24.12%)	47,245 (47.09%)
Date of Birth		
mean ± std (range)	1995±2.6 (1990–1999)	1995±2.6 (1990–1999)
Number Diagnosed with Encephalopathy (ICD-9 Code: 348.3x)	313[4]	3,512

ASD = Autism Spectrum Disorder; ICD-9 = International Code of Disease, 9th revision; std = standard deviation. [1] All persons examined in this study were enrolled from their date of birth for 120 consecutive months. All persons had non-changing dates of birth and gender status. All persons had ≥ 10 outpatient office visits. [2] Persons diagnosed with an ASD (ICD-9 codes of 299.00 or 299.80). [3] Persons were not diagnosed with an autism spectrum disorder (ICD-9 codes 299.00 or 299.80). [4] A total of 241 persons with a diagnosed encephalopathy post-ASD diagnosis were excluded.

Table II. Demographic summary of the persons diagnosed with an encephalopathy evaluated in this study[1].

Parameter Examined	Persons Diagnosed with an Encephalopathy (n=3,825)
Gender (%)	
Male	2,282 (59.66%)
Female	1,543 (40.34%)
Date of Birth	
mean ± std (range)	1995±2.6 (1990–1999)
Age in Years of Initial Diagnosis	
mean ± std (range)	3.61±2.91 (0–10)
Encephalopathy Diagnosis Categories	
Encephalopathy not elsewhere classified (348.3)	3,538 (92.50%)
Encephalopathy unspecified (348.30)	259 (6.77%)
Metabolic encephalopathy (348.31)	1 (0.03%)
Other encephalopathy (348.39)	27 (0.70%)

[1] All persons examined in this study were enrolled from their date of birth for 120 consecutive months. All persons had non-changing dates of birth and gender status. All persons had ≥10 outpatient office visits.

