I, Sarah A Hrabik, hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

It is entitled:
The Clinical Utility of a SNP Microarray in Patients with Epilepsy at a Tertiary Medical Center

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This work and its defense approved by:

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Committee member: Valentina Pilipenko, Ph.D.
Committee member: Christine Spaeth, MS, CGC
The Clinical Utility of a SNP Microarray in Patients with Epilepsy at a Tertiary Medical Center

A thesis submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

Master of Science
In the
College of Medicine
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by

Sarah A. Hrabik
BS, University of Dayton, 2011
Committee Chair: Derek E. Neilson, MD
Committee Members: Christine G. Spaeth, MS, CGC
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Abstract

Background: Microarray testing has revolutionized clinical cytogenetics, as it provides a significantly higher resolution and greater clinical yield than karyotype analysis. Previously, microarray studies have been performed in patients with developmental delay, intellectual disability, autism, and multiple congenital anomalies. This study assessed the clinical utility of Single Nucleotide Polymorphism (SNP) microarray in patients with epilepsy. Methods: Study subjects were patients (between the ages of birth-23 years) who were diagnosed with epilepsy and had a SNP microarray performed at Cincinnati Children’s Hospital Medical Center. Chi-square test and Fisher’s exact test were employed to examine the association of microarray results and the following laboratory or clinical features: brain MRI, seizure type, and functional and structural malformations. Results: One hundred and forty seven individuals met all study criteria. Of the 147 individuals studied, 17.7% (26/147) had an abnormal microarray. However, there was no difference in frequency of abnormal brain MRI or seizure type between the abnormal and normal microarray groups. Musculoskeletal malformations occurred in 38.5% of subjects with abnormal microarray results and 14.1% of subjects with a normal microarray (p<.0035). Cardiovascular malformations occurred in 30.8% of subjects with an abnormal microarray and 10.7% of subjects with normal microarray results (p<.0081). Conclusions: Brain MRI and seizure type are not helpful indicators of an abnormal microarray. Clinicians should consider SNP microarray analysis in individuals who have epilepsy in combination with musculoskeletal malformation or cardiovascular malformation.
Acknowledgements

I would like to acknowledge the hard work and guidance of my research committee, Christine G. Spaeth, MS, CGC, Shannon M. Standridge, MPH, DO, Hansel M. Greiner, MD, Derek E. Neilson, MD, Valentina V. Pilipenko, PhD, Sarah L. Zimmerman, PhD, and Jessica A. Connor, MS. I would also like to thank bioinformatics specialist Jason M. Tillman for his assistance in this project. REDCap electronic data collection tools hosted by the University of Cincinnati are made available through the Center for Clinical and Translational Science and Training grant support (UL1-RR026314-01 NCRR/NIH).
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Introduction

Epilepsy is a common condition that is estimated to affect 2.2 million people in the United States.\(^1\) A genetic etiology underlies epilepsy in approximately 40% of individuals, a majority of which is thought to be due to complex genetic inheritance with multiple interacting genes.\(^2\)\(^-\)\(^3\) There are 200 single gene disorders in which epilepsy is a clinical feature, and approximately 1% of individuals with epilepsy have an identifiable mutation in a single gene.\(^4\)\(^-\)\(^5\) Over 400 chromosomal imbalances and eight chromosomal disorders have been associated with seizures or electroencephalogram abnormalities.\(^5\) Although a majority of epilepsy with a genetic basis is thought to be due to a complex genetic inheritance, tests for multiple interacting genetic variants are not clinically available.

Single Nucleotide Polymorphism (SNP) microarray analysis has revolutionized clinical cytogenetics, as it provides a relatively quick method to scan the entire genome for variations with significantly higher resolution than was previously possible with karyotype analysis.\(^6\) Current SNP microarrays can have over 1,000,000 oligonucleotide probes and provide a resolution of less than .5MB.\(^7\) SNP microarray technology uses whole-genome screening to detect copy number variations including deletions, duplications, and regions of homozygosity (ROH). Large ROH suggest a common ancestor, and raise the risk for an autosomal recessive disorder.

