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Control Groups in Pediatric Epilepsy Research: Are Familial Effects Present in First Degree Cousins?

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Abstract

Aim: To determine whether the first degree cousins of children with idiopathic focal and genetic generalized epilepsies (cases) show any association across measures of cognition, behavior and brain structure. The presence/absence of associations addresses the question of whether and to what degree first degree cousins may serve as unbiased controls in research addressing the cognitive, psychiatric, and neuroimaging features of pediatric epilepsies.

Methods: Participants were children (age 8 – 18) with epilepsy who had at least one first-degree cousin control enrolled in the study (n=37) and all enrolled cousin controls (n=100). Participants underwent neuropsychological assessment, brain imaging (cortical, subcortical and cerebellar volumes) and parents completed the Child Behavior Checklist (CBCL). Data (42 outcome measures) from cousin controls were regressed on the corresponding epilepsy cognitive, behavioral and imaging measures in a linear mixed model and case – control correlations were examined.

Results: Of the 42 uncorrected correlations involving cognitive, behavioral and neuroimaging measures, only 2 were significant ($p < 0.05$). The median correlation was 0.06. A test for whether the distribution of p-values deviated from the null distribution under no association was not significant ($p > 0.25$). Similar results held for the cognition/behavior and brain imaging measures separately.

Conclusions: Given the lack of association between cases and first degree cousin performances on measures of cognition, behavior and neuroimaging, the results suggest a non-significant genetic influence on control group performance. First -degree cousins appear to be unbiased controls for cognitive, behavioral, and neuroimaging research in pediatric epilepsy.

Keywords

epilepsy; cousin controls; research design; cognition; behavior; brain imaging

Introduction

Studies investigating the neurobehavioral comorbidities of pediatric epilepsy have collectively used a variety of control groups that include siblings or other family members, friends, persons with other chronic medical conditions, or individuals from the general population. Approaches to selecting a particular control group vary with the intent and design as well as the constraints of each individual study. Furthermore, each potential choice of control group is associated with a range of unique advantages and disadvantages.

Sibling controls have been a commonly used control group in studies of behavioral, cognitive and neurodevelopmental comorbidities in pediatric epilepsy (Austin et al., 2002; Austin et al., 2001; Austin et al., 2011; Benn et al., 2010; Bennett-Back et al., 2011; Berg et al., 2008; Bourgeois et al., 1983; Dunn et al., 2010; Fastenau et al., 2009; Hesdorffer et al., 2012; Ong et al., 2010; Smith et al., 2012; Sogawa et al., 2010; Zelko et al., 2014). One advantage of siblings as controls is that they allow for better control of unmeasured or unknown family-level environmental factors (Austin et al., 2002; Benn et al., 2010; Riva et al., 2002) given that they share similar sociocultural backgrounds and family experiences. Use of a sibling control group, therefore, can provide an advantage by controlling for differences in environmental experiences that might contribute to cognitive, psychiatric, or behavioral comorbidities. In addition, siblings may provide partial control for genetic factors which may assist in discriminating between disorder-specific sequelae and comorbidities attributed to a genetic predisposition related to the disorder of interest. Research designs that use sibling controls also provide practical benefits for recruitment and retention as siblings commonly live in the same house (or in geographically nearby areas) and are therefore easier to recruit (Gauderman et al., 1999; Witte et al., 1999). Siblings also tend to be more invested in study goals and outcomes than controls from the general population, making them more motivated to participate and maintain longitudinal participation (Gauderman et al., 1999). These practical advantages make the use of sibling control groups an appealing control group.

