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BRIEF REPORT

Interactions Between the Dietary Polyunsaturated Fatty Acid Ratio and Genetic Factors Determine Susceptibility to Pediatric Crohn's Disease

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Increased dietary ratios of ω6/ω3 polyunsaturated fatty acids have been implicated in the pathogenesis of Crohn's disease (CD), but epidemiologic data are limited. We investigated whether variants of genes that control polyunsaturated fatty acid metabolism (*CYP4F3*, *FADS1*, and *FADS2*), along with the dietary ratio of ω6/ω3, confers susceptibility to CD. Based on data from 182 children newly diagnosed with CD and 250 controls, we found that children who consumed a higher dietary ratio of ω6/ω3 were susceptible for CD if they were also carriers of specific variants of *CYP4F3* and *FADS2* genes. Our findings implicate diet–gene interactions in the pathogenesis of CD.

Keywords: DNA; Inflammatory Bowel Disease; Fat Content; Food.

Although many studies suggest that a high dietary ratio of ω6/ω3 polyunsaturated fatty acids (PUFAs) is potentially risk-conferring for CD, evidence across studies is equivocal.[1] Notwithstanding methodologic issues, a key phenomenon overlooked in most studies is that risk or protection from dietary PUFA may depend on an individual's ability to appropriately metabolize them. Recent studies have shown that genes involved in PUFA metabolism are associated with endogenous levels of PUFA metabolites.[2,3] It is thus possible that risk of CD associated with a high ω6/ω3 PUFA ratio depends on variation in the PUFA metabolic genes.

There are 2 main families of PUFA, classified as ω3 and ω6, based on the location of the last double bond relative to the terminal methyl end of the molecule.[4] The human body can produce all but 2 of the PUFAs it requires: linoleic acid (precursor to the ω6 series) and α-linolenic acid (ALA) (precursor to the ω3 series). Lipid mediators generated from long-chain (LCN) PUFAs such as ω-6 arachidonic acid, and ω3 eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), have important roles in immune regulation and inflammation.[5] For the most part, metabolism of ω6 PUFA leads to the production of proinflammatory lipid mediators, whereas those produced via ω3 metabolism are less proinflammatory. As a result of nutritional trends, Western society diets presently are characterized by high ω6 PUFA, as well as an overall low ω3 PUFA intake.[6] Optimal dietary intakes of the ω6/ω3 ratio are suggested to be approximately 1–4:1. However, in Western diets it now is between 10:1 and 20:1.[7] This high ratio could influence the PUFA metabolic pathway in favor of the ω6 path that, in the presence of genetic susceptibility, predisposes to chronic inflammation, which is a characteristic of CD. We explored this gene–diet interaction hypothesis in the present study.

We studied 182 newly diagnosed CD cases and 250 controls of Caucasian origin (Table 1). Most patients had ileocolonic disease (47%). We investigated 15 single-nucleotide polymorphisms (SNPs) across 3 PUFA metabolic genes (*FADS1*, 1; *FADS2*, 10; and *CYP4F3*, 4). The genes/SNPs were chosen based on their significant associations with plasma levels of different PUFA metabolites as reported in the recent meta-analyses of genome-wide association studies (GWAS)[2] (see the Supplementary Materials and Methods section). All genotyped SNPs were in Hardy–Weinberg equilibrium ($P > .01$) in our controls. Usual dietary consumption during the 12 months before disease diagnosis was acquired using a validated food frequency questionnaire as was reported previously.[8]

Logistic regression analysis using energy-adjusted nutrients (using the residual method,[9] energy measured in kilocalories) and accounting for potential confounders such as age, sex, body mass index, family history of inflammatory bowel disease, and study center, showed that a higher ratio of LCN ω6/ω3 was associated with increased risks for CD

Abbreviations used in this paper: **ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LCN, long chain; PUFA, polyunsaturated fatty acid; SNP, single-nucleotide polymorphism.**

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Table 1. Sociodemographic Characteristics of the Study Population