Microarray studies have been conducted in patients with developmental delay, intellectual disability and autism spectrum disorders.\(^8\)\(^-\)\(^10\) Studies have shown that microarray analysis yields an abnormal result in 10-15% of children with intellectual disability and 10-20% in those with multiple congenital anomalies and congenital heart defects.\(^6\)\(^,\)\(^11\)\(^-\)\(^12\) In 2010, a statement from the International Standard Cytogenomic Array (ISCA) Consortium recommended
microarray testing as a first-tier test in patients with unexplained developmental delay, intellectual disability, autism, and multiple congenital anomalies.\textsuperscript{13}

The goal of our study was to assess the clinical utility of using a SNP microarray in patients diagnosed with epilepsy. The first specific aim of our study was to describe the patient population with epilepsy that had an abnormal microarray result. The second aim of our study was to determine whether epileptic patients' other clinical findings were associated with an abnormal microarray. Factors such as brain MRI findings or structural malformations in various body systems could be used to refine indications for SNP microarray testing in cases of epilepsy.

**Methods**

The study conducted was a retrospective medical record review. A bioinformatics query of medical records documented in the electronic medical record system EPIC at Cincinnati Children’s Hospital Medical Center (CCHMC) was used to identify subjects between the ages of birth to 23 years who were diagnosed with epilepsy (ICD9 code 345.X) and had a SNP microarray performed at CCHMC (between January 2009 and February 2012). One hundred and seventy eight subjects met initial inclusion criteria. Of these 178 subjects, 152 were currently followed in the Division of Neurology at CCHMC and had a brain MRI available for review. Encounter and billing diagnoses for each subject were retrieved and stored in a Microsoft Access Database. Subjects were excluded if they had an encounter diagnosis of epilepsy but had chart notes indicating a diagnosis of spells, a change in epilepsy diagnosis to a diagnosis of migraine with aura, or seizures due to breath holding episodes. One hundred and forty seven subjects met all study criteria.

Chart review abstracted the following categories of information: 1) presenting medical indications other than epilepsy, including functional and/or structural abnormalities; 2) epilepsy
diagnosis and seizure type at first and last visits; 3) brain MRI result; and 4) microarray result. All data was recorded and stored in a Redcap database. The study received IRB approval by both CCHMC and the University of Cincinnati.

**Structural Malformations**

Structural malformations for each patient were obtained from a Microsoft Access database created by a CCHMC bioinformatics specialist which included both encounter and billing diagnosis (JMT). Each subject’s diagnoses were further classified into functional and structural malformations. Structural malformations were classified into seven groups including neurological, ophthalmological, otolaryngological, genitourinary, cardiovascular, and gastrointestinal malformations. The specific malformation and total numbers of systems affected were also recorded. Accuracy of the classifications was reviewed by an expert panel consisting of a geneticist and a genetic counselor (DEN and CGS).

**Epilepsy Diagnosis and Seizure Type**

Epilepsy diagnosis and seizure type were classified based on the 2010 International League Against Epilepsy (ILAE) published revised guidelines for the terminology and classification of seizures.\(^{14}\) Seizures were classified into three main categories: generalized, focal, and undetermined. Epilepsy etiologies were classified as structural/metabolic, genetic, or unknown. Epilepsy and seizure diagnosis at both the first visit and the last visit were recorded to determine if the diagnosis evolved over time or remained unchanged.

**Brain MRI results**

Brain MRI results were obtained from the subject’s electronic medical record. Results from the original MRI interpretation were classified by a neurologist/epileptologist as normal,
abnormal and indicative of epilepsy, abnormal and not indicative of epilepsy or inconclusive (HMG).

**Microarray Results**

Microarray results were obtained from CCHMC’s cytogenetic database. Microarrays performed between January 2009 to October 2010 were analyzed with the Illumina® Human610-Quad v1.0 BeadChip, and microarrays performed between October 2010 to February of 2012 were analyzed with Illumina® HumanOmni1-Quad BeadChip (Illumina Inc., San Diego, CA, USA). Beadchips contained approximately 610,000 to 1,140,419 markers, respectively. DNA was isolated using the Qiagen DNA isolation kit (Qiagen, Valencia, CA, USA) and processed using the Illumina Infinium®HD Assay, following manufacturer protocol (Illumina Inc., San Diego, CA, USA). Data was analyzed using the Illumina GenomeStudio v2009.2 and v2011.1 analysis software. DNA copy number changes were examined using cnvPartition Plug-ins v2.3.4 and v3.1.6 software in addition to visualization of the B allele frequency and \( \log_2 R \) ratio.

For the purpose of statistical analysis, microarray results were represented as categorical data. The significance of microarray results was determined by CCHMC Clinical Cytogenetics Laboratory guidelines, which takes into account the presence of genes in the abnormal region, gene content, size, and prior samples with same abnormality. Abnormal microarray results included deletions, duplications, ROH, and variants of unclear clinical significance. Patients with results of unclear clinical significance were re-classified as normal if parental studies determined the variant to be a likely benign change.

