Autism and the 15q11-13 Locus: A Case

CB is a 16-year-old girl whose first developmental concerns arose at four to five months of age. At that time, she was not visually fixing on faces. She did not smile responsively or laugh. She remained extremely quiet through her first year of life, with only minimal babbling. Language did not develop until approximately three years of age. Rather than go through a period of babbling, she exhibited echolalia. She was able to pronounce words clearly, but did not use them in an appropriate context.

CB engaged only in solitary play until she was approximately three years old, when she began to interact with her older sister. It was not much later that she began to interact with her parents. She did not exhibit joint attention until nine years of age. Even after she began interacting, her play was repetitive. She was very rigid in her behaviors and craved structure. CB had poor attention. She developed simple motor tics including throat clearing. She displayed features of sensory integration disorder; she did not allow anyone to touch her hands and there were certain textures of food that upset her greatly. Over time, CB displayed severe obsessive compulsive symptoms. She has never had any seizures.

The family history was limited since the patient was adopted, but her biological mother was known to have cognitive delays and social impairments. The patient has a half sister by the same mother who has a seizure disorder, cognitive delays, and social impairments.

The physical examination was notable for short stature and almond-shaped eyes. She was otherwise non-dysmorphic. The neurological examination was notable for pressured, perseverative speech involving a limited array of topics. She repetitively asked the same questions and dictated the topics of conversation. CB exhibited poor attention, motor tics, and obsessive compulsive behaviors. She insisted that the examiner use the same stethoscope that had been used during previous visits and that the order of the examination not deviate from previous visits. The remainder of the general and neurological examinations was normal.

To evaluate CB’s developmental concerns, she underwent fluorescent in-situ hybridization (FISH) of the 15q11-13 region. This revealed a duplication within 15q12.

The 15q11-13 locus is highly unstable and subject to genomic imprinting. Of 58 CNVs observed in this locus, 45 are associated with disorders, including Prader Willi Syndrome, Angelman Syndrome, autism, intellectual disability, and schizophrenia. A number of genes within this locus, such as UBE3A and members of the GABA receptor gene family, are expressed throughout the central nervous system and have been associated with autism or other neuropsychiatric disorders.

More widespread use of genetic testing for autism would enable earlier detection and therefore intervention in a sizable fraction of patients.

Variations at the 15q11-13 locus are observed in up to one percent of autistic individuals, and the majority of the numerous observed variations are maternally-inherited duplications. Many of CB’s symptoms are in accordance with those described in other patients harboring 15q11-13 duplications, including developmental delay, echolalia, lack of major facial dysmorphisms, and short stature.

As expected, many of CB’s symptoms overlap with ASD symptoms, including delayed development of language, abnormal social interaction, solitary and repetitive play, sensory integration differences, and strong desire for structure. Based on the family history, it is likely that both CB and her half-sister inherited the duplication from their mother.

Of note, a recent physician advisory (http://www.idic15.org/PhysicianAdvisory_Feb2009.pdf) warns that medications that target the GABA-A receptor—including benzodiazepines, phenobarbital, and ethanol derivatives—may increase the risk of sudden death in individuals with 15q11-13 duplications. Further research is necessary to definitively establish a link between these medications and sudden death in these individuals; however, the fact that the 15q11-13 locus includes several GABA receptor genes provides biological plausibility for this clinical concern.

In summary, CB now has a diagnosis of “autism associated with 15q11-13 duplication.” This molecular subtype of autism provides a biological explanation, has relevance to recurrence risk, may have significant implications for treatment (e.g. caution in prescribing benzodiazepine, phenobarbital, or ethanol derivatives) and may help predict the clinical course.

Diagnosis in the Multidisciplinary Clinic: The Advantages of Genetic Testing

Given the wide spectrum of autistic phenotypes, it is fitting that diagnosis and management of this complex disorder is becoming increasingly collaborative. It is common for patients to be seen by a number of professionals, including pediatricians, psychiatrists, neurologists, medical geneticists, and speech and behavioral therapists.

A crucial collaboration is that between clinicians and research geneticists. Although autism remains a behaviorally-defined disorder in DSM-IV-TR, approximately ten percent of ASD cases have known cytogenetic causes and more discoveries are certainly on the way, thanks to the advent of array-CGH technology and the rapid advancement of the field. Discovery of the genetic causes of specific forms of autism will allow these molecular subtypes to be added as descriptors of the behavioral diagnosis, much in the way that MECP2 mutations in Rett’s disorder have provided important biological explanations.

