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# Genetic influences on ketogenic diet efficacy

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## Summary

An average of 15–20% of patients on the ketogenic diet (KD) experience a >50% reduction in seizure frequency; however, 10–40% discontinue the diet due to either a lack of response or adverse side effects. This variability in patient response raises the possibility that genetic factors may influence the efficacy of the KD. As a first step towards identifying these factors, we evaluated the ability of the KD to alter seizure thresholds in 4 commonly used inbred mouse strains: C57BL/6J, FVB/NJ, A/J, and DBA/2J. We observed strain-specific differences, indicating that genetic factors are likely to influence efficacy.

### Keywords

ketogenic diet; sodium channel; SCN1A; severe myoclonic epilepsy of infancy; animal models; epilepsy

The ketogenic diet (KD) has been used as an alternative to antiepileptic drugs (AEDs) for the treatment of refractory epilepsies since the 1920's (Freeman et al., 1998; Maydell et al., 2001). The diet is based on the consumption of three or four times as much fat by weight as carbohydrates and proteins combined. Clinically, it has proved effective at reducing the number of recurring seizures in patients with a range of epilepsy subtypes. The efficacy of the KD has been well documented and is the subject of several recent review papers (Hartman and Vining, 2007; Henderson et al., 2006; Mackay et al., 2005). A review of available clinical data indicates that an average of 15–50% of patients on the KD experience a > 50% reduction in seizures; however, 10–40% of patients on the KD discontinue treatment, due either to unresponsiveness or adverse side effects. This variable efficacy raises the possibility that genetic factors may influence the anticonvulsant effect of the KD.

## Efficacy of the KD in idiopathic epilepsy

Genetic analysis of large pedigrees with rare Mendelian inheritance has led to the identification of a number of dominant epilepsy genes, including several that encode voltage-gated sodium channel (VGSC) subunits (Ragsdale, 2008). Mutations in *SCN1A*, which encodes the α1 subunit of the VGSC, result in a variety of epilepsy subtypes, including severe myoclonic epilepsy in infancy (SMEI) (Claes et al., 2001). SMEI is a debilitating childhood epilepsy characterized by severe febrile and afebrile seizures in the first year of life, mental retardation, and ataxia. *SCN1A* mutations have been identified in > 70% of SMEI patients. Most represent *de novo* mutations and are predicted to abolish

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channel function. SMEI patients are often refractory to AEDs, and there is a pressing need to develop more effective treatments.

To date, there have been three studies examining the efficacy of the KD in patients with SMEI (Caraballo et al., 2005; Fejerman et al., 2005; Korff et al., 2007). In the first of these, 20 children with SMEI were placed on the KD (Caraballo et al., 2005). Patients in this study were not screened for *SCN1A* mutations. After one year, 13 patients remained on the diet. Of these, two (15%) were seizure-free, eight (62%) had a 75–99% decrease in seizure frequency, and the remaining three (23%) had a 50–74% decrease in seizure frequency. Overall, 10 patients (77%) had a > 75% decrease in seizure frequency, illustrating the variable efficacy of the KD in SMEI patients. The most recent study examined 16 patients with SMEI, six of whom were positive for *SCN1A* mutations (Korff et al., 2007). During the course of the study, six patients were placed on the KD as well as other AEDs. Four of the six patients experienced a favorable response.

The fact that some patients achieve seizure control while others have insufficient response demonstrates the variable efficacy of the KD. Our knowledge of the genetic basis of SMEI presents an opportunity to study the genetic factors that influence efficacy. Since a mouse model of SMEI was recently generated by knocking out the mouse *Scn1a* gene (Ogiwara et al., 2007; Yu et al., 2006), the genetic variation observed in the human population could be modeled by crossing SMEI mice to various inbred strains. Such mice could then be evaluated for responses to the KD. Differences in responses would suggest the influence of genetic factors that affect efficacy. Standard mouse genetic approaches could then be used to identify the responsible genetic factors. The SMEI mouse model might also be useful for determining whether early intervention with the KD can alter or prevent the development of SMEI.