**Statistics**
Data were summarized using percentages and frequencies. Chi-square test and Fisher’s exact test were employed to examine the association of microarray results with 1) MRI results; 2) seizure types; 3) functional malformations; and 4) structural malformations. Associations were considered nominally significant at \( \alpha \leq 0.05 \). Bonferroni correction with an \( \alpha \leq 0.007 \) was employed to account for multiple testing error of the seven groups of structural malformations. Data were analyzed in R statistical software (http://cran.r-project.org).

**Results**

**Demographics**

A total of 147 children (between the ages of birth to 23 years) met the inclusion criteria. Out of the 147 participants there were 64 females (43.5%) and 83 males (56.5%). Demographic data outlining the race and ethnicity of the participants is in Table 1.

**Abnormal Microarray Results in Children with Epilepsy**

Out of our patient population, 17.7% (n=26) had an abnormal microarray. Abnormal microarrays of clinical significance included: 1p36 deletion syndrome (n=2); ring chromosome 6 (n=1); Velocardiofacial syndrome (n=1); Williams Syndrome Plus (n=1); mosaic Trisomy 9 (n=1); large chromosomal deletions of 5.9-7.8 Mb (n= 2); Tetrasomy 15 q syndrome (n=1), unbalanced translocation (n=1); and MECP2 duplication syndrome (n=1); (Table 2). Abnormal results also included areas of unknown clinical significance in which there was a deletion or duplication at a susceptibility loci associated with neurodevelopmental issues (n=8), deletions or duplications smaller than previously reported but in areas of known disorders (n=2), deletions or duplications not previously reported (n=3) and areas with a copy number loss of heterozygosity, (n=2; Table 2).

**Comparison among abnormal and normal microarray groups**
Brain MRI

In comparing brain MRI within the abnormal and normal microarray groups, there was not a significant difference between the four MRI classifications (Table 3). In the abnormal microarray population, 53.9% (14/26) had an abnormal MRI, whereas in the normal microarray population 56.2% (68/121) had an abnormal brain MRI. Comparing patients with abnormal or normal microarray resulted in no significant differences in the frequency of abnormal MRI results indicative of epilepsy or not indicative of epilepsy.

Seizure type

In 87.1% of cases (n=128), seizure type (focal, generalized, or undetermined) did not change between first and last visits. Seizure type was compared between the abnormal and normal microarray groups at the last visit. There was not an association between seizure type and microarray status (Table 4).

Presenting medical indications other than epilepsy

Subjects with abnormal microarrays were more likely to have musculoskeletal and cardiovascular malformations than subjects with normal microarrays. Musculoskeletal malformations, including congenital anomaly of the hip joint, congenital anomaly of the shoulder joint, osteoporosis, sacral disorder, disorder of the bone and cartilage, congenital anomalies of the skull and face bone, and lordosis, occurred in 38.5% of subjects with abnormal microarrays and 14.1% of subjects with normal microarrays (p<.0035, OR=3.8) (Table 5). Cardiovascular malformations occurred in 30.8% of subjects with abnormal microarrays and 10.7% of subjects with normal microarrays (p<.0081, OR= 3.7) (Table 5). Although association for cardiac malformations is not statistically significant after correcting for multiple testing, it is nominally significant and may represent a trend in the data. No significant association of cytogenetic
results with neurological, genitourinary, gastrointestinal, ophthalmological, and otolaryngological malformations was detected (Table 5). Comparisons among abnormal and normal microarray groups did not find any difference in the number/prevalence of functional problems. There was a notably high percentage of intellectual disability/developmental delay in our patient population (79.9% or 117/147).