More widespread use of genetic testing for autism would enable earlier detection and, therefore, intervention in a sizable fraction of patients. The benefit of early diagnosis and intervention is highlighted by experiments involving animal models of the monogenic disorders Fragile X syndrome and Rett Disorder, in which early treatment was shown to delay or prevent the onset of autistic symptoms, as well as decrease their severity.

Genetic testing has additional advantages. Firstly, identification of genetic causes of autism not only provides bio-
logical explanations for families but also may help to reduce the stigma around this disorder and prevent the development of false, damaging explanations for autism. Secondly, a specific genetic diagnosis may reveal information important to a patient’s medical future, such as increased risk of sudden death for 15q11-13 duplication patients being treated with GABA-A agonists (http://www.idic15.org/PhysicianAdvisory_Feb2009.pdf) or increased susceptibility to cancer for individuals with PTEN mutations. Thirdly, given the high risk of autism recurrence among siblings, a specific genetic diagnosis in one individual may aid in the identification of autism or related neuropsychiatric disorders such as epilepsy, and/or associated susceptibilities to diseases such as cancer in family members.

A recent consensus statement proposed that microarray analysis should replace G-banded karyotype analysis as the standard genetic test for autistic patients. This recommendation is based on the fact that microarrays have a resolution more than ten times that of karyotypes as well as a diagnostic yield about six times greater. Furthermore, the cost of microarray testing is lower than that of karyotype analysis followed by specific FISH testing.

As the cost of genetic testing decreases and clinical microarrays become more widely available, this technology will almost certainly become a standard aspect of autism diagnosis. As we continue to identify the genetic causes of the numerous subtypes of autism, more and more people on the spectrum can benefit from genetic testing, earlier diagnosis, and earlier intervention. Appreciation of the multidisciplinary nature of autism will enhance the rate of discovery of autism-associated loci and facilitate improved diagnosis and treatment of autistic patients, thus improving the lives of individuals and families affected by autism. While progress in autism genetics has made significant contributions to the clinic, much research remains to be done.

References


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Disclosure of Financial Interest

Eric M. Morrow, MD, PhD, acknowledges research support from the NIMH (1K23MH080954-01), Charles H. Hood Child Health Research Foundation and holds a Career Award for Medical Scientists from the Burroughs Wellcome Fund.

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Myoclonic Astatic Epilepsy and the Role of the Ketogenic Diet
Jessica E. Mulligan, MS, RD, LDN, and David E. Mandelbaum, MD, PhD

CASE PRESENTATION
NG presented at four years of age with two generalized convulsive seizures, each lasting approximately two minutes, on two consecutive days. His EEG was diffusely slow, but with no epileptiform activity. After a third seizure on the following day he was started on oxcarbazepine. Shortly after starting oxcarbazepine he began having generalized, non-convulsive seizures, which were attributed to the oxcarbazepine, and was switched to levetiracetam. The non-convulsive seizures persisted and were refractory to high doses of levetiracetam, and he was switched to zonisamide, still without control. He was diagnosed with Doose Syndrome and was hospitalized two months after his initial presentation for management of intractable, non-convulsive seizures. The EEG revealed frequent, three-cycle per second, spike-wave discharges with clinical and subclinical seizures. Treatment with valproic acid (VPA) failed to control the seizures; his ammonia (NH3) level became elevated with normal liver function tests (LFTs) and the VPA was discontinued. He had a dramatic response to high dose (1 mg/kg) oral diazepam with normalization of the EEG and complete control of the seizures, and was discharged on zonisamide. However, within days he had a recurrence of the non-convulsive seizures, this time refractory to high dose diazepam. He was hospitalized and treated with ethosuximide. Due to persistent clinical and electrographic seizures, VPA was tried a second time; the NH3 remained in the fifties and there were no changes in the LFTs, but he developed a significant encephalopathy, manifesting with marked somnolence, without control of the seizures. The VPA was discontinued with resolution of the encephalopathy (with one rather disconcerting exception; he emerged from the valproate-induced encephalopathy a Yankees fan, having previously been a dedicated member of the Red Sox nation). Rufinamide was added to his AED regimen and the ketogenic diet was initiated. There was no benefit from the rufinamide, which was discontinued. The child had difficulty tolerating the ketogenic diet, becoming quite nauseated when acidicotic, to the point of requiring NG tube feeding for adequate nutrition. Addition of bicarbonate to his diet resolved the severe acidosis. Levo-carnitine was then added. There was a dramatic improvement in the EEG and complete cessation of clinical seizures. NG’s mental status was back to baseline, though there was a report of a need for a daytime nap. After tapering the zonisamide and ethosuximide, with complete seizure control on the ketogenic diet alone, the daytime lethargy resolved. He developed a better tolerance for the diet and was taking all his nutrition by mouth, without difficulty. After quite a few months he was, again, a Red Sox fan.