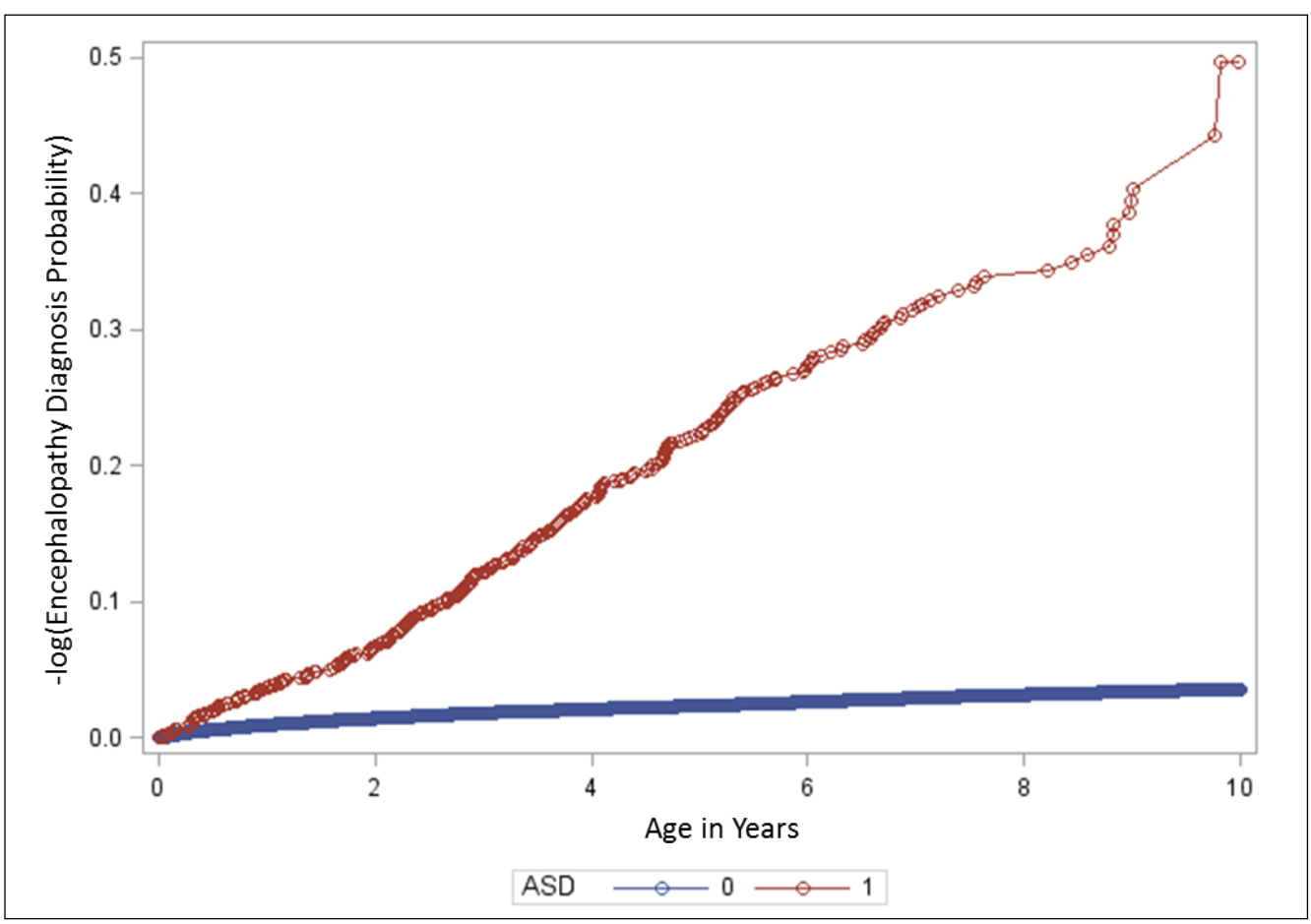

Fig. 2. A Cox proportional hazards survival plot evaluating the incidence rate of encephalopathy diagnosed over the period examined in the ASD diagnosed cohort (1) in comparison to the undiagnosed cohort (0).

Table III. Cox proportional hazards model results examining the incidence rate of encephalopathy diagnoses in the ASD diagnosed and undiagnosed cohorts.

Model	Outcome	Variable	Hazard Ratio	95% CI	p-value	χ^2
I						
	Encephalopathy (Diagnosed at any age)					
		ASD (Diagnosed vs. Undiagnosed)	*9.65*	*8.59 to 10.84*	*<0.0001*	*1,449*
II						
	Encephalopathy (Diagnosed at any age)					
		ASD (Diagnosed vs. Undiagnosed)	*8.98*	*7.98 to 10.11*	*<0.0001*	*1,325*
		Gender (Female vs. Male)	*0.82*	*0.76 to 0.87*	*<0.0001*	*38*
		County of Residence	*0.99*	*0.99 to 0.99*	*<0.0001*	*207*
		Date of Birth	1.00	1.00 to 1.00	0.92	0.01

Italicized results are statistically significant. Model I = unadjusted, Model II = adjusted for gender, county of residence, and date of birth. CI=confidence interval

Table IV. An evaluation of the impact of the age of an encephalopathy diagnosis in ASD diagnosed and undiagnosed cohorts using Cox proportional hazards modeling.

Model	Outcome	Variable	Hazard Ratio	95% CI	p-value	χ^2
I						
	Encephalopathy (Diagnosed at <1 year-old)					
		ASD (Diagnosed vs. Undiagnosed)	*3.91*	*2.94 to 5.19*	*<0.0001*	*88*
	Encephalopathy (Diagnosed at >1 year-old)					
		ASD (Diagnosed vs. Undiagnosed)	*13.22*	*11.62 to 15.03*	*<0.0001*	*1,547*
II						
	Encephalopathy (Diagnosed at <1 year-old)					
		ASD (Diagnosed vs. Undiagnosed)	*3.65*	*2.74 to 4.87*	*<0.0001*	*78*
		Gender (Female *vs.* Male)	0.91	0.80 to 1.03	0.14	2.20
		County of Residence	*0.99*	*0.99 to 0.99*	*<0.0001*	*22*
		Date of Birth	*1.00*	*1.00 to 1.00*	*0.018*	*5.63*
	Encephalopathy (Diagnosed at >1 year-old)					
		ASD (Diagnosed vs. Undiagnosed)	*12.30*	*10.8 to 14.02*	*<0.0001*	*1,423*
		Gender (Female vs. Male)	*0.78*	*0.73 to 0.85*	*<0.0001*	*40*
		County of Residence	*0.99*	*0.99 to 0.99*	*<0.0001*	*192*
		Date of Birth	1.00	1.00 to1.00	0.13	2.27

Italicized results are statistically significant. Model I = unadjusted, Model II = adjusted for gender, county of residence, and date of birth. CI=confidence interval.

