Phytosterolemia on the island of Kosrae: founder effect for a novel *ABCG8* mutation results in high carrier rate and increased plasma plant sterol levels

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Abstract **Screening of 932 adults on the Pacific island of Kosrae for plasma plant sterol levels disclosed three subjects, two of them asymptomatic, with phytosterolemia. Sequencing the ATP binding cassette subfamily G member 8 (ABCG8) gene revealed a novel exon 2 mutation that causes a change in codon 24 from glutamine to histidine and a frame shift followed by a premature stop codon, precluding the formation of a functional ABCG8 protein. Genotyping of 1,090 Kosraens revealed 150 as carriers, a 13.8% carrier rate. DNA sequencing of 67 carriers revealed the same mutation as in the probands. In carriers, plasma campesterol and sitosterol levels were 55% and 30% higher, respectively, than in noncarriers. Moreover, compared with noncarriers, carriers showed 21% lower plasma levels of lathosterol, a surrogate marker for cholesterol biosynthesis. There was no difference between the groups in plasma total cholesterol, triglycerides, apolipoprotein B, or apolipoprotein A-I levels.** **In summary, on the island of Kosrae, a strong founder effect of a mutant ABCG8 allele results in a large number of carriers with increased plasma plant sterol levels and decreased lathosterol levels. The latter finding suggests that heterozygosity for a mutated ABCG8 allele results in a modest increase in dietary cholesterol absorption and a decrease in cholesterol biosynthesis.**—Sehayek, E., H. J. Yu, K. von Bergmann, D. Lutjohann, M. Stoffel, E. M. Duncan, L. Garcia-Naveda, J. Salit, M. L. Blundell, J. M. Friedman, and J. L. Breslow. **Phytosterolemia on the island of Kosrae: founder effect for a novel *ABCG8* mutation results in high carrier rate and increased plasma plant sterol levels.** ***J. Lipid Res.*** **2004.** 45: **1608–1613.**

Supplementary key words ATP binding cassette subfamily G member 8 • cholesterol absorption • population genetics

Plasma concentrations of plant sterols correlate with and are widely considered a marker for dietary cholesterol absorption (1–3). Better understanding of the genes that regulate plant sterol metabolism should provide important insights into cholesterol metabolism and perhaps even atherosclerosis susceptibility. In mammals, the diet is the only source of plasma plant sterols, and the metabolism of these sterols has much in common with that of cholesterol. For example, dietary plant sterols and cholesterol partition into the same intestinal compartments and compete with each other for uptake by the intestine. In addition, both sterols are transported in plasma by the same lipoproteins from which they are taken up by the liver and excreted into bile. However, there are important differences in the metabolism of these two classes of sterols (4). In humans, on average, ~50% of the daily cholesterol intake is absorbed from the intestines, whereas for plant sterols absorption is only 5–15% (5). The mechanisms that regulate the similarities and differences in plant sterol and cholesterol metabolism are only partially understood, and much recent knowledge has come from studies of the disorder phytosterolemia.

Phytosterolemia is a rare autosomal recessive disease characterized by extremely high levels of plasma plant sterols. It was shown to be caused by homozygosity or compound heterozygosity for mutations in ATP binding cassette subfamily G member 5 or 8 (*ABCG5* or *ABCG8*) (6–8). These genes are adjacent to each other on the short arm of human chromosome 2 and code for two hemitransporters, which heterodimerize with each other to transport sterols in the intestines and the liver. In addition to severely increased plasma plant sterol levels, attributable to increased plant sterol absorption and decreased biliary excretion, patients also have tendon xanthomas, arthritis,

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Abbreviations: *ABCG5* and *ABCG8*, ATP binding cassette subfamily G members 5 and 8.

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hemolysis, and coronary heart disease at a young age (5). The effect of heterozygosity for an *ABCG5* or *ABCG8* mutation on plasma plant sterol levels has been controversial, with mixed results reported (5). In association studies in Caucasians, common sites of variation in the *ABCG5* and *ABCG8* genes account for only a small proportion of the population variation in plasma plant sterol levels (9).