Despite these benefits, the close genetic relationship between siblings can be problematic for interpreting results of cognitive and behavioral comorbidities as well as neuroimaging anomalies, particularly when the primary disorder under investigation (i.e., childhood epilepsies) may have genetic associations to the behaviors under investigation--thereby making "unaffected siblings" a potentially less than optimal or biased control group. When cases have disorders with genetic correlates and also have a large amount of shared genetic variance with controls, it can reduce bias but require larger sample sizes to delineate the genetic contributions to the disorder under examination. In such cases, the effect of genetic differences may be less easily identified due to fewer genetic differences between participants and controls, therefore, siblings may mask true genetic effects that might otherwise be revealed using a control group that is more genetically distant (Heins et al.,

2011). Consistent with these concerns, past studies have shown that unaffected siblings of those with epilepsy tend to be more similar on a number of neurocognitive, behavioral and neuroimaging measures compared to general population controls (Alhusaini et al., 2015; Aronu and Illoeje, 2011; Badawy et al., 2013; Chowdhury et al., 2014; Hesdorffer et al., 2012; Iqbal et al., 2015; Iqbal et al., 2009; Singhi et al., 1992; Tsai et al., 2013; Verrotti et al., 2013; Wandschneider et al., 2014; Wandschneider et al., 2010).

The general population, on the other hand, is usually considered ideal as a potential control group across different areas of scientific research (Ho et al., 2008). Using a sample from the general population as a control group provides for more genetic variability in the study sample, but may also lead to confounding due to genetic admixture. The concern is that a putative correlate of disease status may not be a result of the disease at all, but rather may arise owing to unmeasured population variation in genes that influence both disease occurrence and the manifestation of the putative correlate. While considered ideal in most research, general population controls may be more difficult to recruit compared to siblings, making the recruitment process more time and cost intensive (Gauderman et al., 1999). Additionally, as individuals from the general population may be more likely to have differing life experiences and sociocultural backgrounds, general population controls may provide less protection from environmental and demographic confounds compared to siblings (Gauderman et al., 1999; Gur et al., 2015; Riva et al., 2002). Lastly, and most importantly, general population controls may be less motivated to maintain participation in research studies compared to siblings (Gauderman et al., 1999), making longitudinal retention rates lower, reducing the study's statistical power and possibly introducing selection bias.

First degree cousin controls provide an alternative to siblings and population-based controls. Cousins have been used in a variety of fields but not frequently employed in epilepsy research. In genetics and child development research, family-based association studies use cousins to reduce sociocultural and genetic variance and confounding (Gauderman et al., 1999; Geronimus et al., 1994; Turley, 2003; Witte et al., 1999) and utilization of such family-based designs has extended into diverse fields including schizophrenia, ADHD, and behavioral and social psychology research (D'Onofrio et al., 2010; Giordano et al., 2015; Gur et al., 2015; Gur et al., 2007; Kendler et al., 2015; Larsson et al., 2014; Ljung et al., 2013; McIntosh et al., 2005; Skoglund et al., 2014). Cardy et al. (2007) used a pure cousin control group to examine risk of a congenital disorder, while Riva et al. (2002) used a mixed cousin and sibling control group to study the effect of a surgical cancer treatment on cognitive functioning in a pediatric population.

Using cousins as controls represents one potential approach to combine the practical advantages of sibling controls with the strengths of general population controls. In fact, some studies (Giordano et al., 2015; Skoglund et al., 2014) have shown that general population controls share more similarities with cousins than siblings in terms of psychiatric outcomes, suggesting that cousins are genetically distant enough from participants to serve as a substitute for general population controls. Additionally, there is typically a larger pool of cousins available for recruitment and they are easier to age match than siblings

(Gauderman et al., 1999; Witte et al., 1999), making recruitment of cousins more efficient for control groups.

Cousin controls may have other advantages over general population controls as longitudinal studies require multiple follow-up assessments and retention of a related control group is usually better compared to unrelated controls (Gauderman et al., 1999). Similar to siblings, cousin controls tend to be more invested in the research given their close connection with an affected family member (Gauderman et al., 1999) with stronger incentive to participate in the study and cooperate with study tasks; all serving to reduce the amount of time, effort and financial resources needed to enroll and retain participants in the investigation (Gauderman et al., 1999).