DISCUSSION

The results of this retrospective longitudinal cohort study of prospectively collected healthcare data provide important and compelling new epidemiological quantitative data regarding the relationship between progressive childhood encephalopathy diagnoses and the long-term risk of an ASD diagnosis. It was revealed among persons with an ASD diagnosis relative to persons not diagnosed with an ASD that the incidence rate of diagnosed encephalopathy was significantly increased and the relationship was significantly mediating by age of an encephalopathy diagnosis. Namely, among persons diagnosed with an ASD relative to persons not diagnosed with an ASD, the incidence rate of diagnoses encephalopathy made at >1 year-old were significantly increased relative to the incidence rate of diagnoses encephalopathy made at <1 year-old. Finally, the observed effects remained significant when considering such covariates as gender, date of birth, and county of residence.

A literature search identified only one epidemiological study that examined the relationship between childhood encephalopathy and the subsequent risk for an ASD diagnosis (Badawi et al., 2006). In that study, Badawi et al (2006), examined 276 cases with an encephalopathy and 564 randomly selected controls from Western Australia. All cases and controls underwent comprehensive neurobehavioral and cognitive follow-up at 3 and 5 years of age. At 5 years-old, it was observed that 5% of cases with an encephalopathy were diagnosed with an ASD, whereas only 0.8% of controls were diagnosed with an ASD. Overall, when comparing cases to controls, cases were 5.9-fold significantly more likely to be diagnosed with an ASD than controls.

The results of the present study are consistent with those observed in the aforementioned study (current study risk=9.65 *vs.* previous study risk=5.9), but are differentiated in several important aspects. First, a longitudinal cohort study design was utilized in this study, whereas the aforementioned study utilized a cross-sectional case-control study design. Second, diagnoses generated from prospectively recorded medical records generated by healthcare providers on a longitudinal basis were examined in the present study, whereas the aforementioned study utilized comprehensive neurobehavioral and cognitive follow-up evaluations at specific ages. Third, the present study examined gender, county of residence, and date of birth were considered as covariates in statistical models, whereas the aforementioned study did not examine covariates. Fourth, this study examined members of the cohorts for a much longer period of follow-up (10 years) than the aforementioned study (5 years). The length of follow-up is important because it can take many years for an ASD diagnosis to become fully clinically apparent (Geier et al., 2018), and studies that fail to consider this phenomenon may underestimate the true ASD diagnosis rate (Geier et al., 2013). Fifth, the current study evaluated the importance of the age of an encephalopathy diagnosis on the long-term risk of an ASD diagnosis, whereas the aforementioned study did not consider the age of an encephalopathy diagnosis. Finally, the present study examined time as a variable in the Cox proportional hazards models constructed, whereas the aforementioned study did not. A simple comparison of the incidence rate of diagnosed encephalopathy in the ASD cohort in comparison to the undiagnosed cohort revealed a significantly increased risk ratio=6.39 (95% confidence interval=5.76 to 7.08) that was quite comparable to the risk=5.9 observed in the aforementioned study.

The results observed in this study also revealed that the age of an encephalopathy diagnosis is an important mediating factor on the long-term risk of a child being diagnosed with an ASD. Specifically, it was observed that among those children diagnosed with an encephalopathy after the first year of life (adjusted hazard ratio=12.30), the risk was significantly greater for an ASD diagnosis than for those children diagnosed with an encephalopathy within the first year of life (adjusted hazard ratio=3.65). As described previously, investigators hypothesized that many children diagnosed with an ASD undergo a progressive childhood encephalopathy associated with a developmental regression manifested by a loss of verbal, nonverbal, and social abilities starting at about 1 year-old (Kern et al., 2013). The results observed in this study are supported by a number of other investigators observing developmental regression in the second year of life among children subsequently diagnosed with an ASD. For example, investigators recently undertook a longitudinal study of children to examine the onset patterns of ASD (Ozonoff et al., 2018). These investigators described that regressive onset of ASD was observed on a prospective basis in 88% of children evaluated by medical examiners and in 69% as described by parents. The investigators concluded that a regressive onset pattern is common among children diagnosed with an ASD. Other investigators validated the phenomenon of regression among persons diagnosed with an ASD by examining home videotapes (Werner et al., 2005).