To shed additional light on genes that affect plasma plant sterol levels, a study was done on the Micronesian island of Kosrae. This island is 2,500 miles northeast of Australia and was first inhabited by Southeast Asians 2,000–3,000 years ago. Westerners, largely whalers and missionaries of Caucasian ancestry, first visited Kosrae in 1824 and in subsequent years intermarried with native Kosraens. In the 1800s, there was a severe decline in the native population attributable to Western diseases. This resulted in a population bottleneck that was most severe in the 1880s, with relatively few survivors giving rise to modern Kosraens. In 1945, immediately after World War II, Kosrae became a United States Trust Territory, and most citizens received sedentary civil service jobs and high-fat, high-calorie surplus foods. This resulted in a major lifestyle change, which has caused an epidemic of obesity and diabetes (10–13). In the last 10 years, the Kosraen population has been studied for genes that cause obesity, diabetes, hypertension, and dyslipidemia (14, 15). As a consequence, population screenings were carried out for syndrome X-related phenotypes in 1994 and again in 2001/2002. A total of 3,300 adult Kosraens have been phenotyped and their DNA taken for genotyping. In addition, a multigenerational family tree of the islanders has been constructed.

This resource was used to identify loci in Kosraens that modify plasma plant sterol levels. Measurements were made on frozen plasma samples from a subset of individuals who participated in the 1994 screen. This resulted in the identification of two siblings and one additional subject with extremely high plasma plant sterol levels, compatible with the diagnosis of phytosterolemia. Sequencing efforts revealed a novel mutation in the *ABCG8* gene, and testing for the frequency of this mutation disclosed a high carrier rate among Kosraens consistent with a strong founder effect. Kosraen carriers had increased plasma plant sterol levels and a decrease in a sterol marker for cholesterol biosynthesis. These findings indicate that in Kosrae, the *ABCG8* locus is an important modifier of plasma plant sterol levels with a moderate effect on cholesterol biosynthesis, presumably attributable to changes in intestinal cholesterol absorption.

MATERIALS AND METHODS

Sample collection and analysis

The first public health screening to identify risk factors associated with syndrome X was conducted in 1994 among 2,188 adult Kosraens (age range, 20–85 years) under a protocol approved by both The Rockefeller Hospital Institutional Review Board and the Kosrae Department of Health. Informed consent was obtained from all participants. Recruitment was through open meetings and radio announcements, and it was estimated that more than 90% of adult Kosraens then residing on the island participated. Participants filled out questionnaires, including family data, which were subsequently used to construct a family tree of the island population. A detailed description of the study has been published (14). Of relevance to the present study, body weight and height were recorded and body mass index was calculated (body weight in kilograms divided by squared height in meters). Subjects were designated as diabetics in the presence of at least one of the following three criteria: *i*) fasting plasma glucose levels $\geq$ 126 mg/dl; *ii*) 2 h oral glucose tolerance test levels $\geq$ 200 mg/dl; or *iii*) treatment with insulin or oral hypoglycemic drugs. Blood was drawn after 12 h of fasting into EDTA tubes, and plasma was immediately separated by low-speed centrifugation and stored in aliquots from 1994 to 2002 at −20°C before sterol analysis. Genomic DNA was extracted from whole blood, plated at a random order on 384-well plates, and kept at −20°C until analysis. The levels of plasma sterols, including campesterol, sitosterol, lathosterol, and cholesterol, were measured in a cohort of 932 Kosraens for whom complete genome scan data were available. Plasma sterol levels were measured by gas-liquid chromatography as described previously (16). In addition, plasma triglyceride levels were measured with a commercially available enzymatic kit (Sigma Diagnostics, St. Louis, MO), and plasma apolipoprotein B and apolipoprotein A-I levels were measured by ELISA.