The purpose of the current study is to evaluate potential biases associated with use of first degree cousin controls in research examining cognitive, psychiatric, and neuroimaging comorbidities of pediatric epilepsy. As noted, previous research has shown that unaffected siblings may share cognitive, behavioral or imaging “anomalies” with their affected siblings, manifesting as correlations between case and sibling controls. That is, there is greater relatedness between patients with epilepsy and unaffected siblings compared to population based controls. Examination of the relatedness of children with epilepsy to first degree cousins would similarly speak to the bias associated with a cousin control group. In the current study we examined whether outcomes of youth with recent onset epilepsy predict their healthy cousins outcomes across a wide range of neurocognitive, behavioral, and neurobiological (i.e. MRI) measures. Our primary assumption is that the degree of bias in a control group, such as first degree cousins, will be reflected in the number and strength of associations between the experimental and control group across dependent measures of interest. The more correlations observed between epilepsy and cousin controls, the more biased the control group. The fewer and weaker the associations between groups, the more the controls approach the status of an unbiased comparison group.

Methods

Participants

We recruited children with recent and new-onset epilepsy and their healthy first-degree cousin controls, aged 8 to 18 years, from pediatric neurology clinics at three Midwestern medical centers (University of Wisconsin-Madison, Marshfield Clinic, Dean Clinic). The following inclusion criteria were used for children with epilepsy: 1) a diagnosis of epilepsy within the past 12 months, 2) no other developmental disabilities or neurological disorders, 3) normal neurological examination, and 4) normal neuroimaging (magnetic resonance imaging or computed tomography) results. Prior to recruitment, the medical records for all patients were independently reviewed by a board-certified pediatric neurologist to confirm that participants had epilepsy, to provide confirmation of syndrome diagnosis, and to ensure that all other study criteria (e.g. normal neuroimaging) were met. Specific syndromes were classified using the modified diagnostic criteria of the International League Against Epilepsy Task Force on Classification and Terminology (Berg et al., 2010).

Participants in the control group were first-degree cousins and met the following inclusion criteria: 1) no history of seizures, 2) no early initial precipitating injuries (e.g. febrile convulsions), 3) no developmental or neurological disease, or 4) no history of loss of consciousness greater than five minutes. To obtain first degree cousin controls, the mother of each participating child with epilepsy was asked to identify associated families with a potential first degree cousin with epilepsy who met the standard inclusion and exclusion criteria. Potential candidate control families were then contacted by the study investigator who confirmed study requirements. More details regarding subject selection criteria have been published previously by our group (Hermann et al., 2006).

Procedures

This study was reviewed and approved by the Institutional Review Boards of all participating institutions. Families and children gave informed consent and assent on their study participation day. All procedures were consistent with the Declaration of Helsinki (1991). The children subsequently underwent neuropsychological testing and an MRI scan and parents completed the Child Behavior Checklist (CBCL).

Neuropsychological assessment.—Participants received comprehensive neuropsychological testing using a test battery that included standardized measures of intelligence, academic achievement, executive function, processing speed and memory. Test selection was based on domains of interest and applicability to a broad age range to ensure the same test versions could be administered to children of varying ages (Table 1). A parent completed measure of emotional and behavioral problems was also included and is listed in Table 1.

MRI acquisition.—Images were obtained on a 1.5T GE Signa MRI scanner (GE Healthcare, Waukesha, WI, U.S.A.). Sequence acquired for each participant was a T1-weighted, three-dimensional (3D) spoiled gradients recall (SPGR) using the following parameters: TE = 5ms, TR = 24ms, flip angle = 40 degrees, NEX = 1, slice thickness = 1.5mm, slices = 124, plane = coronal, field of view (FOV) = 200mm, matrix = 256 × 256. All MR images were inspected before image processing. Image quality was rated on a five-point (0 – 4) scale and we required a minimum quality of 3 for the scan to be included in this analysis.