Another important aspect of the findings of the present study was that it was possible to determine the risk difference (attributable rate) of encephalopathy in the ASD cohort. It was observed that 22.35% of persons in the ASD cohort were diagnosed with an encephalopathy, whereas only 3.50% of persons in the un-

diagnosed cohort were diagnosed with an encephalopathy. As a consequence, the risk difference was 18.86% (95% confidence interval=16.67 to 21.04) for diagnosed encephalopathy in the ASD cohort. Therefore, almost 1 out of 5 children diagnosed with an ASD were diagnosed with an encephalopathy apparently attributably associated to their ASD diagnosis. This significant and common observation highlights the importance of brain damage (apparently manifesting as an encephalopathy diagnosis) in the etiology of an ASD for many children.

The results observed in the present study are biologically plausible. Brain pathology studies among persons diagnosed with an ASD indicate marked and ongoing inflammatory reactivity with concomitant neuronal damage, neurodegeneration and cerebral hypoperfusion (Kern et al., 2013; Bjorklund et al., 2016; 2018) rather than some type of developmental mishap of potential genetic or fetal origin (Bauman et al., 1997). A developmental mishap does not explain the extensive and consistent evidence of neuroinflammatory reactivity, neuronal damage, and neurodegeneration which includes: activated microglia and astrocytes, elevated levels of glial fibrillary acidic protein, increased oxidative stress, elevated 8-oxo-guanosine levels, elevated proinflammatory cytokines, aberrant expression of nuclear factor kappa-light-chain-enhancer of activated B cells, and neuronal cell loss (Laurence and Fatemi, 2005; Kern and Jones, 2006; Rodriguez and Kern, 2011; Kern et al., 2013).

One of the hallmarks of neuroinflammation and the potential for neurodegeneration is microglial activation. Microglial activation is reported in autism in many studies (Rodriguez and Kern, 2011; Kern et al., 2012; 2015; 2016). In addition, evidence indicates that the microglial activation in autism is sustained or chronic. Chronic microglial activation is implicated in the pathology of numerous neurodegenerative diseases including: Parkinson's disease, Alzheimer's disease, prion diseases, multiple sclerosis, and HIV-dementia (Dheen et al., 2007; Lull and Block, 2010). Chronic microglial activation can cause neuronal loss by directly attacking brain tissue, especially axons and synapses (Kern et al., 2016); however, microglial activation can also cause neuronal damage through the release of potentially cytotoxic molecules such as proinflammatory cytokines, reactive oxygen intermediates, proteinases and complement proteins (Dheen et al., 2007). Evidence suggests that the adaptive immune system can orchestrate an attack against central nervous system tissue by driving microglia to act like "Pac-Man", eating up synapses, oligodendrocytes, neurons, and other healthy material (Zindler and Zipp, 2010; Piore, 2016). This process leads to cumulative neuronal loss over time (Lull and Block, 2010) and eventually becomes clinically evident.

The combined evidence in ASD is suggestive of a progressive childhood encephalopathy (Kern et al., 2013). Encephalopathy, defined as a disease, damage, or malfunction of the brain, consists of various types, e.g., mitochondrial encephalopathy, hepatic encephalopathy, toxic encephalopathy, Hashimoto's encephalopathy, metabolic encephalopathy, toxic-metabolic encephalopathy, neonatal encephalopathy, etc. A recent review of the literature shows that the clinical manifestation of sequelae following insult can be delayed from weeks to years. Therefore, it is biologically plausible for a childhood encephalopathy associated with developmental neurotoxicant exposures, especially among males, could later manifest as ASD sequelae (Kern et al., 2017).

Strengths / Limitations

An important strength of this study was that retrospective observations made in the IHRD were derived from eligibility and claims records prospectively generated as part of the routine healthcare provided for persons in the Florida Medicaid system. Therefore, the data examined were generated completely separate from the current study design. The healthcare providers submitting claims for ASD and encephalopathy diagnoses were most likely not thinking about the possible relationship between ASD and encephalopathy diagnoses.

The study design utilized to examine the IHRD was another important strength of the present study. All persons examined in this study were eligible for Florida Medicaid from birth for 120 months (no gaps in eligibility were allowed). In addition, in order to ensure that the cohort of persons examined was actively utilizing healthcare services from the Florida Medicaid system, all persons examined in this study had to have ≥ 10 outpatient office visit claims submitted (that averages to at least one outpatient office visit per person per year). These requirements helped to significantly reduce possible enrollment factors or difference in healthcare-seeking behaviors among the persons examined in this study.