Genotyping

The *ABCG5* and *ABCG8* genes were sequenced using genomic DNA in one proband and one of her parents with primers described by Hubacek et al. (17). Resequencing of exon 2 of the *ABCG8* gene was done with two new primers (forward primer, 5′-AGATGGGCCCTTGTCAGCACTCCTATTT-3′; reverse primer, 5′-GCCATTGATGACACCTATTGCACCTGACA-3′), yielding an amplicon of 464 bp. For *ABCG8* exon 2 genotyping, we applied two strategies. In the first strategy, the entire pedigrees of the sequenced phytosterolemic proband and 362 additional adult Kosraens (whose DNA has been plated on a single 384-well plate) were genotyped using the same primer pair and the PCR product was digested with the restriction enzyme *Pfo*I (Fermentas, Inc., Hanover, MD). This enzyme fails to digest the product of the mutated allele but digests the normal allele into two fragments of 267 and 197 bp. The PCR was done in 20 μl consisting of 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 2 mM $MgCl_2$, 800 μM deoxyribonucleoside triphosphates, 0.4 μM of each primer, 20 ng of genomic DNA, and 0.4 μl of 50× BD Advantage 2 Polymerase Mix (Clontech, Palo Alto, CA). After ascertainment, through sequencing, that all homozygote and heterozygote subjects identified by *Pfo*I restriction enzyme genotyping were carriers of the same mutated allele, we devised a high-throughput genotyping strategy using fluorescently labeled allele-specific primers that amplified the normal and mutated alleles. This strategy was applied for the genotyping of an additional 717 adult Kosraens (whose DNA has been plated on two additional 384-well plates). The normal allele was PCR amplified with the forward primer 5′-FAM-TGTCTTCCACAGGGCCTCCAG-3′ and the reverse primer 5′-GCTTCTGTCTCTGACCTCCAGGGTGTTGG-3′, yielding a 105 bp product, whereas the mutated allele was PCR amplified with the forward primer 5′-VIC-TCTCCCACAGGGCCTCCACA-3′ and the reverse primer 5′-GCTTCTCTGTCCCTGCCTGCTCCTCTCC-3′, yielding a 162 bp product. PCR products were analyzed by capillary electrophoresis by using the Applied Biosystems 3700 DNA sequencer, and allele scores were analyzed by using Applied Biosystems GENOTYPE 3.6 NT software.

Statistical analysis

Differences in plasma sterol levels, triglycerides, and apolipoproteins B and A-I between carriers and noncarriers were tested using a two-tailed unpaired Student's *t*-test. Differences in plasma sterols after stratification of carriers and noncarriers by either sex and diabetes-affected status (four strata) or sex and body mass index tertiles (six strata) were tested by two-tailed unpaired Student's *t*-test or one-way ANOVA with Tukey's posttest, respectively.

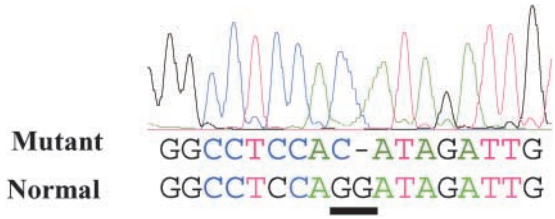

Fig. 2. Electropherogram and sequences of the normal and mutated alleles of *ABCG8*.

RESULTS

Levels of campesterol and sitosterol were measured in a cohort of 932 adult Kosraens. This disclosed two siblings, a 46 year old male and a 48 year old female, as well as another 50 year old female, with campesterol and sitosterol levels 25- to 50-fold higher than normal (plasma campesterol levels of 3.9–5.6 mg/dl and plasma sitosterol levels of 7.5–11.6 mg/dl compared with mean levels of 0.18 ± 0.08 and 0.22 ± 0.13 mg/dl, respectively, in the other Kosraens). The clustering in one nuclear family of two subjects with exceptionally high plasma plant sterol levels strongly suggested the diagnosis of phytosterolemia. Of these two siblings, one had symptoms consistent with stable angina, but neither sibling had a clinical history of arthritis or hemolytic episodes, nor did physical examination reveal xanthelasma or tendon xanthomas. Clinical data for the other 50 year old affected female were not available.

As shown in **Fig. 1**, the two siblings were born to a consanguineous marriage between two second degree cousins, compatible with autosomal recessive inheritance. The exons and exon-intron splice junctions of *ABCG5* and *ABCG8* were sequenced in one parent and one proband. As shown in **Fig. 2**, the proband was homozygous for a novel *ABCG8* exon 2 mutation, and the parent was heterozygous for the same mutation. As detailed in Fig. 2, this mutation entails a G→C substitution followed by a single base deletion. This mutation would result in a change in codon 24 from Gln to His and a frame shift followed by eight new codons and a premature stop codon, precluding the formation of a functional *ABCG8* protein. The entire pedigree was genotyped for this mutation by amplifying exon 2 and digesting with the *Pfo*I restriction enzyme, which fails to cut the mutant allele. As shown in Fig. 1, the two probands displayed a single high molecular weight band (464 bp), corresponding to the nondigested allele. In contrast, the obligate heterozygous parents displayed both the high molecular weight band and two lower molecular weight bands, corresponding to the products of the digested normal allele (267 and 197 bp). The seven siblings of the probands were also genotyped, and as