Characteristic	Cases (n = 182)	Controls (n = 250)	*P* value
Mean age, *y* (±SD)	12.8 (3.2)	13.3 (3.2)	.05
Sex, n (%)			
Males	111 (61.0)	122 (48.8)	
Females	71 (39.0)	128 (51.2)	.01
Study site, n (%)			
Montreal	83 (45.6)	150 (60.0)	
Ottawa	78 (42.9)	83 (33.2)	
Vancouver	21 (11.5)	17 (6.8)	.009
Mean energy intake (±SD)	2437.3 (681.1)	2214.9 (615.0)	<.001
Mean BMI (±SD)	21.5 (10.9)	20.5 (4.7)	.23
Family history, n (%)[a]			
No	145 (79.7)	231 (92.4)	
Yes	37 (20.3)	19 (7.6)	<.001

BMI, body mass index.
[a]Inflammatory bowel disease in first- or second-degree relatives.

(Q4 vs Q1–3: odds ratio, 1.63; 95% confidence interval, 1.01–2.64; $P = .044$). None of the 15 SNPs were associated independently with CD (Supplementary Table 1), as previously reported.[10] When interactions were examined, no SNP/ω6 interactions were evident, whereas interaction between 1 *FADS2* SNP rs11230815 and dietary ω3 was observed ($P = .042$). When the dietary ratio of LCN ω6/ω3 was considered, we observed significant interactions ($P < .05$) involving 6 SNPs (Table 2), suggesting that associations between the dietary ratio and CD varied according to *CYP4F3* and *FADS2* genotypes. For example, in children who carried the GG genotype in SNP rs1290617 or the CC genotype in SNP rs1290620 in the *CYP4F3* gene, a higher LCN ω6/ω3 dietary ratio was associated with a greater risk for CD. Because the G and C alleles of these 2 SNPs are associated with high levels of DPA[2] and because the *CYP4F3* gene is involved in the metabolism of DHA, children who are carriers of these alleles and consume a higher LCN ω6/ω3 dietary ratio may produce and metabolize DHA less efficiently into anti-inflammatory resolvins and protectins, resulting in a higher risk for CD. Similarly for the *FADS2* gene, the alleles of 3 SNPs (rs11230815, rs968567, and rs174627) involved in the interactions (Table 2) are associated with high levels of ALA and low levels of EPA and DPA.[2] We hypothesize that in carriers of these alleles, a higher LCN ω6/ω3 dietary ratio might divert the pathway to the metabolism of ω6 PUFA and further compromise the metabolism of ω3 PUFA. This may result in an increased production of potentially proinflammatory mediators and a greater risk of CD. However, given that these effects are not consistent for the *FADS2* SNP rs17831757, complex interactions are likely to be in play.

The *CYP4F3* gene is an important modulator of the inflammatory process via its ability to inactivate leukotriene B4 (LTB_4).[11] The CYP4F3 pre–messenger RNA is spliced into 2 mature transcripts (CYP4F3B and CYP4F3A), of which

Table 2. Association Between the Dietary PUFA Ratio and CD According to Genotypes in the *CYP4F3* and *FADS2* Genes

SNP (gene)	OR (95% CI) associated with the ratio (low ratio as reference)	Interaction, *P* value	Association with PUFA plasma levels[a]
rs1290617 (*CYP4F3*)			G allele
TT	0.92 (0.44–1.89)	.04	DPA: high
GT	1.37 (0.78–2.42)		
GG	2.91 (0.90–9.39)		
rs1290620 (*CYP4F3*)			C allele
TT	0.52 (0.13–2.04)	.025	DPA: high
CT	0.98 (0.54–1.77)		
CC	2.53 (1.29–4.96)		
rs11230815 (*FADS2*)[b]			G allele
CC + CG	0.50 (0.20–1.22)	.007	ALA: high
GG	1.77 (1.11–2.82)		EPA: low
			DPA: low
rs17831757 (*FADS2*)[b]			T allele
CC + CT	0.52 (0.21–1.27)	.011	ALA: low
TT	1.75 (1.10–2.78)		EPA: high
			DPA: high
rs968567 (*FADS2*)[b]			T allele
CC	0.99 (0.62–1.60)	.028	ALA: high
CT + TT	2.68 (1.17–6.19)		EPA: low
			DPA: low
rs174627 (*FADS2*)[b]			A allele
GG	1.08 (0.68–1.71)	.029	ALA: high
AG + AA	2.99 (1.12–7.99)		EPA: low
			DPA: low

NOTE. Associations were adjusted for age, sex, family history of inflammatory bowel disease, study site, and body mass index.
AA, arachidonic acid; CI, confidence interval; OR, odds ratio.
[a]Published and unpublished data from Lemaitre et al.[2]
[b]Genotypes were combined owing to small numbers.