Discussion

This study demonstrated a large range of abnormal microarray findings in a patient population with epilepsy. There were a total of 26/147 patients with abnormal microarrays, which included 11 clinically significant results representing 10 different diagnoses and 15 different variants of unclear significance classified into four different subgroups. Although the studied population represents a highly selected group of children with epilepsy whose presentation created adequate clinical suspicion to warrant a microarray, the complexity of the data set illustrates the intricacy of the relationship between genotype and phenotype. A report from the 2010 ILAE Genetic Commission states that the complexity of genetic testing should be explored fully in genetic counseling prior to testing.\textsuperscript{15} Findings such as copy number variation and regions of homozygosity can be complicated to interpret and communicate to patients and families. Also, many of the clinically significant diagnosis may require medical management by a geneticist. Taken as a whole, this study provides support for the utility of a geneticist or a genetic counselor in the evaluation of patients with epilepsy

More than half (8/15) of the abnormal results with unclear clinical significance involved variants in the 15q13.3, 16p11.2, and 1q21.1 susceptibility loci comprising, the largest category of abnormal results. Variations in these areas have been reported with a wide range of neurodevelopmental disorders including autism, intellectual disability, schizophrenia, and
These susceptibility loci are thought to increase an individual’s predisposition to a certain disorder. Unique features to these susceptibility loci include incomplete penetrance and moderate to high odds ratios for risk. However, a number of environmental factors have also been identified which increase risks for neurodevelopmental disorders. Further studies, including epidemiologic, genomic, metabolic and proteomic, will be necessary to explore gene-environment interactions related to epilepsy associated with these susceptibility loci.

Two recent studies explored the use of microarray technology in individuals diagnosed with epilepsy. Ezugha et al. conducted a retrospective chart review on 82 subjects with neurodevelopmental disorders and a normal karyotype and found that 23.5% of the subjects had an abnormal array [bacterial artificial chromosome comparative genomic hybridization (BAC) and SNP]. Only 22 of the patients had a diagnosis of epilepsy and eight of these patients had an abnormal microarray. The larger sample size of our study allowed us to obtain a wider range of abnormal results with 26/147 patients diagnosed with epilepsy having an abnormal microarray. In 2007, Kim and colleagues conducted a microarray study (BAC) in Korea on 60 subjects with epilepsy and identified chromosomal aberrations in 30%. The most frequently altered loci included copy number gains of 1p, 5p, 8q, 10q, and copy number losses of 7q. The researchers reported variable phenotypes among the groups, but did not record what these phenotypic features were. Also, because this study used BAC array technology, researchers were unable to detect areas of homozygosity that could be detected by a SNP array. These studies reported a similar yield of microarray results in patients with epilepsy but did not provide data regarding features or phenotype. Our study provides a distinct advantage over previous studies because it was larger, able to detect regions of homozygosity, and more detailed in its
description of other findings. This information is useful when trying to establish clinical guidelines of if/when to order a microarray in patients with epilepsy.

When comparing patients with abnormal microarrays to patients with normal microarrays we found that brain MRI result and seizure type were not helpful indicators of when to order a SNP microarray. Other structural malformations present in the patient, however, may be helpful in this decision. In our study, musculoskeletal and cardiac malformations were particularly prevalent in children with abnormal microarrays. Although cardiac malformations did not reach statistical significance after correcting for multiple tests, it is a trend that is consistent with previous literature and may be clinically relevant. Presence of a cardiac or musculoskeletal malformation in addition to epilepsy appears to increase the pre-test likelihood of an abnormal result on microarray.

The limitations of this study include its retrospective design and ordering provider bias. Our study recognizes the current challenges in a retrospective design, such as misclassification bias and selection bias. Severity of epilepsy and/or developmental problems was likely to impact the decision of provider to order microarray. Although we did not assess medical intractability as a part of the chart review, our patient population may represent a more medically refractory or severe population than pediatric epilepsy patients as a whole. In contrast, children with idiopathic epilepsy syndromes and relatively intact cognitive function may be under-represented in this sample. To address selection bias, each child’s phenotypic presentation was recorded so that a full picture of the population characteristics could be documented. Because there are no guidelines for ordering genetic testing in cases of epilepsy, different physicians may order SNP microarrays at different rates and for different indications. Only subjects whose physicians have sent genetic testing have been captured. To limit ordering provider bias, our subject population
was obtained from a tertiary medical center with over 783 physicians. A cross-sectional study of all children with epilepsy would be needed to generalize our results to the entire patient population with epilepsy.