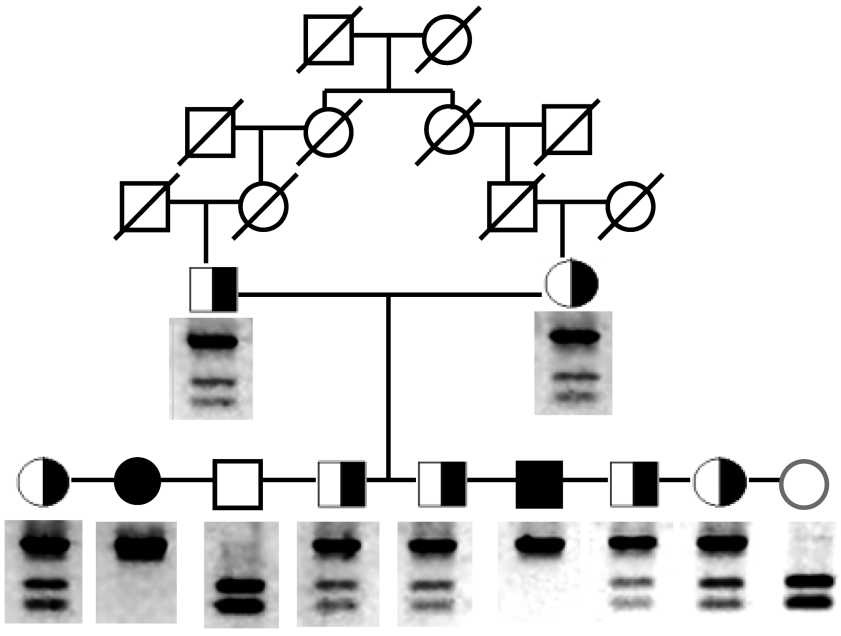

Fig. 1. Family tree of a phytosterolemia pedigree on the island of Kosrae. Shown is the *Pfo*I genotyping of the ATP binding cassette subfamily G member 8 (*ABCG8*). The PCR-amplified product (464 bp) was subjected to *Pfo*I digestion as described in Materials and Methods. The enzyme fails to digest the mutated allele and digests the normal allele, producing two fragments of 267 and 197 bp.

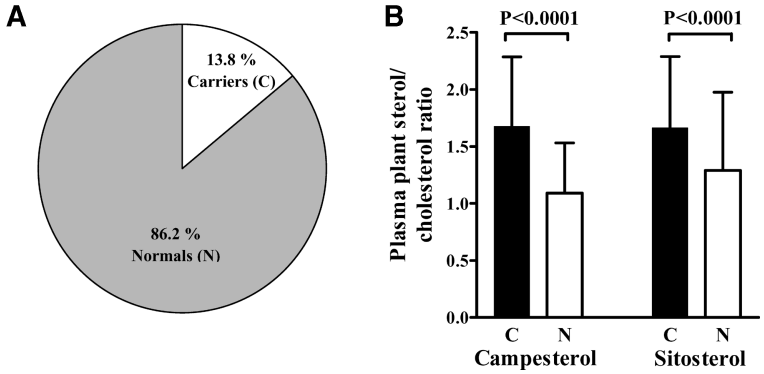

Fig. 3. Frequency of the *ABCG8* mutation in Kosrae (A) and the mutated allele affect on plasma plant sterol levels (B). A random sample of 1,090 adult Kosraens was genotyped using either the *Pfo*I restriction enzyme or allele-specific primers as described in Materials and Methods. Shown are the frequency of carriers (C) and noncarriers (N) and the genotypic effect on plasma campesterol and sitosterol-to-plasma total cholesterol ratio in 84 carriers and 546 noncarriers for whom the *ABCG8* genotype and plasma plant sterol measures were available. Error bars are SD.

shown, five were carriers and two were noncarriers. This distribution is compatible with the Mendelian segregation of an autosomal recessive mutation of *ABCG8* in this family causing phytosterolemia. Sequencing studies in the other 50 year old female disclosed that she was homozygous for the same ABCG8 mutation.

