neurogastroenterology & motility

Neurogastroenterol Motil (2009) **21,** 936–e72 doi: 10.1111/j.1365-2982.2009.01305.x

Are pediatric and adult-onset cyclic vomiting syndrome (CVS) biologically different conditions? Relationship of adult-onset CVS with the migraine and pediatric CVS-associated common mtDNA polymorphisms 16519T and 3010A

R. G. BOLES,*,† E. A. ZAKI,* T. LAVENBARG,‡ R. HEJAZI,‡ P. FORAN,‡ J. FREEBORN,* S. TRILOKEKAR§ & R. MCCALLUM‡

*Division of Medical Genetics and the Saban Research Institute, Childrens Hospital Los Angeles, CA, USA
†Department of Pediatrics, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA
‡Division of Gastroenterology, University of Kansas, Kansas City, KS, USA
§Ossining High School, Ossining, NY, USA

Abstract *Pediatric cyclic vomiting syndrome (CVS) is associated with a high prevalence of co-morbid migraine and other functional disorders, and with two adult migraine-associated mitochondrial DNA (mtDNA) polymorphisms: 16519T and 3010A. These potential associations have not been studied in adult CVS. The objective of this study is to determine the prevalence of 16519T and 3010A mtDNA polymorphisms and other functional disorders in adult CVS patients. Adults with CVS recruited from the University of Kansas meeting Rome III criteria and a population control group completed a self-reported survey that included questions relating to the diagnostic criteria for several functional disorders. DNA was isolated from blood or saliva and genotyping was performed by standard methodologies. Adult CVS subjects, compared to controls, had significantly more symptoms consistent with several other functional disorders. 16519T was present in 22/31 cases (71%) of child-onset (<12 years) and 9/31 (29%) cases of adult-onset (18+ years) CVS (P = 0.01), vs 27% of controls. Among subjects with 16519T, 3010A was present in 30% of child-onset vs 0% of adult-onset CVS (P = 0.05) and 2% of controls. The conclusions drawn were: (i) unlike pediatric CVS, adult CVS is not associated with the 16519T and 3010A mtDNA polymorphisms, suggesting a degree of genetic distinction and (ii) similar to the pediatric setting, adult CVS is associated with a substantial burden of co-morbid functional disorders.*

Keywords *16519T, 3010A, cyclic vomiting syndrome, functional symptomatology, mitochondrial DNA.*

Address for correspondence
Richard G. Boles, MD, Division of Medical Genetics, Box 90, Childrens Hospital Los Angeles, Los Angeles, CA 90027, USA.
Tel: +1 323 361 2178; fax: +1 323 361 1172;
e-mail: rboles@chla.usc.edu

Presented as an abstract at Neurogastroenterology & Motility Joint International Meeting 6–9 November 2008 Lucerne, Switzerland.
Received: 26 December 2008
Accepted for publication: 17 February 2009

INTRODUCTION

Cyclic vomiting syndrome (CVS) is defined by recurrent stereotypical episodes of nausea and vomiting separated by relatively symptom-free intervals. Although first described in the nineteenth century, the vast majority of published information on CVS involves children and post-dated the first international symposium on CVS in 1994.[1] CVS in children is being recognized more commonly, being present in as many as 2% of school-aged children.[2] In the last few years, it has become apparent that CVS in adults may be more common than in children, but often it is not being recognized by physicians.

Recent articles have started to characterize CVS among adults.[3–5] The data suggest that adult and pediatric CVS differ in certain respects, including longer hospitalizations and more severe abdominal pain during vomiting episodes, and a higher incidence of nausea between the cycles among adults. While CVS in children is well defined and easily recognized by trained observers in the vast majority of cases, some have suggested that CVS in adults is more heterogeneous, a different condition, or does not truly exist as an entity separate from other functional gastrointestinal disorders.