In conclusion, this study provided insight into a complex patient population of individuals with epilepsy. When comparing abnormal and normal microarrays across a variety of structural features, clinicians should consider a microarray in epileptic patients who also have a diagnosis of a musculoskeletal malformation or a cardiovascular malformation.
Bibliography


Table 1. Demographic Data

<table>
<thead>
<tr>
<th>Race</th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American</td>
<td>8 (5.4%)</td>
<td>15 (10.2%)</td>
<td>23 (15.6%)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>0</td>
<td>1 (0.7%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>56 (38.1%)</td>
<td>67 (45.6%)</td>
<td>123 (83.7%)</td>
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</table>

Ethnicity

<table>
<thead>
<tr>
<th>Race</th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic</td>
<td>5 (3.4%)</td>
<td>2 (1.4%)</td>
<td>7 (4.8%)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>59 (40.1%)</td>
<td>81 (55.1%)</td>
<td>140 (95.2%)</td>
</tr>
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</table>

Table 2. Description of Abnormal Microarray Results

<table>
<thead>
<tr>
<th>ID</th>
<th>Microarray Result</th>
<th>Interpretation/ Clinical significance</th>
<th>Structural Malformations</th>
<th>Brain MRI Results</th>
<th>Seizure Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>arr 9p24.3q34.3(590-140,233,916)x2-3</td>
<td>Clinically Significant Mosaic trisomy 9 (20% of cells)</td>
<td>1. Neurological- arachnoid cyst 2. Cardiovascular- hemangioma</td>
<td>Cystic lesion along the left medial temporal region</td>
<td>Undetermined</td>
</tr>
<tr>
<td>27</td>
<td>arr 1p36.33p36.32(72,017-4,121,459)x1</td>
<td>Clinically Significant 4.0 Mb deletion (1p36.33-1p36.32) 1p36 deletion syndrome</td>
<td>1. Neurological- gray matter heteropia 2. Musculoskeletal- sacral disorder 3.ENT- congenital anomaly of larynx, trachea, and bronchus 4. Cardiovascular- atrial septal defect</td>
<td>Gray matter heteropia</td>
<td>Undetermined</td>
</tr>
<tr>
<td>34</td>
<td>arr 15q11.2q13.2(21,235,224-28,560,060)x4;15q13.2(28,570,072-30,501,109)x3</td>
<td>Clinically Significant 7.33 Mb quadruplication (15q11.2-15q13.2) Tetrasomy 15q syndrome 1.93 Mb duplication (15q13.2-15q13.3) overlaps region associated with neuropsychiatric and neurological conditions</td>
<td>Normal</td>
<td>Generalized</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>arr 4q34.3q35.1(178,219,662-184,166,575)x1</td>
<td>Clinically Significant 5.9 Mb deletion (4q34.3-4q35.1)</td>
<td>Normal</td>
<td>Focal</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>arr 7q11.23q21.11(75,001,105-82,820,129)x1</td>
<td>Clinically Significant 7.8 Mb deletion (7q11.23-7q21.11)</td>
<td>Normal</td>
<td>Generalized</td>
<td></td>
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<tr>
<td>80</td>
<td>arr 16p13.3(1,451-143,254)x1;12q13.3</td>
<td>Clinically Significant Unbalanced</td>
<td>Normal</td>
<td>Focal</td>
<td></td>
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<tr>
<td>89</td>
<td>arr 1p36.33p36.31(37,7 14-6,582,459)x1</td>
<td>Clinically Significant 6.5 Mb deletion (1p36.33-1p36.31) 1p36 deletion syndrome</td>
<td>1. Neurological- polymicrogyria 2. Musculoskeletal Features- congenital deformity of hip (joint) 3. Ophthalmological- adhesions and disruptions of pupillary membranes 4. Genitourinary- congenital cystic kidneys 5. Cardiovascular- left ventricular non-compaction</td>
<td>Polymicrogyria</td>
<td>Focal</td>
</tr>
<tr>
<td>110</td>
<td>arr 6p25.3(5,107- 533,950)x1.6q27(16 7.971,017- 170,896,711)x1</td>
<td>Clinically Significant Ring Chromosome; 2.9 Mb deletion (6p25.3); 529 Kb deletion (6q27)</td>
<td>1. Neurological- obstructive Hydrocephalus 2. Musculoskeletal- scoliosis, craniosynostosis 3. Ophthalmological- macular degeneration</td>
<td>Hydrocephalus with dilatation of the lateral ventricles</td>
<td>Focal</td>
</tr>
<tr>
<td>129</td>
<td>arr 7q11.23(72,660,998-73,162,143)x1</td>
<td>Clinically Significant 501 Kb deletion (7q11.23) William’s syndrome</td>
<td>1. Musculoskeletal- pectus excavatum 2. Cardiovascular- congenital Anomaly of the heart, cardiomegaly</td>