The frequency of the *ABCG8* mutation on Kosrae was estimated through either *Pfo*I restriction enzyme digestion of PCR-amplified DNA (373 subjects) or allele-specific PCR genotyping (717 subjects) from a random sample of 1,090 participants in the 1994 screening. As shown in **Fig. 3**, this revealed 150 carriers of the *ABCG8* mutation, or a carrier frequency of 13.8%. Because the mutation in the probands involved a base change followed by a single base deletion, the second exons of 67 carriers, identified through *Pfo*I restriction enzyme digestion, were sequenced, and in each case the mutation was the same as in the probands. The high frequency of this mutation in Kosraens indicates a strong founder effect.

The effect of the Kosraen *ABCG8* mutation on plasma plant sterol levels was determined in 84 carriers and 546 noncarriers (subsets of the 1,090 genotyped subjects) who were both genotyped for the *ABCG8* mutation and phenotyped for plasma plant sterol levels. As shown in **Table 1** and Fig. 3, carriers of the mutation had significantly higher plasma campesterol and sitosterol levels than noncarriers both in absolute levels (0.29 ± 0.11 and 0.28 ± 0.11 mg/dl vs. 0.18 ± 0.08 and 0.22 ± 0.13 mg/dl for campesterol and sitosterol, respectively; $P < 0.0001$) and in the ratio of plasma campesterol or plasma sitosterol to cholesterol (1.68 ± 0.61 and 1.67 ± 0.63 vs. 1.09 ± 0.44 and 1.28 ± 0.68 for campesterol and sitosterol, respectively; $P < 0.0001$). In addition, as shown in Table 1, carriers showed significantly decreased absolute concentrations of plasma lathosterol (0.23 ± 0.12 vs. 0.28 ± 0.14 mg/dl; $P < 0.001$), a cholesterol precursor that has been used as a surrogate marker for whole-body cholesterol biosynthesis. Furthermore, data analysis after stratification by sex and diabetes-affected state or sex and body mass index tertiles revealed that the effect of the ABCG8 mutation on plasma plant sterol and lathosterol levels is independent of these variables (data not shown). Finally, plasma levels of total cholesterol, triglycerides, apolipoprotein B, and apolipoprotein A-I were compared in 144 carriers and 925 noncarriers (for whom the ABCG8 exon 2 genotype and plasma levels of these measures were available), and, as shown in Table 1, no differences were found.

DISCUSSION

In a population screen for plasma plant sterol levels on the Micronesian island of Kosrae, three subjects were identified with phytosterolemia. Genetic analysis revealed that they were homozygous for a novel mutation in the *ABCG8* gene that rendered it completely dysfunctional. Analysis of the nuclear family of two of these three subjects revealed that both parents and five of seven siblings were heterozygous for this mutation; in the general Kosraen population, 13.8% were carriers. Carriers had higher levels of plant sterols and lower levels of the cholesterol precursor lathosterol but equal total cholesterol, triglycerides, apolipoprotein A-I, and apolipoprotein B levels compared with noncarriers. These findings raise some inter-

TABLE 1. Subject characteristics, plasma lipids and apolipoproteins levels, and ratio of plasma sterols to total cholesterol in carriers and noncarriers of the ATP binding cassette subfamily G member 8 mutation

Variable	Carriers	Noncarriers	*P*
	(N = 84)	(N = 546)	
Males/females (n)	40/44	242/304	
Age (years)	45 ± 16	43 ± 14	
Diabetes (%)	28	23	
BMI	29.5 ± 5.3	30.7 ± 5.4	
Campesterol (mg/dl)	0.29 ± 0.11	0.18 ± 0.08	<0.0001
Sitosterol (mg/dl)	0.28 ± 0.11	0.22 ± 0.13	<0.0001
Campesterol/cholesterol	1.68 ± 0.61	1.09 ± 0.44	<0.0001
Sitosterol/cholesterol	1.67 ± 0.63	1.28 ± 0.68	<0.0001
Lathosterol/cholesterol	1.30 ± 0.63	1.65 ± 0.75	<0.0001
	(N = 144)	(N = 925)	
Males/females (n)	64/80	405/520	
Age (years)	44 ± 16	43 ± 14	
Diabetes (%)	26	24	
BMI	30.0 ± 5.3	30.9 ± 5.5	
Cholesterol (mg/dl)	180 ± 37	177 ± 37	NS
Triglycerides (mg/dl)	91 ± 48	103 ± 114	NS
apoB (mg/dl)	91 ± 21	88 ± 23	NS
apoA-I (mg/dl)	118 ± 25	117 ± 25	NS

apoB, apolipoprotein B; BMI, body mass index. Values shown are means ± SD unless indicated otherwise.

esting questions with regard to phytosterolemia and its carrier state.

