Null Results in Brief

KRAS Mutations Are Not Predictive for Progression of Preneoplastic Gastric Lesions[1]

Jay D. Hunt,[2] Robertino Mera, Anna Strimas, A. Tod Gillespie, Bernardo Ruiz, Pelayo Correa, and Elizabeth T. H. Fontham

Departments of Biochemistry and Molecular Biology [J. D. H., A. S., A. T. G.], Pathology [R. M., P. C.], and Public Health and Preventive Medicine [E. T. H. F.], and the Stanley S. Scott Cancer Center [J. D. H., R. M., P. C., E. T. H. F.], Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112

Abstract

Individuals with atrophic gastritis ($n = 863$) were recruited to participate in a chemoprevention trial in Nariño, Columbia. The volunteers were randomly assigned to intervention therapies, which included antibiotic treatment for *Helicobacter pylori* infection, and then daily dietary supplementation with antioxidant micronutrients in a 2^3 factorial design. Biopsies were obtained according to a specified protocol from designated areas in the stomach for each individual at baseline (before intervention therapy), at year 3, and at year 6. A systematic sample of 160 participants was selected from each of the eight treatment combinations, and the first exon of *KRAS* was examined for mutations. At year 3, the data indicated that individuals with *KRAS* mutations in their baseline premalignant stomach biopsies were 3.74 times as likely to progress to a higher premalignant stage than those who lacked baseline mutations ($P = 0.04$; C. Gong *et al.*, Cancer Epidemiol. Biomark. Prev. *8*:167–171, 1999). However, after 6 years, baseline *KRAS* mutations failed to predict histological progression. Also, *KRAS* mutation in 72-month biopsies did not predict histological progression.

Introduction

Sequential steps of precancerous changes often precede intestinal-type gastric carcinoma. These steps include atrophic gastritis, IM[3] (type I, complete, or small intestinal metaplasia; and type III, incomplete, or colonic intestinal metaplasia), and dysplasia (reviewed in Ref. 1). Mutations in *KRAS* are detected in many types of human tumors and have been associated with development and progression (2). Recently, we analyzed the *KRAS* mutation status of premalignant stomach biopsies taken at baseline and at 36 months from 160 individuals from Colombia who had a high risk for developing gastric cancer and were enrolled in a 2^3 factorial intervention trial. In that study, we found that those with baseline *KRAS* mutations were 3.76 times more likely to progress from either atrophy to metaplasia or from complete metaplasia (type I) to incomplete metaplasia (type III; $P = 0.05$; Ref. 3). When the odds ratio was adjusted for intervention therapy, the estimate was unchanged and statistically significant (odds ratio, 3.74; $P = 0.04$). The presence of baseline *KRAS* mutations was a significant predictor of progression at 36 months.

In the present study, biopsies were taken from 142 of the 160 study subjects who returned for 72-month follow-ups. They were analyzed for *KRAS* mutation and for progression/regression of stomach lesions. The hypothesis: baseline *KRAS* mutations predict histological progression of premalignant stomach lesions.

Materials and Methods

Study Population. Eight hundred and sixty-three individuals with chronic multifocal atrophic gastritis were recruited from the towns of Pasto and Tuquerres of Nariño in the southern Colombian Andes. In this region, the incidence of gastric cancer ranks as one of the highest in the world (150/100,000; Ref. 4). The estimated *Helicobacter pylori* prevalence is >90% among asymptomatic adults (5). The volunteers were agricultural or blue-collar workers of Spanish-Indian ("mestizo") extraction. The demographic characteristics of the volunteer population have been described previously (6). Endoscopic evaluations of individuals from the community who volunteered to participate in the study were performed in the Hospital Departmental (Pasto, Colombia) after obtaining informed consent approved by the local Human Subjects Committee and the Louisiana State University Health Sciences Center Institutional Review Board. Infection with *H. pylori* was detected by the Steiner modification of the Warthin-Starry staining method using baseline biopsies.

Study Design. Volunteer individuals were randomized into treatment groups using a 2^3 factorial design as described previously (3). A systematic sample of 160 participants was selected from each of the eight treatment combinations for this study. Of these 160 individuals, samples were available from 142 at 72 months. The anti-*Helicobacter* treatment consisted of a 2-week course of amoxicillin (500 mg, three times per day), metronidazole (400 mg, three times per day), and bismuth subsalicylate (262 mg, four times per day). Ascorbic acid (1-g tablet, twice per day) and/or β-carotene (30-mg capsule, once per day) or matched placebos for these two drugs (provided by Hoffman-La Roche, Inc.) were given throughout the study. Compliance was assessed by quarterly pill-counts as well as by measurement of serum antioxidant levels at the time of the third endoscopy. Compliance was consistently >90%, as measured by pill-count. Biopsies from 160 individuals (20 from each

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[2] To whom requests for reprints should be addressed, at Louisiana State University Health Sciences Center, Department of Biochemistry and Molecular Biology, 533 Bolivar St., CSB-4-18, New Orleans, LA 70112. Phone: (504) 568-4734; Fax: (504) 599-1014; E-mail: jhunt@lsuhsc.edu.
[3] The abbreviation used is: IM, intestinal metaplasia.

Table 1 *KRAS* mutations and progression of preneoplastic lesions

KRAS mutation	Progression after 72 mo[a]			Total
	Progressed	No change	Regressed	
Baseline				
Absent	10	68	44	122
Present	2	13	5	20
Total ($P = 0.934$)	12	81	49	142
72 Mo				
Absent	10	65	39	114
Present	1	13	8	22
Total ($P = 0.446$)	11	78	47	136[b]

[a] Progression includes the following situations: atrophy → IM; within IM: small intestinal metaplasia → colonic intestinal metaplasia.
[b] *KRAS* mutation status was determined from 136 of 142 biopsies.

group) were used to detect *KRAS* mutations in a double-blinded study at 36 months (3). Biopsies, DNA isolation, mutation detection by denaturing gradient gel electrophoresis, DNA sequencing, and statistical analyses have all been described previously in detail (3).

Results and Conclusions

Of the 160 original volunteers, histological diagnoses were available for 142 individuals after 72 months of follow-up. As seen in Table 1, 12 of 142 (8.4%) progressed to a higher histological stage, 81 of 142 (57.0%) did not change, and 49 of 142 (34.5%) regressed. There was no association between histological change at the end of 6 years of follow-up and *KRAS* mutation status at baseline ($P = 0.934$). This was somewhat surprising, given our previous finding that baseline *KRAS* mutations strongly predicted histological progression after 36 months of follow-up (3).

Of the 142 individuals for whom histological diagnoses were available, *KRAS* mutation status was determined in 136 after 72 months of follow-up. Again, as seen in Table 1, there was no association between *KRAS* mutation status at 72 months and histological progression or regression.

Given our encouraging preliminary study that indicated that the presence of detectable *KRAS* mutations at baseline was a strong predictor of future progression (3), it is disappointing that after longer follow-up the association did not hold. Given the worldwide frequency of gastric cancer and the poor 5-year survival rate associated with this disease, a reliable prognostic indicator for the development of stomach cancer is badly needed.

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