CYP4F3A is expressed in neutrophils/monocytes and in the intestines, and is more than 25 times as efficient as CYP4F3B for the ω-hydroxylation and inactivation of LTB_4 to 20-OH LTB_4.[4] CYP4F3A also ω-hydroxylates fatty acid epoxides and lipoxins A and B. This ability of the gene to detoxify fatty acids that are associated intimately with inflammation makes it a relevant candidate for CD.[12–15] However, we noted that variation in this gene appears to impact the association between the dietary ratio of LCN ω6/ω3 and CD, whereby only children with certain genotypes are at higher risk for CD if they consume a high ratio. In such individuals a higher LCN ω6/ω3 dietary ratio conceivably may lead to inefficient clearance of LTB_4, promoting prolonged tissue inflammation.

The *FADS2* gene is a key PUFA metabolic gene influencing the pathway at multiple steps. It is a desaturase that catalyzes the first step in the conversion of both ω6 linoleic acid and ω3 ALA into longer chain ω6 and ω3 PUFAs. Not surprisingly, variations in the gene are associated with endogenous plasma/serum levels of EPA, DHA, and DPA,[2] and different PUFA ratios.[3] The higher risk for CD that is

associated with a higher dietary ratio of LCN $\omega 6/\omega 3$ in individuals with particular *FADS2* SNPs suggest that lower endogenous production of $\omega 3$ PUFAs may influence risk when combined with a higher LCN $\omega 6/\omega 3$ dietary ratio. Certainly any postulated mechanism can be considered speculative at this time. Nonetheless, taken together, our findings do support the possibility of an altered fatty acid metabolic status that facilitates chronic inflammation in response to diet. These findings suggest that preventive dietary intervention could be targeted to specific subgroups (based on PUFA metabolic genes) that might benefit the most. Studies to replicate these findings are needed.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.12.034.

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Conflicts of interest
The authors disclose no conflicts.

Supplementary Materials and Methods

We performed a case-control study including children with CD diagnosed before age 19, recruited from 3 pediatric gastroenterology clinics across Canada. Details of the study have been reported previously.[1] In brief, for this study children newly diagnosed with CD using standard criteria were included. As controls, children visiting the orthopedic clinics of the respective study hospitals (Montreal and Vancouver) for minor fractures/scoliosis or population-based controls (Montreal and Ottawa) were recruited. Both cases and controls were restricted to those of Caucasian ancestry (self-report). Dietary consumption during the 12 months before disease diagnosis (for cases) and before the date of recruitment (for controls) was acquired using a self-administered, validated, food-frequency questionnaire Youth Adolescent Questionnaire.[2,3] The Youth Adolescent Questionnaire has been validated and tested for reproducibility in children. Good correlations have been reported between 24-hour dietary recalls and the Youth Adolescent Questionnaire–based consumption levels of different fatty acids including PUFAs ($r = 0.30$), saturated fatty acids ($r = 0.54$), monounsaturated fatty acids ($r = 0.52$), and total fats ($r = 0.56$).[3] Children reporting major changes in diet (eg, reducing fat intake) in the 12-month period were excluded. Peripheral blood and/or saliva samples were collected as a source for DNA. The DNA was genotyped for SNPs in the *CYP4F3* ($n = 4$), *FADS1* ($n = 1$), and *FADS2* ($n = 10$) genes using the Sequenom-based platform (San Diego, California). The 15 SNPs were selected based on their reported associations with plasma/serum levels of key PUFA metabolites (such as EPA, DPA, and DHA) as reported in a recent meta-analysis of GWAS[4] that examined genome-wide associations with $\omega 3$ fatty acids. In this genome-wide association study, 28 *CYP4F3* SNPs were reported to be associated with serum levels of either EPA, DPA, or DHA ($P < .05$). We selected 4 SNPs that showed the highest linkage disequilibrium with other associated SNPs for genotyping.[5] For the *FADS2* gene, in the same study, 38 SNPs were found to be associated with serum levels of either EPA, DPA, or DHA ($P < 10^{-5}$). We randomly selected 10 of these SNPs for genotyping. For the *FADS1* gene, 9 SNPs were reported to be associated similarly with the earlier-mentioned serum PUFA levels. We chose for genotyping the single SNP that was in maximum linkage disequilibrium with the other associated SNPs. Informed consent was acquired from all participants and the ethics boards of the 3 study hospitals approved the study.