<td>Mild to moderate third ventricular enlargement</td>
<td>Undetermined</td>
</tr>
<tr>
<td>135</td>
<td>arr 22q11.21(17,255,956-19,796,715)</td>
<td>Clinically Significant 2.5 Mb deletion (22q11.21) VelocardioFacial syndrome</td>
<td>1. Cardiovascular- ventricular septal defect</td>
<td>Normal</td>
<td>Focal</td>
</tr>
<tr>
<td>134</td>
<td>arr 5q33.3q35.2(156,445,760- 173,549,379)x2 hnz,6p12.1q21(53,509,958- 108,299,478)x2 hnz,10q21.3q23.31(70,975,273- 89,998,548)x2 hnz</td>
<td>Unknown Clinical Significance Multiple regions showing loss of heterozygosity. This raises a concern for the potential for unmasking an autosomal recessive disorder in these regions</td>
<td>1. Musculoskeletal- osteoporosis</td>
<td>Normal</td>
<td>Generalized</td>
</tr>
<tr>
<td>24</td>
<td>arr 1p35.1p34.1(34,343,79345,267,337)x2h nz,1q32.1q42.13(201,150,686227,871,496)x2hnz,2q21.2q 24.1(132,206,79715 6,192,075)x2hnz,3p21.3p14.2(45,136,866-63,412,898)x2 hnz,6q16.1q23.2(9 3,778,968- 132,475,753)x2hnz</td>
<td>Unknown Clinical Significance Multiple regions showing loss of heterozygosity. This raises a concern for the potential for unmasking an autosomal recessive disorder in these regions</td>
<td>1. Cardiovascular- congenital anomaly of pulmonary artery</td>
<td>Normal</td>
<td>Focal</td>
</tr>
<tr>
<td>Locus</td>
<td>Description</td>
<td>Clinical Significance</td>
<td>Status</td>
<td></td>
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<tr>
<td>15q13.2(28,148,879-28,607,058)x1</td>
<td>Unknown Clinical Significance 458 Kb deletion (15q13.2); overlaps 15q13.3 microdeletion syndrome</td>
<td>Normal</td>
<td>Undetermined</td>
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<tr>
<td>6q15(89,978,501-89,983,681)x0,7q36.1(147,700,415-147,715,565)x0</td>
<td>Unknown Clinical Significance 5.18 Kb deletion (6q15), GABRR1 gene; 15.15 Kb deletion (7q36.1) CNTNAP2 gene</td>
<td>Normal</td>
<td>Generalized</td>
<td></td>
<td></td>
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<tr>
<td>1q21.1(144,800,611-145,858,238)x1</td>
<td>Unknown Clinical Significance 1.1 Mb deletion (1q21) overlaps microdeletion region that may predispose to neurodevelopmental problems and congenital anomalies</td>
<td>Abnormal signal in the left posterior temporal and periatral white matter is nonspecific and may represent gliosis</td>
<td>Undetermined</td>
<td></td>
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<tr>
<td>16p13.11(14,956,144-16,260,667)x1</td>
<td>Unknown Clinical Significance 1.3 Mb deletion (16p13.11) overlaps neurocognitive susceptibility locus, includes NDE1 gene</td>
<td>1. Neurological-encephalomalacia</td>
<td>Focal</td>
<td></td>
<td></td>
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<tr>
<td>Xp21.1(32,554,912-32,555,407)x0</td>
<td>Unknown Clinical Significance 496 Bp deletion (Xp21.1) intron of DMD gene</td>
<td>1. Neurological-heterotopic gray matter</td>
<td>Gray matter Heterotopia</td>
<td>Focal</td>
<td></td>
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<tr>
<td>1q21.1(143,649,677-144,459,282)x3</td>
<td>Unknown Clinical Significance 810 Kb duplication (1q21.1) overlaps neurodevelopmental susceptibility locus</td>
<td>1. Neurological-congenital malformation of brain</td>
<td>Normal</td>
<td>Undetermined</td>
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<tr>
<td>1q21.1(143,812,534)</td>
<td>Unknown Clinical Significance</td>
<td>Normal</td>
<td>Focal</td>
<td></td>
<td></td>
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<tr>
<td>Microarray Result</td>
<td>Abnormal MRI Not Indicative of Epilepsy</td>
<td>Abnormal MRI Indicative of Epilepsy</td>
<td>Inconclusive</td>
<td>Normal</td>
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<tr>
<td>Abnormal (n=26)</td>
<td>30.8% (8/26)</td>
<td>23.1% (6/26)</td>
<td>0% (0/26)</td>
<td>46% (12/26)</td>
<td></td>
</tr>
<tr>
<td>Normal (n=121)</td>
<td>23.1% (28/121)</td>
<td>33.1% (40/121)</td>
<td>5% (6/121)</td>
<td>38.8% (47/121)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Brain MRI results for subjects with normal and abnormal microarray results
Table 4. Seizure types for subjects with normal and abnormal microarray results