In children, most cases of CVS are associated with co-morbid and/or a close family history of migraine[6] and other 'functional disorders' (e.g. depression, irritable bowel syndrome, etc.).[7] In addition, most cases demonstrate a maternal inheritance bias for migraine and other functional disorders, suggesting that at least some of the pre-disposing genetic factor is on the maternally-inherited mitochondrial DNA (mtDNA).[8] Recently, the common mtDNA polymorphism, 16519T, was found to be six-fold more common in pediatric CVS than in control populations.[9] Another common mtDNA polymorphism, 3010A, was noted to increase the odds ratio for developing CVS in subjects with 16519T by as much as 17-fold. Twenty-five of the 30 CVS cases in that study[8] had the pediatric-onset (before the 18th birthday) of vomiting episodes. In German adults with 'common migraine' (migraine without aura) the odds ratios for 16519T and 16519T+3010A were 3.6 and 15, respectively.[9] The effects of these polymorphisms are substantial, with migraine predicted in 11% of individuals with 16519C, 28% with 16519T and 74% with 16519T+3010A. In pediatric CVS, the higher odds ratios translate into even greater effects of these polymorphisms.

In order to determine if adult and pediatric CVS are phenotypically and genetically similar or distinct, we studied a large group of adult CVS patients to determine the prevalence of migraine, other functional disorders, and the 16519T and 3010A mtDNA polymorphisms. In order to minimize background mtDNA sequence variability, all molecular data reported herein, and in the studies mentioned above, have mtDNA haplogroup H, which is found in about one half of peoples of heritage from Northern Europe.

METHODS

Adult CVS subjects were recruited from the University of Kansas, including 39 with adult (18+ years) onset of stereotypical episodes of nausea and vomiting, 11 with adolescent onset (12–17 years) and 5 with child (pre-pubertal) onset (<12 years). This cohort of 55 patients had a mean age of 31 years at the time of the study (range 18–63); 29 were male. In addition, predominantly child-onset subjects with CVS were recruited from the CVS Association database and a pediatric genetics clinic at Childrens Hospital Los Angeles (25 out of 30 were adolescent and child-onset). CVS was defined by the established Rome III criteria.[10] Regardless of mtDNA haplogroup, the 55 Kansas CVS subjects completed a self-reported survey that includes questions relating to the accepted diagnostic criteria for several functional disorders.[10] The diagnostic criteria used for each condition are listed in the legend of Table 1. As the control group, 73 mothers of high school students enrolled in Biology classes in Ossining High School in Ossining, New York also self-completed the same survey. Both groups completed the surveys in the first half of 2008.

The survey also contained the six questions from the Karolinska Scales of Personality (KSP) comprising the 'KSP6'.[11] This brief battery was constructed from those KSP questions in which answers correlated with the mitochondrial ATP production rate in muscle among adults with major depressive disorder (MDD).[12] Questions are answered on a four-point scale, and the KSP6 measure is considered to be positive if the subject self-reports the highest response, 'Applies Completely', to at least two of the six questions. The KSP6 was found to be sensitive and highly specific for mitochondrial dysfunction in the above MDD group.[11]

For molecular studies, we divided all CVS subjects into groups based on the age-of-onset of stereotypical episodes of nausea and vomiting, and not based on current age or place of ascertainment. Most adult-onset (vomiting episodes began after their 18th birthday) CVS subjects were in the Haplogroup H Kansas group (26/31). Most child-onset (vomiting episodes began before their 12th birthday) CVS subjects were previously and randomly recruited from the Cyclic Vomiting Syndrome Association database,[8,9] with a few cases recruited from the clinic of the first author.[13] A small number of adolescent-onset subjects (vomiting episodes began between their 12th and 18th birthdays) were recruited from all three sources. Molecular data in most of the non-Kansas cases were previous reported.[8]

DNA was isolated from blood by standard methods or from saliva using a commercially-available kit (Oragene; DNA Genotek Inc., Ottawa, ON, Canada). Haplogroup H was defined in the conventional manner as the presence of a C at mtDNA position 7028. Genotyping at 7028, 16519 and 3010 was performed by standard PCR-restriction fragment length polymorphism methodology, confirmed by random sequencing.[9] Pseudogene amplication was excluded by the absence of any product in rho negative cell DNA. For molecular analyses, the control group consisted of 444 and 231 individuals in regards to positions 3010 and 16519, respectively.[9] Only haplogroup H sequences from individuals ascertained as part of a population or control study from Europe or North America were included as controls.