Phytosterolemia in the adult population is usually diagnosed in patients with tendon xanthomas and normal or moderately increased plasma cholesterol levels (5). In this situation, the alert clinician might order gas chromatographic analysis of plasma sterols, and the finding of markedly increased plasma plant sterol levels confirms the diagnosis. Through a general population screening, our finding of two individuals with phytosterolemia in their late 40s but without tendon xanthomas and minimal other signs of disease raises the possibility that many other patients with this disease go undetected in the general population. The frequency of phytosterolemia in the general population is not entirely clear; however, based on the number of patients who have been described worldwide (about 70 patients by 2002), it is thought that this is a rare disease. Although current methods for measuring plasma plant sterol levels are too cumbersome, general population screening might identify many more phytosterolemics.

In Kosrae, if the mutant phytosterolemia allele is in Hardy-Weinberg equilibrium, the carrier rate of 13.8% requires an allele frequency of ~7% and an expected frequency of affected homozygotes of ~0.47%. Of the 932 Kosraens initially screened for plasma plant sterol levels, 3 were found to have phytosterolemia, corresponding to a frequency of 0.32%, which is close to what would have been expected.

The high carrier rate of the novel *ABCG8* mutation on Kosrae indicates a founder effect. This is presumably attributable to a carrier surviving the severe population bottleneck that occurred in the 1880s and the subsequent contribution of this survivor to the gene pool of the island. Estimates of linkage disequilibrium and heterozygosity in present-day Kosraens indicates relatively few founders of this population. This is compatible with the large founder effect observed for the mutant *ABCG8* allele. With regard to whether the founder allele was Micronesian or Caucasian, we cannot be certain. The construction of haplotypes based on microsatellite markers 1 Mb proximal and 7 Mb distal to the *ABCG8* gene reveals the mutation to have occurred on the most common haplotypes, which are present in both Micronesians and Caucasians. Denser mapping with single nucleotide polymorphisms (SNPs) in this region would be necessary to identify subhaplotypes to distinguish the origin of the mutant allele. Based on Y chromosome haplotypes and mitochondrial DNA sequences in Kosrae, it is estimated that ~75% of the autosomal genes are Micronesian and 25% are Caucasian (D. Shmulewitz, J. M. Friedman, and M. Stoffel, unpublished data). Nevertheless, because many Caucasian phytosterolemia mutations have been documented without detecting this mutation, it is likely that the Kosraen mutation is either of Micronesian origin or private to one of the founders.

In the current study, there were sufficient numbers of individuals carrying the mutant *ABCG8* allele to ask whether having only one dysfunctional *ABCG5/ABCG8* allele has metabolic consequences. In this comparatively homogeneous population carrying only one type of mutation at the *ABCG5/ABCG8* locus, it was possible to discern that carriers had higher plasma plant sterol levels compared with noncarriers. It is possible that in previous studies a more variable environment or heterogeneity of mutations provided conflicting results (5). Thus, we conclude that *ABCG8* is one locus that influences plasma plant sterol levels, albeit in Kosraens. Berge et al. (9) have found that common sites of variation in the *ABCG5/ABCG8* locus, including nonsynonymous SNPs, have relatively little affect on plasma plant sterol levels in the general population. The most likely explanation is that these common sites of variation have mild or no effects on gene function and that a fully dysfunctional allele is required to see affects.

The effect of haploinsufficiency of the *ABCG8* gene on other parameters related to sterol metabolism and risk factors for cardiovascular disease could also be evaluated in Kosraens because of the high carrier rate. For example, several studies have shown a correlation between dietary cholesterol absorption and plasma plant sterol levels and suggested that the latter could be used as a marker for the former (1–3). If this is the case, one would expect that Kosraen carriers of the *ABCG8* mutation with increased plasma plant sterol levels would have increased dietary cholesterol absorption compared with noncarriers. Although this could not be assessed directly, Kosraen carriers had decreased levels of a surrogate measure of whole-body cholesterol synthesis, the cholesterol biosynthetic intermediate lathosterol (Table 1). Decreased whole-body cholesterol biosynthesis could be the result of a moderate increase in dietary cholesterol absorption in the carriers. Thus, the results in Kosrae are compatible with the notion that plasma plant sterol levels are a marker of dietary cholesterol absorption. With regard to risk factors for cardiovascular disease, the Kosraen carriers did not have altered levels of such classic risk factors as plasma total cholesterol or triglycerides, apolipoprotein B, or apolipoprotein A-I levels. It was recently suggested that plasma plant sterol levels may be an independent risk factor for cardiovascular disease (18), but this could not be assessed in the context of the Kosrae population screening.