The reported volumetric data were produced using Freesurfer 5.3. Post-processed data were visually inspected, and manual interventions were used to correct surface errors. In addition to the standard Freesurfer processing stream, we also extracted the cortical gray matter surface volume using a larger lobar parcellation scheme.

Analytic Plan.—For this analysis, we included only children with epilepsy with at least one related cousin in the study (n=37) and all enrolled cousins (n=100). Cognitive and behavioral scores used in this analysis are specified in Table 1, and quantitative MRI measures used are listed in Table 2.

Population controls would be expected to differ *on average* from epilepsy (case) participants on many of the measures, but the concern of interest here is the extent to which cousin

controls will differ from population controls owing to their genetic, socio-cultural, and environmental proximity to the cases. To test this, we exploited the variability in the association of the foregoing measures in the cases. The logic was as follows. If the cousins tend to be a biased sample from the general population perspective on these measures, owing to having a cousin case (or proband) with epilepsy, then, within our sample, we would expect the control cousins of more impaired cases to perform more poorly and for those of less impaired cases to perform better. That is, we expect the cousin control measures to be positively correlated with the epilepsy case measures.

To test the degree to which this is true, the cousin measures were regressed on their corresponding epilepsy case measures in a linear mixed model (Laird and Ware, 1982)

$$\text{Cous}_{ij} = \beta_0 + \beta * \text{Epi}_i + U_i + \epsilon_{ij}.$$

In this model, for a given measure, the cousin control and epilepsy case measures are given by Cous_{ij} and Epi_i , U_i is the nuclear family-level random effect, and ϵ_{ij} is the residual error. The family random effect accounts for the fact that in our design more than one cousin control could be linked to a given case; the multiple cousins are often siblings with one another in and thus correlated. In the model, the variance of the cousin measures is $\beta^2 * \text{Var}_{\text{Epi}} + \text{Var}_U + \text{Var}_\epsilon$, and the covariance between the control and case measures is $\beta * \text{Var}_{\text{Epi}}$. The intercept β_0 accounts for the fact that the mean control response is on average better than the mean case response.

For each measure, analyses included computing the empirical mean and variance of both the epilepsy cases and cousin controls. The mixed effects model was then fitted to the data; the fitted model-based variance of the cousins was compared to the empirical variance to ensure a close match (which always occurred). Using the fitted model, the correlation between the cases and their cousin controls was computed and tabulated, and the p-values testing $\beta = 0$ were obtained. Aggregate analyses compared the distribution of p-values across all measures to the uniform (0,1) distribution that would be expected if there were no association in the measures between cases and controls. We also examined the distribution of correlation values for the degree to which they were centered on zero. Mixed models were estimated using PROC MIXED and aggregate summaries were constructed using PROC UNIVARIATE and PROC SGPLOT in SAS version 9.4 (Cary, NC).

3. Results

Table 3 provides information regarding the epilepsy and cousin control participants. We fitted the model to the 42 distinct measures (13 cognitive, 4 behavioral, 25 imaging). Table 1 and Table 2 provide descriptive statistics and correlation coefficients for the combined 17 cognitive and behavioral scores and the 25 brain volume measurements respectively. All correlations ranged between -0.28 to 0.43 (median= 0.06). P-values obtained from the mixed models were plotted against a Uniform (0, 1) distribution (Figure 1). The P-values were found to be concentrated near the 45 degree diagonal line, which means their distribution was close to to the uniform (0, 1) distribution which would be expected under the null hypothesis of no association. Out of the 42 P-values, only 2 were <0.05 and

approximately 2 of 42 tests can be expected to be significant at 0.05 level. A Kolmogorov-Smirnov (KS) test examining whether the distribution of P-values deviated from the Uniform (0,1) was not significant (KS = 0.12, $p > 0.25$). Associations were also examined as a function of domain of interest (cognition and behavior vs. brain measurements). Figures 2 and 3 show p-values plotted against a Uniform (0, 1) distribution for cognitive and behavioral (Figure 2) and imaging (Figure 3) measures. Again, there was no overall significant association of the domain specific measurements (KS for cognition and behavior = 0.15, $p > 0.25$; KS for brain measurements = 0.19, $p > 0.25$). The correlations ranged between -0.17 to 0.24 (median=0.08) for the cognition and behavior measures, and -0.28 to 0.43 (median= 0.05) for the brain measurements.