Data Analysis

The children's responses to the Youth Adolescent Questionnaire were analyzed for acquiring information on the daily consumption of specific nutrients. For nutrients of relevance to the current study (PUFA), the daily intake (grams or micrograms as the case may be) was estimated. The raw intakes of the LCN ω-3 (EPA, DPA, and DHA) and LCN ω-6 (arachidonic acid) were adjusted for energy intake using the residual method.[6] The ratio of arachidonic acid/(EPA, DPA, DHA) then was calculated. The quartiles were used for studying independent associations and the median was used for analyzing gene–diet interactions.

The genotyping data on the 15 SNPs first was examined for deviation from Hardy–Weinberg equilibrium using chi-square tests. SNPs in Hardy–Weinberg equilibrium in the controls were retained.

Associations between the arachidonic acid/(EPA, DPA, DHA) ratio and CD adjusting for age, sex, family history, study center, and body mass index were estimated using logistic regression. Associations between the 15 SNPs and CD were examined separately (1 model for each SNP) using univariate logistic regression models assuming additive inheritance. Subsequently, multivariate analysis was performed to assess for interactions. Single models (per SNP) that included the variables representing the ratio (low, below the median; high, above the median), the SNP (coded as 0, 1, or 2), an interaction term comprising the ratio and the SNP, along with potential confounders, were fit. Models wherein the interaction term was statistically significant ($P < .05$) were considered to represent important interactions. Because the study was exploratory, no corrections for multiple comparisons were made. For SNP-ratio interactions that appeared to be prominent, the odds ratios, and their respective 95% confidence intervals for the association between the ratio and CD were estimated based on stratification of specific genotypes in the study SNPs.