In summary, phytosterolemia has been described on the Micronesian island of Kosrae as a result of a novel *ABCG8* gene mutation. Because of a founder effect, a high carrier rate of the mutant allele was documented and found to influence plasma plant sterol levels and whole-body cholesterol synthesis and possibly dietary cholesterol absorption but not plasma total cholesterol or triglycerides, apolipoprotein B, or apolipoprotein A-I levels.

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REFERENCES

1. Tilvis, R. S., and T. A. Miettinen. 1986. Serum plant sterols and their relation to cholesterol absorption. *Am. J. Clin. Nutr.* **43:** 92–97.
2. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesaniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
3. Von Bergmann, K., D. Lutjohann, B. Lindenthal, and A. Steinmetz. 2003. Efficiency of intestinal cholesterol absorption in humans is not related to apoE phenotype. *J. Lipid Res.* **44:** 193–197.
4. Sehayek, E. 2003. Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences. *J. Lipid Res.* **44:** 2030–2038.
5. Bjorkhem, I., K. M. Boberg, and E. Leitersdorf. 2001. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. *In* The Metabolic and Molecular Bases of Inherited Disease. Vol. 2. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 2961–2988.
6. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science.* **290:** 1771–1775.
7. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, A. K. Srivastava, G. Salen, M. Dean, and S. B. Patel. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
8. Lu, K., M. H. Lee, S. Hazard, A. Brooks-Wilson, H. Hidaka, H. Kojima, L. Ose, A. F. Stalenhoef, T. Mietinnen, I. Bjorkhem, E. Bruckert, A. Pandya, H. B. Brewer, Jr., G. Salen, M. Dean, A. Srivastava, and S. B. Patel. 2001. Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. *Am. J. Hum. Genet.* **69:** 278–290.
9. Berge, K. E., K. von Bergmann, D. Lutjohann, R. Guerra, S. M. Grundy, H. H. Hobbs, and J. C. Cohen. 2002. Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *J. Lipid Res.* **43:** 486–494.
10. Irwin, G. 1994. The Prehistoric Exploration and Colonisation of the Pacific. Cambridge University Press, London.
11. Hezel, F. 1983. The First Taint of Civilization: A History of the Caroline and Marshall Islands in Pre-Colonial Days. University of Hawaii Press, Honolulu. 1521–1885.
12. Segal, H. 1989. Kosrae: The Sleeping Lady Awakens. Kosrae Tourist Division, Department of Conservation and Development, Kosrae State Government, Kosrae, Federated States of Micronesia.
13. Cordy, R. 1993. The Lelu Stone Ruins (Kosrae, Micronesia) 1978–1981. Historical and Archaeological Research. University of Hawaii Press, Honolulu.
14. Shmulewitz, D., S. B. Auerbach, T. Lehner, M. L. Blundell, J. D. Winick, L. D. Youngman, V. Skilling, S. C. Heath, J. Ott, M. Stoffel, J. L. Breslow, and J. M. Friedman. 2001. Epidemiology and factor analysis of obesity, type II diabetes, hypertension, and dyslipidemia (syndrome X) on the island of Kosrae, Federated States of Micronesia. *Hum. Hered.* **51:** 8–19.
15. Han, Z., S. C. Heath, D. Shmulewitz, W. Li, S. B. Auerbach, M. L. Blundell, T. Lehner, J. Ott, M. Stoffel, J. M. Friedman, and J. L. Breslow. 2002. Candidate genes involved in cardiovascular risk factors by a family-based association study on the island of Kosrae, Federated States of Micronesia. *Am. J. Med. Genet.* **110:** 234–242.
16. Heinemann, T., G. Axtmann, and K. von Bergmann. 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur. J. Clin. Invest.* **23:** 827–831.
17. Hubacek, J. A., K. E. Berge, J. C. Cohen, and H. H. Hobbs. 2001. Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. *Hum. Mutat.* **18:** 359–360.
18. Sudhop, T., B. M. Gottwald, and K. von Bergmann. 2002. Serum plant sterols as a potential risk factor for coronary heart disease. *Metabolism.* **51:** 1519–1521.