Taken across the 42 measures, and within each of the sub-domains, the minimal association between cousins and cases suggests no selection bias in the cousin group relative to the general population.

4. Discussion

The results of this study reveal minimal correlative relationships between youth with epilepsy and their unaffected first degree cousins across a diverse array of neuropsychological, behavioral, and neuroimaging measures, even after accounting for differences due to having epilepsy versus unaffected by epilepsy. The landscape of dependent measures was quite broad, with the neuropsychological measures representing classic domains of cognitive ability (intelligence, language, learning and memory, executive function, processing speed), the behavioral measures assessing broad and commonly used measures of externalizing and internalizing disorders and social competence, and the neuroimaging measures examining cortical, subcortical, and cerebellar volumes. This lack of association between the epilepsy participants and cousin controls suggests a lack of selection bias in the latter, which is especially important as some epilepsies with genetic contributions were included. The obtained pattern of results is the pattern that would be expected if epilepsy participants were compared to population based controls, although such comparisons were not possible in the current study.

In the current study, participants with epilepsy were not required to supply a cousin control in order to meet study inclusion criteria. Whereas this protects against introducing a sampling bias in the patient group, it does present a possible sampling bias threat in the cousin control group. Thus, our cousin control group may not be representative of all cousins of youth with epilepsy. Additionally, we were not able to directly examine the relationship between cousin controls and general population controls on any of the measures collected in this study. Such a comparison would provide insight into the validity of substituting cousins for general population controls in epilepsy research and should be considered in future research. Additionally, our behavioral measures were limited in scope compared to the wide range of cognitive and quantitative MRI variables; however, it is important to note that correlations on these measures were not significant. The current study provides preliminary evidence that use of first degree cousins as controls is defensible. Given the associated advantages associated with this group it may be worthwhile to consider recruiting first degree cousins in medical and neurological research.

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Questions

1. Control groups used in pediatric epilepsy behavioral research include: a) siblings, b) general population, c) cousins, d) all of the above.
Correct answer: d
2. Children with epilepsy and their first degree cousins show several significant correlations across measures of cognition, behavior and brain imaging: True or False
Correct answer: False
3. An advantage of sibling and cousins control groups is easier recruitment and better retention compared to population controls. True or False
Correct answer: True

Figure 1.
QQ plot for all 42 P-values obtained from the mixed models against a uniform (0, 1) distribution.

Figure 2. QQ plot for 17 cognition and behavior measures p-values obtained from the mixed models against a uniform (0, 1) distribution.

Figure 3.

QQ plot for 25 brain measures p-values obtained from the mixed models against a uniform (0, 1) distribution.

Table 1.
Descriptive statistics and correlation coefficients for cognitive and behavioral variables by assessment

Assessment	Variable	Epilepsy Cases			Cousin Controls			rho
		N	Mean	Variance	N	Mean	Variance	
WASI ¹	Full-scale IQ	37	106	13	100	110	11	0.09
	Performance IQ	37	105	14	100	108	12	0.04
	Verbal IQ	37	106	13	100	109	13	0.08
WRAT-3 ²	Arithmetic Standard Score	37	102	10	100	107	12	0.22
	Reading Standard Score	37	107	11	100	105	10	0.18
	Spelling Standard Score	37	105	13	99	105	12	-0.04
CMS ³	Learning Scaled Score	27	10	3	81	10	3	-0.17
	Letter Fluency Scaled Score	37	10	3	100	11	3	0.05
D-KEFS ⁴	Category Fluency Scaled Score	37	12	3	100	12	3	0.02
	Category Switching Accuracy Scaled Score	37	9	3	100	10	3	0.08
	Color-word Inhibition Scaled Score	35	10	3	100	11	2	-0.04
	Total Confirmed Correct Sorts Scaled Score	37	10	2	100	10	2	-0.02
WISC-III ⁵	Digit Symbol Coding Scaled Score	32	9	2	94	10	3	-0.09
	Total Competence T-score	37	50	12	98	52	9	0.10
CBCL ⁶	Internalizing Problems T-score	37	55	12	100	49	10	0.24
	Externalizing Problems T-score	37	49	12	100	46	10	0.10
	Total Problems T-score	37	52	13	100	46	11	0.24