<table>
<thead>
<tr>
<th>Microarray Result</th>
<th>Focal</th>
<th>Generalized</th>
<th>Undetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal (n=26)</td>
<td>50% (13/26)</td>
<td>19.2% (5/26)</td>
<td>30.8% (8/26)</td>
</tr>
<tr>
<td>Normal (n=121)</td>
<td>54.5% (66/121)</td>
<td>30.6 % (37/121)</td>
<td>14.9% (18/121)</td>
</tr>
</tbody>
</table>

Table 5. Other presenting structural malformations for subjects with normal and abnormal microarray results

<table>
<thead>
<tr>
<th>Structural Malformation</th>
<th>Abnormal Microarray (n=26)</th>
<th>Normal Microarray (n=121)</th>
<th>p Value</th>
<th>Odds Ratio</th>
<th>CI Lower</th>
<th>CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological</td>
<td>38.5% (10/26)</td>
<td>40.5% (49/121)</td>
<td>p=.85</td>
<td>0.9233</td>
<td>0.3726</td>
<td>2.1955</td>
</tr>
<tr>
<td>Musculoskeletal†**</td>
<td>38.5% (10/26)</td>
<td>14.1 (17/121)</td>
<td>p=.0035</td>
<td>3.7861</td>
<td>1.4355</td>
<td>9.8046</td>
</tr>
<tr>
<td>Ophthalmological</td>
<td>7.7% (2/26)</td>
<td>3.3% (4/121)</td>
<td>p=.287</td>
<td>2.4996</td>
<td>0.2956</td>
<td>14.4211</td>
</tr>
<tr>
<td>Otolaryngological</td>
<td>7.7% (2/26)</td>
<td>5% (6/121)</td>
<td>p=.635</td>
<td>1.6634</td>
<td>0.2101</td>
<td>8.0362</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0 (0/26)</td>
<td>4.1% (6/121)</td>
<td>p=.591</td>
<td>Undetermined</td>
<td>Undetermined</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>7.7% (2/26)</td>
<td>9.9% (12/121)</td>
<td>p=1.00</td>
<td>0.9771</td>
<td>0.1329</td>
<td>3.9619</td>
</tr>
<tr>
<td>Cardiovascular†</td>
<td>30.8% (8/26)</td>
<td>10.7% (13/121)</td>
<td>p=.0081</td>
<td>3.664</td>
<td>1.2744</td>
<td>10.1459</td>
</tr>
</tbody>
</table>

†Nominally Significant
*Significant after correcting for multiple comparisons
### Supplemental Table

Table 6. All medical diagnosis including functional and structural malformations

<table>
<thead>
<tr>
<th>System with diagnosis</th>
<th>Abnormal Microarray (n=26)</th>
<th>Normal Microarray (n=121)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental Delay/ Intellectual Disability</td>
<td>80.8% (21/26)</td>
<td>79.3% (96/121)</td>
<td>p=.870</td>
</tr>
<tr>
<td>Autism/Autistic Like Features</td>
<td>19.2% (5/26)</td>
<td>16.5% (20/121)</td>
<td>p=.739</td>
</tr>
<tr>
<td>Psychological Conditions</td>
<td>46.5% (12/26)</td>
<td>47.9% (58/121)</td>
<td>p=.869</td>
</tr>
<tr>
<td>Respiratory Conditions</td>
<td>38.5% (10/26)</td>
<td>43.8% (53/121)</td>
<td>p=.618</td>
</tr>
<tr>
<td>Immunological Conditions</td>
<td>11.5% (3/26)</td>
<td>4.13% (5/121)</td>
<td>p=.150</td>
</tr>
<tr>
<td>Endocrine Conditions</td>
<td>50.0% (13/26)</td>
<td>44.6% (54/121)</td>
<td>p=.618</td>
</tr>
<tr>
<td>Neurological Conditions</td>
<td>80.8% (21/26)</td>
<td>80.2% (97/121)</td>
<td>p=.944</td>
</tr>
<tr>
<td>Musculoskeletal Conditions</td>
<td>57.7% (15/26)</td>
<td>44.6% (54/121)</td>
<td>p=.226</td>
</tr>
<tr>
<td>Ophthalmological Conditions</td>
<td>50.0% (13/26)</td>
<td>39.7% (48/121)</td>
<td>p=.332</td>
</tr>
<tr>
<td>Otolaryngological Conditions</td>
<td>50.0% (13/26)</td>
<td>47.1% (57/121)</td>
<td>p=.789</td>
</tr>
<tr>
<td>Gastrointestinal Conditions</td>
<td>46.2% (12/26)</td>
<td>54.5% (66/121)</td>
<td>p=.447</td>
</tr>
<tr>
<td>Genitourinary Conditions</td>
<td>23.1% (6/26)</td>
<td>24.0% (29/121)</td>
<td>p=.923</td>
</tr>
<tr>
<td>Cardiovascular Conditions</td>
<td>42.3% (11/26)</td>
<td>36.4% (44/121)</td>
<td>p=.570</td>
</tr>
</tbody>
</table>
Appendix 1