## Genetic variation and the efficacy of the KD

Several studies have demonstrated that inbred mouse strains vary in their thresholds to seizures induced by a number of paradigms, including maximal electroshock, 6-Hz seizure induction, kainic acid, and pilocarpine (Frankel et al., 2001; Freund et al., 1987; Kosobud and Crabbe, 1990). These differences are likely due to sequence variation in genes that are responsible for either setting an endogenous excitability threshold or a specific response to a stimulus. This variation between inbred strains can be exploited to identify the genetic factors that may contribute to the efficacy of the KD.

In a pilot study, we evaluated the ability of the KD to alter seizure thresholds in four commonly used inbred mouse strains: namely, C57BL/6J, FVB/NJ, A/J, and DBA/2J. DBA/2J and C57BL/6J were chosen because they often demonstrate opposite responses in seizure-induction paradigms; DBA/2J are typically more seizure susceptible, whereas C57BL/6J are often more seizure resistant compared with other inbred strains (Engstrom and Woodbury, 1988; Frankel et al., 2001). FVBN mice exhibit spontaneous seizures (Goelz et al., 1998), and A/J mice exhibit spontaneous spike-wave activity during sleep (Strohl et al., 2007).

We divided males from each strain at eight weeks of age into a control group and an experimental group. The control animals were given Purina rodent chow (5001), and the experimental animals were given a 4.3:1 KD (Harlan TD.96355). The diet was initiated following a 12-h overnight fast, and all mice were maintained on their diets *ad libitum* for 14 days. We then determined thresholds to flurothyl-induced seizures by measuring the latency to onset of the generalized tonic clonic seizure (GTCS). In addition, we also evaluated body weights at the time of seizure induction as well as β-hydroxybutyrate (βHB) and glucose levels.

As shown in Figure 1A, seizure thresholds were unaltered in C57BL/6J and DBA/2J mice following 14 days on the KD. However, the KD-fed FVB/NJ mice exhibited an increased latency to the GTCS (5.5 min) compared with their control littermates (4.6 min) ($p = 0.004$). In contrast, A/J mice on the KD exhibited reduced latency to the GTCS (7.25 min) compared with their control littermates (8.25 min) ($p = 0.003$), suggesting an adverse effect. We observed differences in weights between the control and experimental groups in FVB/NJ mice only (Figure 1B). FVB/NJ mice on the control diet weighed an average of 25 grams, compared with 28 grams for these mice on the KD. βHB levels were elevated for all groups on the KD (Figure 1C). Glucose levels were unaltered following KD treatment (Figure 1D).

## Conclusion

Here, we report results supporting the hypothesis that genetic factors influence the efficacy of the KD. Identification of the underlying genes would give us a better understanding of the mechanism of the KD and an opportunity to further improve upon the diet.

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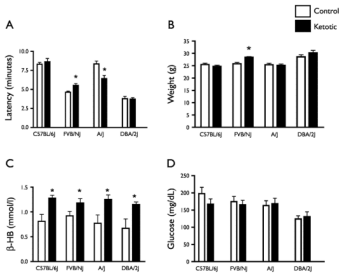


**Figure 1.**

The effects of the KD on seizure thresholds, body weight, and glucose and β-hydroxybutyrate levels are strain dependent.

Following the administration of either the KD or standard chow for two weeks, (A) thresholds to flurothyl-induced seizures were determined. There were no differences in the time to the GTCS in C57BL/6J and DBA/2J mice on either the KD or control diet. FVB/NJ mice on the KD diet exhibited increased latency to GTCS, whereas A/J mice showed reduced latency. (B) No difference in weight was observed between C57BL/6J, A/J, and DBA/2J mice on the KD compared with their respective control groups. However, FVB/NJ mice on the KD showed a statistically significant increase in weight compared with their littermates on standard rodent chow. (C) βHB levels in KD-fed mice were elevated compared with their control littermates. (D) All mice on KD maintained glucose levels that were comparable to their control littermates. Error bars represent SEM. * indicates significance of $p < 0.05$ compared with controls.