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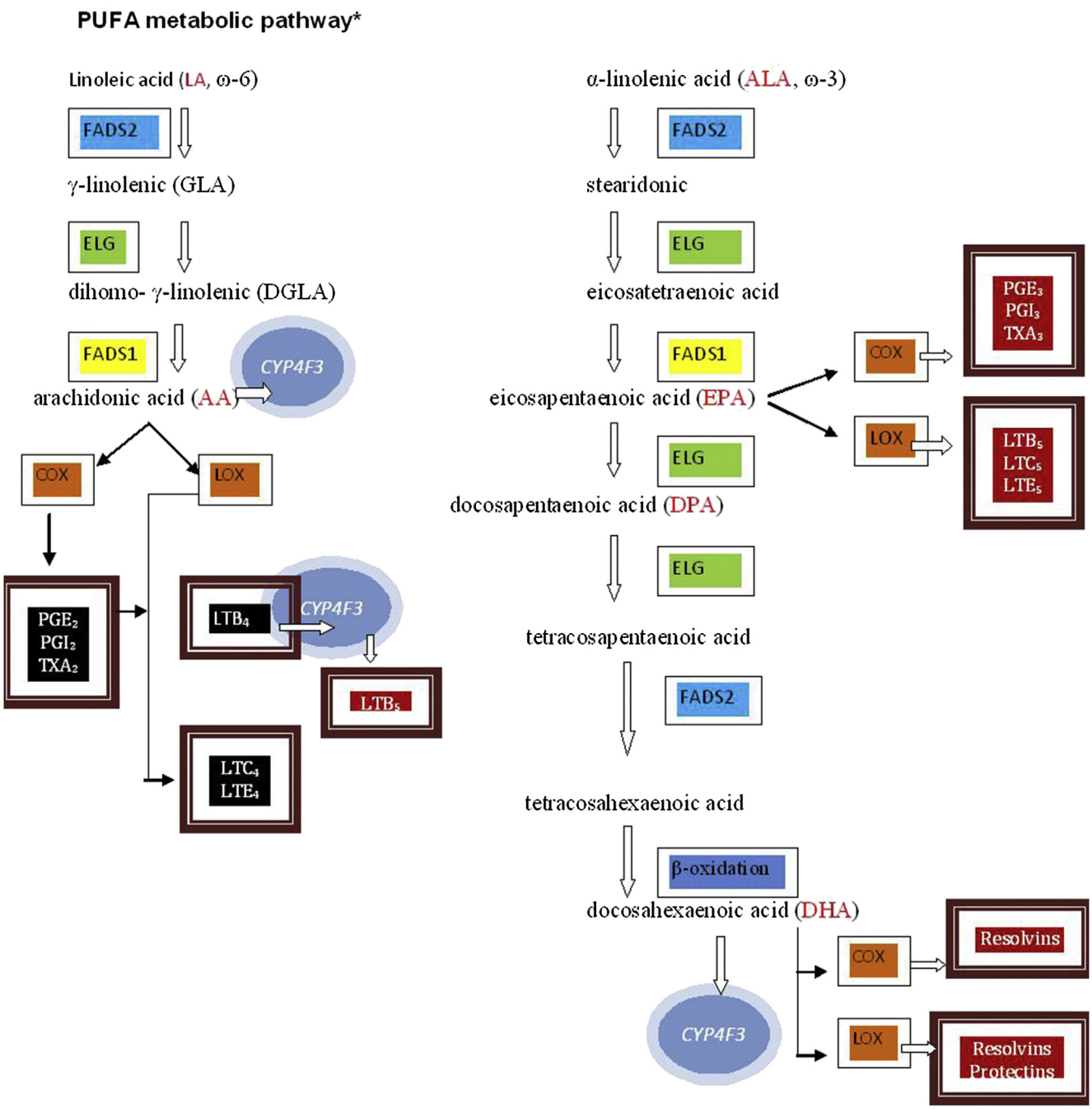
Supplementary Table 1. Associations Between SNPs in the *CYP4F3*, *FADS1*, and *FADS2* Genes and CD

SNP (gene)	Odds ratio (95% confidence interval)	*P* value
rs1290617 (*CYP4F3*)	1.17 (0.88–1.55)	.28
rs1290620 (*CYP4F3*)	1.08 (0.82–1.44)	.57
rs2283612 (*CYP4F3*)	1.36 (0.92–1.99)	.11
rs8106799 (*CYP4F3*)	1.14 (0.87–1.52)	.34
rs11230815 (*FADS2*)	0.72 (0.45–1.14)	.16
rs17831757 (*FADS2*)	0.68 (0.42–1.08)	.11
rs968567 (*FADS2*)	1.01 (0.67–1.53)	.95
rs174579 (*FADS2*)	1.01 (0.71–1.43)	.94
rs174627 (*FADS2*)	1.09 (0.72–1.66)	.68
rs174577 (*FADS2*)	1.03 (0.76–1.38)	.86
rs174601 (*FADS2*)	0.99 (0.74–1.33)	.96
rs174602 (*FADS2*)	0.92 (0.66–1.27)	.60
rs498793 (*FADS2*)	1.03 (0.77–1.39)	.82
rs174547 (*FADS2*)	0.91 (0.68–1.22)	.54
rs174575 (*FADS1*)	0.94 (0.68–1.29)	.69



Supplementary Figure 1. Major PUFA metabolites are shown in red (LA, ALA, AA, EPA, DPA, DHA). COX, cyclooxygenase; ELG, elongase; LOX, lipoxygenase; LT, leukotriene. *To reduce complexity, some metabolites are not shown.