Demographic Information

ID number ________________________         MRN _______________________________
DOB ________________________________         Sex _________________________________
Ethnicity ____________________________

Data Collection

1. Epilepsy Diagnosis (first visit)
   - I. Genetic (Idiopathic)
   - II. Structural/Metabolic (symptomatic)
   - III. Unknown Cause (cryptogenic)

2. Type of Seizure (first visit)
   - I. Generalized seizures
     - A. Tonic–clonic (in any combination)
     - B. Absence
       - Typical
       - Atypical
       - Absence with special features
         - Myoclonic absence
         - Eyelid myoclonia
     - C. Myoclonic
       - Myoclonic
       - Myoclonic atonic
       - Myoclonic tonic
     - D. Clonic
     - E. Tonic
     - F. Atonic
   - II. Focal seizures
     - Without impairment or responsiveness
     - With observable motor or autonomic components
     - Involving the sensory psych phenomena only
     - With impairment of consciousness or responsiveness
     - Evolving bilateral, convulsive seizure
   - III. Undetermined

3. Epilepsy Diagnosis (last visit)
   - I. Genetic (Idiopathic)
   - II. Structural/Metabolic (symptomatic)
   - III. Unknown Cause (cryptogenic)
4. Type of Seizure (last visit)
   □ I. Generalized seizures
      □ A. Tonic–clonic (in any combination)
      □ B. Absence
         □ Typical
         □ Atypical
         □ Absence with special features
            □ Myoclonic absence
            □ Eyelid myoclonia
      □ C. Myoclonic
         □ Myoclonic
         □ Myoclonic atonic
         □ Myoclonic tonic
      □ D. Clonic
      □ E. Tonic
      □ F. Atonic
   □ II. Focal seizures
      □ Without impairment or responsiveness
         □ With observable motor or autonomic components
         □ Involving the sensory psych phenomena only
      □ With impairment of consciousness or responsiveness
         □ Evolving bilateral, convulsive seizure
   □ III. Undetermined

5. Presenting features other than epilepsy
   □ Developmental Delay/Intellectual Disability
   □ Autism/ Autistic Like Features
   □ Cardiovascular Problems Specify______________________
   □ Respiratory Problems Specify______________________
   □ Gastrointestinal Problems Specify______________________
   □ Psychological Problems Specify______________________
   □ GU Problems Specify______________________
   □ Endocrine Problems Specify______________________
   □ Musculoskeletal Problems Specify______________________
   □ Ophthalmology Problems Specify______________________
   □ Otolaryngological Problems Specify______________________
   □ Neurological Problems Specify______________________
   □ Immune Problems Specify______________________

6. Structural Malformations
   □ Cardiovascular Malformations Specify______________________
   □ ENT Malformations Specify______________________
   □ Gastrointestinal Malformations Specify______________________
   □ Genitourinary Malformations Specify______________________
   □ Ophthalmology Problems Specify______________________
7. How many systems had malformations?
- □ 1
- □ 2
- □ 3
- □ 4
- □ 5

8. What result was the most recent Brain MRI?
- □ Normal
- □ Abnormal/Indicative Specify_____________________
- □ Abnormal/Not Indicative Specify_____________________
- □ Inconclusive

9. Microarray Results
- □ Abnormal (Clinically Significant)
- □ Abnormal (Unknown Clinical Significance)
- □ Normal (Not Significant)

10. If clinically significant
    Specify______________________________________________________

11. If unknown clinical significance
    Specify______________________________________________________