Informed consent was obtained from each CVS subject/parent and clinical control subject (all molecular control subjects were anonymous), and all aspects of the study were approved by the Childrens Hospital Los Angeles, the University of Kansas and/or the Ossining High School Institutional Review Boards. Statistics were performed by WINSTAT Statistics for Windows, (Kalmia Co. Inc., Cambridge, MA) and by custom-made software. Significance was set at $P < 0.05$.

RESULTS

None of the mothers of high school students self-reported symptoms consistent with CVS. That control group was older (46 ± 6 *vs* 35 ± 12 years,

Table 1 Functional and other neuropsychiatric conditions in the Kansas CVS group

	Kansas CVS	High school mothers	*P*	Odds ratio (95% confidence interval)
Irritable bowel syndrome[1]	34/52 (65%)	3/71 (4%)	2.7×10^{-13}	36 (10–130)
Depression[2]	29/53 (55%)	11/71 (15%)	3.8×10^{-6}	6.3 (2.7–15)
Migraine	26/47 (55%)	10/73 (14%)	1.2×10^{-6}	7.5 (3.1–18)
Syncope[3]	20/55 (36%)	5/71 (7%)	4.3×10^{-5}	7.0 (2.4–20)
Photophobia[3]	16/55 (29%)	7/71 (10%)	0.0056	3.6 (1.4–9.5)
Tinnitus[3]	14/55 (25%)	4/71 (6%)	0.016	5.2 (1.6–17)
ADHD[2,4]	12/55 (22%)	1/71 (1%)	1.9×10^{-4}	13.5 (1.7–100)
Chronic fatigue syndrome[5]	10/55 (18%)	1/72 (1%)	0.0011[6]	11 (1.3–87)
Seizure[3]	9/55 (16%)	3/71 (4%)	0.021	4.0 (1.03–16)

[1]The survey responses were consistent with the diagnosis based upon the Rome III criteria (reference 10).
[2]The subject reported being given that diagnosis in the past by a health care provider.
[3]The subject self-reported this symptom on the survey.
[4]Attention deficit hyperactivity disorder.
[5]The survey responses were consistent with the diagnosis based upon the 1994 Centers of Disease Control criteria (reference 17).
[6]Fisher exact test (all other statistics in this column are chi square).
CVS, cyclic vomiting syndrome.

$P = 2.2 \times 10^{-7}$), and more female [100% *vs* 44% (24/55), $P = 8.9 \times 10^{-15}$] than the adult CVS group, both of which would tend to support the null hypothesis.

Self-reported survey responses consistent with a diagnosis of migraine and several other functional conditions were present at substantially increased prevalence rates in the adult Kansas CVS subjects than in the high school mothers control group (Table 1). Adult CVS subjects, compared to controls, had significantly more symptoms consistent with the following functional disorders: irritable bowel syndrome (65% *vs* 4%, $P < 0.0003$), migraine (55% *vs* 14%, $P < 0.0001$), depression (55% *vs* 15%, $P < 0.0004$) and chronic fatigue syndrome (18% *vs* 1%, $P < 0.0001$) (odds ratio range 6–36).

The KSP6 test was positive in 30/54 (56%) of the Kansas adult-CVS subject group *vs* in 2/73 (3%) of high school mothers ($P = 1.2 \times 10^{-11}$, odds ratio = 36, 95% confidence interval = 8–160, sensitivity = 56%, specificity = 97%).

Unlike child-onset CVS, adult-onset CVS was not associated with 16519T or 3010A (Table 2). The mtDNA genotype 16519T was present in 22/31 (71%) of child-onset, 3/6 cases (50%) of adolescent-onset, and 9/31 (29%) cases of adult-onset ($P = 0.01$ for child *vs* adult-onset). The other polymorphism, 3010A, was present in 10/31 (32%) child-onset and 7/31 (23%) adult-onset CVS patients (P = ns). Among those subjects with 16519T, 3010A was present in 6/20 (30%) of child-onset and 0/9 of adult-onset (0%) CVS, *vs* 1/63 (2%) normal controls ($P = 0.08$ adult CVS *vs* child CVS, Fisher exact test).