Further, the diagnosis status was determined with precision for each person because detailed information regarding outcomes using ICD-9 diagnosis coding and dates of service for claims submitted on behalf of each person were examined. In order for a person to be recognized as having an ASD diagnosis, the initial date of service specifying an ASD diagnosis was identified. Similarly, in order for a person to be recognized as having an encephalopathy diagnosis, the initial date

of service specifying an encephalopathy diagnosis was identified.

Finally, the use of Cox proportional hazards survival plot modeling to evaluate cases of encephalopathy diagnosed over a period of many years in the ASD diagnosed and undiagnosed cohorts allowed for us to draw inferences regarding the relationship between exposures and outcomes. Specifically, it was observed that the relationship was significant when considering the time variable in terms of person-years of follow-up. In order to further consider the Cox proportional hazards survival plot modeling results, logistic regression modeling was undertaken (no consideration of the time variable), and as revealed in Table V, the results remained significant for the risk of an encephalopathy diagnosis in the unadjusted (odds ratio=7.96) and adjusted (odds ratio=7.46) models.

It is possible that a potential limitation of this study was that the findings observed were the result of statistical chance or cofounders/unknown biases in the data. Statistical chance seems unlikely given that a limited number of statistical tests were performed and most results were highly statistically significant. In addition, it was observed that the significant effects observed in unadjusted models remained significant even when adjusting for potential covariates such as gender, date of birth, and county of residence. The results observed this study were also consistent with previous epidemiological observations on different populations and are biologically plausible.

It is also be possible that some of the persons examined in the IHRD may have had symptoms of encephalopathy that were so slight that they were not noted by their healthcare providers, or healthcare providers may have misdiagnosed or misclassified exposure status for some persons. Further, encephalopathy is an imprecise diagnosis, and, as such, there may be differences in symptomology between persons. However, these potential limitations, while possible, should not have affected the results appreciably because it is uncertain how differential application would have occurred in the exposed and unexposed cohorts examined. Importantly, any misclassification with respect to diagnostic or exposure status, would in all likelihood bias the findings towards the null hypothesis because persons examined would have been put into the wrong exposure and/or diagnostic category, and result in diminished statistical power to establish the accurate relationship between exposures and outcomes.

Another potential limitation of the present study is that the underlying direct etiological basis for an ASD diagnosis was not examined. It was hypothesized that the results observed in this study were the result of a neuroinflammation-associated progressive encephalopathy resulting in an ASD diagnosis. However, no direct brain pathological evidence was examined to support this hypothesis. It is recommended in future studies that direct brain pathological evidence be examined among children diagnosed with an ASD.

CONCLUSION

This retrospective cohort study of prospectively collected healthcare data from the IHRD provides new evidence that the incidence rate of childhood encephalopathy diagnoses are significantly more frequent in a cohort of children diagnosed with an ASD in comparison to a cohort of children not diagnosed with an ASD. Furthermore, the risk was greatest for persons diagnosed with a childhood encephalopathy at >1 year-old as compared to those diagnosed with a childhood encephalopathy at <1 year-old. Finally, the IHRD based upon the results obtained in this study, is an important epidemiological resource to help quantitatively evaluate public health issues.

Overall, the results of the present longitudinal epidemiological study provide evidence that a significant

Table V. Logistic regression model results examining the relationship between an encephalopathy diagnosis in the ASD diagnosed and undiagnosed cohorts.

Model	Variable	Odds Ratio	95% Odds Ratio CI	p-value	χ^2
I					
	ASD (Diagnosed vs. Undiagnosed)	*7.96*	*6.99 to 9.07*	*<0.0001*	*975*
II					
	ASD (Diagnosed vs. Undiagnosed)	*7.46*	*6.54 to 8.52*	*<0.0001*	*888*
	Gender (Female vs. Male)	*0.81*	*0.76 to 0.86*	*<0.0001*	*40*
	County of Residence	*0.99*	*0.98 to 0.99*	*<0.0001*	*210*
	Date of Birth	1.00	1.00 to 1.00	0.86	0.03

Italicized results are statistically significant. Model I = unadjusted, Model II = adjusted for gender, county of residence, and date of birth. CI=confidence interval.

number of children diagnosed with an ASD undergo a developmental regression as manifested by a childhood encephalopathy diagnosis with an onset at >1 year-old. Future studies should further evaluate the relationship between childhood encephalopathy and ASD by determining potential postnatal factors contributing to childhood encephalopathy diagnoses.

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