DOSE SYNDROME
Myoclonic astatic epilepsy (MAE), also known as Doose Syndrome, was classified by the International League Against Epilepsy, based on the seizure type, as one of a group of cryptogenic or symptomatic generalized epilepsies and syndromes. A subsequent task force on classification terminology of epilepsy syndromes re-classified MAE as a generalized epilepsy, distinguishing it from Lennox-Gastaut Syndrome, Severe Myoclonic Epilepsy of infancy (SMEI or Dravet Syndrome) and Atypical Benign Partial Epilepsy/Pseudo-Lennox Syndrome (ABPE/PLS), with the latter classified as epileptic encephalopathies. The distinction between the different generalized, myoclonic epilepsy syndromes was based on the conclusion that, in cases of the epileptic encephalopathies, the epileptiform abnormalities contribute to the progressive disruption of brain function, whereas MAE has a course that is highly variable, with instances of complete remission of the epilepsy and normal cognitive development. The EEG findings in MAE are significantly different from the other syndromes. MAE typically presents with rhythmic, generally spike and wave activity. MAE, as well as SMEI, can present with obtundation due to nonconvulsive status. The cognitive outcome appears to be dependent on whether the epilepsy resolves. MAE is considered an idiopathic generalized epilepsy syndrome, as opposed to the epileptic encephalopathies, which are considered cryptogenic or symptomatic. Prior to the onset of epilepsy, usually between 18 and 50 months of age, children with MAE have normal development. The initial seizure types consist of myoclonic, atactic seizures; ongoing seizures include generalized convulsive seizures, absence seizures, and, less often, tonic and febrile seizures. Medications reported to be beneficial in MAE include: valproate, lamotrigine, ethosuximide and benzodiazepines (all of which, with the exception of lamotrigine, were tried in our case, without persistent control of the seizures). The variability in the choice of treatment and prognosis between the different myoclonic epilepsy syndromes warrants careful attention to correctly classifying children presenting with myoclonic seizures, 

HISTORY OF THE KETONIC DIET
Fasting as a method to control seizures has been recognized since at least 500 B.C. The first ‘modern’ use of fasting for the treatment of epilepsy was reported by Guelpa and Marie in 1911. The impracticability of fasting led to the development of the ketogenic diet (KD), described by Wilder in 1921, an effort to mimic the metabolic effects of fasting, yet more tolerable and sustainable. The diet, high in fats and low in carbohydrates, has been utilized with little variation since. Over the next twenty years, the KD grew in popularity and was a recognized treatment for epilepsy. However, the development and introduction of antiepileptic drugs in the United States resulted in diminished use of the diet. In 1993, a 20-month old boy, Charlie Abrahams, was treated with the KD for intractable complex partial seizures after the failure of multiple AEDs and surgery. Within days of the initiation of the diet, the seizures...
were completely controlled. Charlie’s father, Jim Abrahams, created The Charlie Foundation to promote research, awareness and availability of the KD.

Over the last sixteen years there has been an enormous growth of interest in the KD. The KD is now available in over 50 countries and in all continents except Antarctica, and is available at most major medical centers. The KD has become a well-established treatment, supported by extensive research, for the treatment of certain medically refractory epilepsies.

Proposed Mechanisms of Action

The KD has been used successfully to treat a variety of epilepsy syndromes, of known and unknown etiology, including Infantile Spasms, Lennox-Gastaut Syndrome, Landau-Kleffner Syndrome, myoclonic-astatic epilepsy (Doose and Dravet Syndromes) and has been successful in epilepsy attributable to genetic syndromes such as Tuberous Sclerosis, and Rett Syndrome. Yet, in spite of the duration and widespread nature of its use, relatively little is known about the mechanism behind its efficiency. The diet’s ability to treat a diverse range of epilepsy syndromes suggests that it may have multiple mechanisms of action, each of which may be more relevant to a specific disease state. In the case of glucose-transport disorders, when glucose cannot be transported across the blood-brain barrier, the generation of ketones, which can be used by the brain as an energy source, provides an alternative fuel source for the CNS. It has been proposed that ketone bodies may serve a similar function in other neurodegenerative disorders.