DISCUSSION

It has been noted over the years that individuals frequently suffer from more than one functional disorder, as do their relatives. Several hundred studies have demonstrated co-morbidity among the functional disorders, and the literature is so numerous as to preclude any comprehensive review here.[14,15] The functional disorder of CVS has been associated with migraine and other functional disorders in several studies,[7,8,13] but these studies were performed predominately in

Table 2 mtDNA genotype *vs* age of onset of stereotypical vomiting episodes

	Child onset 0–11 years	Adolescent onset 12–17 years	Adult onset 18+ years	Population controls (reference 9)
16519T	22T, 9C 71% T	3T, 3C 50% T	9T, 22C 29% T	63T, 168C 27% T
3010A	10A, 21G 32% A	1A, 3G 25% A	7A, 24G 23% A	143A, 301G 32% A
3010A in subjects with 16519T	6A, 14G 30% A	1T, 3C 25% T	0A, 9G 0% A	1A, 62G 1.6% A

16519, child-onset *vs* adult-onset: $P = 9.6 \times 10^{-4}$, odds ratio = 5.6, 95% confidence interval (C.I.) = 1.9–17.
16519, child-onset *vs* control: $P = 1.1 \times 10^{-6}$, odds ratio = 6.3, 95% C.I. = 2.7–14.
16519, adult-onset *vs* control: $P = 0.67$, odds ratio = 1.1, 95% C.I. = 0.48–2.6.
3010 in individuals with 16519T, child-onset *vs* control: $P = 6.1 \times 10^{-4}$ (Fisher exact test), odds ratio = 19, 95% C.I. = 2.1–170.
All comparisons not shown are non-significant ($P > 0.05$).
Most of the child onset and all of the control data were previously published (reference 9).

children. The current data suggest that adult-onset CVS is also strongly associated with migraine and other functional disorders, including irritable bowel syndrome, syncope, photophobia, tinnitus and chronic fatigue syndrome. In general, these conditions can also be considered as dysautonomias. Furthermore, like pediatric CVS,[7,8,13] adult CVS is also associated with neurocognitive conditions, including depression, seizures and attention deficit hyperactivity disorder.

The prevalence rates in the control group, including 15% for depression, 14% for migraine and 1% for chronic fatigue syndrome are well within the generally-reported background prevalence rates for these disorders, and further validate our survey and approach. The prevalence rate of 4% for IBS is low for a control group, probably reflecting difficulty in understanding the Rome III criteria when presented in our questionnaire setting or format. The majority of Kansas CVS cases had a positive score on the KSP6 screening battery, which reflects the substantial degree of functional/dysautonomic-symptom co-morbidity in adult-onset CVS discussed in the preceding paragraph. The KSP6 was designed to detect specific functional co-morbidity as a marker for potential underlying mitochondrial dysfunction.[11] Thus, the KSP6 data suggests that a high proportion of adult-onset CVS cases may have a degree of mitochondrial dysfunction, as is the case in pediatric-onset CVS,[16] and additional investigation is needed.

Only haplogroup H CVS and control subjects were genotyped at 16519 and 3010. The mtDNA haplogroups denote sets of ancient matrilineal ancestry tens of thousands of years old. The West-Eurasian haplogroup H is well suited for genetic association studies due to the relative lack of intra-group sequence variability (mean of only 8 polymorphisms per genome, *vs* up to ten-fold greater sequence variability in subjects of mixed haplogroups) and high prevalence (about 45% of Northern Europeans).[9] A genetically more homogeneous population obviously facilitates the search for pre-disposing sequence variants in complex disorders.