Note.

¹WASI: Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999)

²WRAT-3: Wide Range Achievement Test - 3 (Wilkinson, 1993)

³CMS: Children's Memory Scale (Cohen, 1997)

⁴D-KEFS: Delis-Kaplan Executive Function System (Delis et al., 2001)

⁵WISC-III: Wechsler Intelligence Scale for Children - III (Wechsler, 1991)

⁶CBCL: Child Behavior Checklist for Ages 6–18 (Achenbach and Rescorla, 2001)

Table 2.

Descriptive statistics and correlation coefficients for brain volume measurements

Measurement (Volume)	Epilepsy Cases			Cousin Controls			rho
	N	Mean	Variance	N	Mean	Variance	
Total Cortical White Matter	23	443344	73474	58	423439	49493	0.25
Total Gray	23	736460	84282	58	728926	55685	0.28
Left Cerebellum Cortex	23	60833	5826	58	60278	5204	-0.12
Right Cerebellum Cortex	23	62070	6348	58	61091	5316	-0.06
Left Cerebellum White Matter	23	13444	1984	58	13609	1433	-0.28
Right Cerebellum White Matter	23	13451	1981	58	13561	1462	-0.05
Total Cerebellum	23	149798	15017	58	148539	12410	-0.17
Left Cingulate	23	12355	2031	58	11837	1450	0.43
Right Cingulate	23	11726	1849	58	11340	1419	0.02
Left Frontal	23	103482	12929	58	103027	9438	0.23
Right Frontal	23	103225	12641	58	102943	9162	0.25
Left Insula	23	7941	1205	58	7779	775	0.04
Right Insula	23	7779	1003	58	7558	880	-0.01
Left Occipital	23	25449	3052	58	25458	2757	0.22
Right Occipital	23	26249	2572	58	25967	2563	0.23
Left Parietal	23	67247	9640	58	66305	7022	0.30
Right Parietal	23	68028	10021	58	67825	6910	0.32
Left Temporal	23	61380	9540	58	60397	5708	0.37
Right Temporal	23	60664	9461	58	60031	5641	0.36
Left Thalamus Proper	23	8006	944	58	8067	650	0.00
Right Thalamus Proper	23	7920	892	58	8066	669	0.05
Total Thalamus	23	15925	1802	58	16133	1262	0.03
Left Hippocampus	23	4282	494	58	4308	551	-0.07
Right Hippocampus	23	4435	544	58	4407	465	0.16
Total Hippocampus	23	8717	1005	58	8714	919	0.04

Table 3.

Participant demographics

	Epilepsy Cases		Cousin Controls	
	IGE (n=21)	LRE (n=16)	Total	Total
Age in Months: M (SD)	157.62 (45.76)	135.5 (28.18)	148.05 (40.22)	150.51 (35.25)
Gender:				
Male	9 (42.9%)	11 (68.7%)	20 (54.1%)	49 (49.0%)
Female	12 (57.1%)	5 (31.3%)	17 (45.9%)	51 (51.0%)
Specific Syndrome:	Absence: 7 (18.9%)	BECTS: 6 (16.2%)		
	JME: 10 (27%)	BOE: 1 (2.7%)		
	PGE NOS: 4 (10.8%)	TLE: 3 (8.1%)		
		Focal NOS: 4 (10.8%)		
		Frontal: 2 (5.4%)		