A study by Oguni et al. compared the response of myoclonic astatic epilepsy to the KD vs. medications. In this study, the KD was the most effective treatment modality, eliminating myoclonic and astatic seizures in fifteen of 26 patients. Medications with GABAergic actions were not as successful as the diet in this study; ACTH and ethosuximide were moderately effective. The greater success of the KD suggests the diet is not merely acting as a GABA-ergic agent in MAE. The relative success of the KD compared to ACTH indicates that the mechanism is something other than a hormonal effect, however, given that the mechanism of action of ACTH is also not understood, this does not add to our understanding of the mechanism of action. Further comparisons of the KD and medications with known mechanisms of action may help better the understanding of the mechanism of action.

Efficacy of the Ketogenic Diet

Early studies reported a 75-90% reduction in seizures with the KD. More recent publications, involving larger subject populations, have not confirmed this level of response. When compared to the vagus nerve stimulation, another non-pharmacologic treatment for intractable epilepsy, the KD appears to work more quickly, usually within two to four weeks compared to several months.

Four meta-analyses of the efficacy of the KD provide compelling evidence for benefit of the KD, despite the lack of blinded, controlled trials. In 2000, Lefevre and Aronson reviewed 11 studies and found a 56% responder rate (more than 50% reduction in seizures), 32% of whom had more than 90% reduction in seizures. They concluded, “although controlled trials are lacking, the evidence is sufficient to determine that the ketogenic diet is efficacious in reducing seizure frequency in children with refractory epilepsy.” In 2003, Levy and Cooper reviewed 14 studies, and concluded, “for those with a difficult epilepsy on multiple antiepileptic drugs, we consider the ketogenic diet a possible option.” In 2006, a meta-analyses of 19 studies, encompassing a total of 1,084 patients, found that the KD reduced seizures by more than 90% in a third of the patients and by more than 50% in half. There was no clear evidence of an effect of age, seizure type, or etiology on seizure reduction.

In 2008, a clinical trial by the Institute for Child Health in London by Neal and colleagues, randomized children to start the KD, after either a one-month (treatment group) or four-month delay (control group), with no additional changes in AEDs. The seizure frequency after four months was significantly lower in the 54 children on the KD (38% decrease in seizures), compared to the 49 controls (37% increase in seizures; p < 0.0001). The control group had no children with a greater than 90% reduction in seizures, compared to five children on the KD (P=0.06).

The KD is a promising therapy for MAE, with over half of children showing a greater than 50% reduction in seizures, and 18% achieving seizure-freedom. In drug resistant MAE, the diet should be considered early in the disease course.

Initiation of the Ketogenic Diet

Upon determination by the neurologist that a patient may be a candidate for the KD, a registered dietitian with expertise in the diet should be consulted to review the child’s growth history, dietary intake, fluid adequacy, evaluate complicating factors (swallowing and/or chewing difficulties, history of kidney stones, dyslipidemia, liver disease, failure to thrive, gastroesophageal reflux, constipation, cardiomyopathy, and chronic metabolic acidosis) and to begin to educate the family on the basics of the KD. The family’s willingness/ability to adhere to the very rigid requirements of the diet should be ascertained. All medications should be reviewed and changed to the lowest carbohydrate formulations to minimize their potential source of carbohydrates. Laboratory studies should be obtained to determine baseline nutrition status and to rule out disorders of fatty acid metabolism (Table 1).

If the neurologist and the dietitian determine that the patient (and family) is a good candidate for the KD, a hospital admission is scheduled to initiate the KD. According to two recent studies, it is no longer considered necessary to fast a child prior to initiation of the diet, however, without the fasting period prior to starting the diet, it may take one to two days longer to reach full ketosis. Even without fasting, hypoglycemia, acidosis, nausea,

<table>
<thead>
<tr>
<th>Table 1. Baseline Laboratory Recommendations Prior to Initiation of the KD</th>
</tr>
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<tbody>
<tr>
<td>Chemistry panel: electrolytes, glucose, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, liver function tests, lipid panel, acylcarnitine panel, carnitine level, urine organic acids, urine metabolic screen, plasma amino acids, uric acid, lactate, and pyruvate</td>
</tr>
</tbody>
</table>
Table 2. Sample Ketogenic Menu at 4:1 Ratio, 1400 calories