Our data suggest that the previously-reported[9] strong association of CVS with the 16519 and 3010 mtDNA migraine-associated polymorphisms is restricted to pediatric-onset CVS and absent in adult-onset CVS. While our data shows that pediatric-onset and adult-onset CVS cases are genetically distinct, in this aspect, the KSP data suggests that adult-onset CVS may also be associated with energy metabolism in ways that are yet to be discovered and will require future research.

While we conclude that adult CVS is genetically distinct from pediatric CVS, our data also suggests a strong association of adult CVS with co-morbid migraine and multiple other functional disorders. Many functional disorders, including pediatric CVS, migraine and chronic fatigue syndrome, are often treated with the tricyclic 'anti-depressant' amitriptyline. Our observations of high prevalence rates of functional disorders in adult CVS is thus consistent with the current practice of utilizing amitriptyline as first line therapy in adult CVS, and anti-migraine medications are also utilized when indicated.

ACKNOWLEDGMENTS

Many thanks to Savio Reddymasu, MD, for his role in the excellent care of patients; to Kathy Roeser, BA, for detailed assistance and to our esteemed colleague, Irene Sarosiek, MD.

REFERENCES

1 Li B. Cyclic vomiting: the pattern and syndrome paradigm. *J Pediatr Gastroenterol Nutr* 1995; **21**(Suppl 21): 6–10.
2 Abu-Arafeh I, Russell G. Cyclical vomiting syndrome in children: a population-based study. *J Pediatr Gastroenterol Nutr* 1995; **21**: 454–8.
3 Fleisher DR, Gornowicz B, Adams K, Burch R, Feldman EJ. Cyclic vomiting syndrome in 41 adults: the illness, the patients, and problems of management. *BMC Med* 2005; **3**: 20.
4 Namin F, Patel J, Lin Z *et al.* Clinical, psychiatric and manometric profile of cyclic vomiting syndrome in adults and response to tricyclic therapy. *Neurogastroenterol Motil* 2007; **19**: 196–202.
5 Abell TL, Adams KA, Boles RG *et al.* Cyclic vomiting syndrome in adults. *Neurogastroenterol Motil* 2008; **20**: 269–84.
6 Li BU, Murray RD, Heitlinger LA, Robbins JL, Hayes JR. Is cyclic vomiting syndrome related to migraine? *J Pediatr* 1999; **134**: 567–72.
7 Boles RG, Powers AL, Adams K. Cyclic vomiting syndrome plus. *J Child Neurol* 2006; **21**: 182–8.
8 Boles RG, Adams K, Li BU. Maternal inheritance in cyclic vomiting syndrome. *Am J Med Genet* 2005; **133**: 71–7.
9 Zaki EA, Freilinger T, Klopstock T *et al.* Two common mitochondrial DNA polymorphisms are highly associated with migraine headache and cyclic vomiting syndrome. *Cephalagia* in press 2009 [Epub ahead of print].
10 Drossman DA, Corazziari E, Delvaux M *et al. Rome III: The Functional Gastrointestinal Disorders*, 3rd edn. McLean, VA: Degnon Associates, 2006: 1–1048.
11 Gardner A, Boles RG. Symptoms of somatization as a rapid screening tool for mitochondrial dysfunction in depression. *Bio Psycho Soc Med* 2008; **2**: 7.
12 Gardner A, Boles RG. Mitochondrial energy depletion in depression with

somatization. *Psychother Psychosom* 2008; **77**: 127–9.
13 Boles RG, Adams K, Ito M, Li BU. Maternal inheritance in cyclic vomiting syndrome with neuromuscular disease. *Am J Med Genet* 2003; **120**: 474–82.
14 Yunus MB. Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. *Semin Arthritis Rheum* 2007; **36**: 339–56.
15 Hudson JL, Mangweth B, Pope HG Jr *et al.* Family study of affective spectrum disorder. *Arch Gen Psychiatry* 2003; **60**: 170–7.
16 Boles RG, Williams JC. Mitochondrial disease and cyclic vomiting syndrome. *Dig Dis Sci* 1999; **44**: 103S–7S.
17 Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994; **121**: 953–9.