<table>
<thead>
<tr>
<th>Breakfast – Scrambled eggs, bacon, and fruit with whipped cream</th>
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</thead>
<tbody>
<tr>
<td>38 grams 40% cream (whipped)</td>
</tr>
<tr>
<td>25 grams fresh strawberries</td>
</tr>
<tr>
<td>17 grams scrambled eggs</td>
</tr>
<tr>
<td>13 grams bacon</td>
</tr>
<tr>
<td>28 grams butter</td>
</tr>
<tr>
<td>Lunch – Tuna salad roll-up and baby carrots</td>
</tr>
<tr>
<td>29 grams fresh baby carrots</td>
</tr>
<tr>
<td>24 grams tuna fish</td>
</tr>
<tr>
<td>28 grams mayonnaise</td>
</tr>
<tr>
<td>25 grams iceberg lettuce</td>
</tr>
<tr>
<td>Dinner – Hamburger patty with Jell-O, fruit, and whipped cream</td>
</tr>
<tr>
<td>25 grams beef 70% lean</td>
</tr>
<tr>
<td>27 grams soybean oil</td>
</tr>
<tr>
<td>39 grams 40% cream (whipped)</td>
</tr>
<tr>
<td>22 grams watermelon</td>
</tr>
<tr>
<td>86 grams Jell-O (sugar-free)</td>
</tr>
</tbody>
</table>

dehydration, or metabolic complications, either due to an undetermined metabolic disorder or the diet itself, may occur. Therefore, the KD is typically administered under careful medical supervision in a hospital setting.

The initiation of the diet at the hospital is usually done gradually, over a course of three to five days. The diet can be introduced in incremental doses, such as advancing by one third of the caloric goal each day over a three-day period. Another method is to begin at full calories and increase the fat content daily (starting at a ratio of 1:1, then 2:1, 3:1, and ending at 4:1). The KD is calculated in a ratio of grams of fat to grams of protein plus carbohydrate. The most common ratio is four grams of fat to one gram of protein plus carbohydrate (described as 4:1). At this ratio, 90% of the calories come from fat and 10% from protein and carbohydrate combined. Sometimes it is necessary to provide a lower ratio (such as 3:1) to increase protein or carbohydrate intake. See Table 2 for a sample ketogenic diet at a 4:1 ratio.

During the initiation, the family and other caregivers are thoroughly trained in food preparation, use of the gram scale to weigh all food and/or formula ingredients, vitamin and mineral supplementation, fluid goals, sick day management, and monitoring for adverse effects of the diet. The patient is discharged once they have reached moderate to high ketosis, labs are within normal limits, and have tolerated three ketogenic meals or feedings at the target ratio. The patient is discharged with carbohydrate-free medication prescriptions and follow-up appointments are arranged.

**Adverse Effects**

Although there have been numerous research studies of the KD, adverse effects have not been consistently reported in these trials. However, side effects of the KD do occur. The most common short-term side effects of the KD are constipation, acidosis, excessive ketosis, hypoglycemia, and dehydration. Possible long-term effects of the diet that have been reported include growth retardation, gastrointestinal symptoms, carnitine deficiency, and elevated lipids. Other, rare but serious, adverse effects include cardiac abnormalities due to selenium deficiency, Fanconi renal tubular acidosis and kidney stones. Lipid levels, including cholesterol, were noted to increase slightly, but not to a clinically significant degree, and over a two year period, while still on the diet, these elevated levels improved. Long-term studies of children who have been on the KD more than six years found that they are at risk for bone fractures, growth disturbances, and kidney stones. A study of patients who had discontinued the diet after six years found that the majority continued to have greater than 50% reduction in seizures; previously mildly elevated lipids had normalized, and growth was normal as well.

An analysis of the KD (at a 4:1) found that despite the inclusion of nutritious foods, the diet was inadequate for 19 of 23 micronutrients. It is essential that all patients on the KD receive appropriate vitamin and mineral supplementation to meet the dietary reference intake (DRI).

**Summary**

The ketogenic diet remains one of the most effective treatments for medically refractory childhood epilepsy. In spite of the long history of its use, relatively little is known about the mechanism of action. The diet’s efficacy in a wide range of epilepsy syndromes suggests that it may have multiple mechanisms of action, each of which may be more relevant to a specific disease state. Further research is necessary to define the mechanism of action, which may, in turn, lead to easier means to provide the therapeutic benefit.

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Disclosure of Financial Interests
The authors and/or spouses/significant others have no financial interests to disclose.

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