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Fish and marine fatty acids intakes, the *FADS* genotypes and long-term weight gain: a prospective cohort study

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ABSTRACT

Objective We tested whether genetic variants near fatty acid desaturases gene (*FADS*) cluster, which were recently identified to be signatures of adaptation to fish-rich and n-3 polyunsaturated fatty acids (PUFAs)-rich diet, interacted with these dietary factors on change in body mass index (BMI).

Design Three *FADS* variants were examined for gene-diet interactions on long-term (~10 years) changes in BMI and body weight in four prospective cohort studies.

Setting Population based study.

Participants 11 323 women from the Nurses' Health Study (NHS), 6833 men from the Health Professionals Follow-up Study (HPFS) and replicated in 6254 women from the Women's Health Initiative (WHI) and 5 264 Chinese from the Singapore Chinese Health Study (SCHS).

Main outcomes Long-term (~10 years) changes in BMI and body weight.

Results In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the *FADS* rs174570 on changes of BMI (P for interaction=0.02 in NHS, 0.05 in HPFS and 0.007 in combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for interaction=0.04 in WHI, 0.02 in SCHS and 0.001 in combined). The genetic associations of the *FADS* rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P for interaction=0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association with long-term changes in BMI among the rs174570 T carriers (beta=0.79 kg/m^2 per g, p=3×10^{-5}) than the rs174570 non-T carriers (beta=0.16 kg/m^2 per g, p=0.08). Similar results were observed for fish intake.

Conclusions Our hypothesis-driven analyses provide replicable evidence that long chain n-3 PUFAs and fish intakes may interact with the *FADS* variant on long-term weight gain. Further investigation is needed to confirm our findings in other cohorts.

Strengths and limitations of this study

► This is the first study with consistent results from four well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings.

► Other major strengths include the prospective design, the large sample size, use of long-term change of body mass index and replication of the results.

► Dietary fatty acids, fish and adiposity measures were self-reported, measurement errors in these variables are inevitable.

► Confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors.

► We acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses.

acids (PUFAs) has shown beneficial effects on cardiometabolic health.[1][2] However, data from population studies on the associations between such diet and body weight are inconsistent.[3][4] Emerging evidence suggests genetic variations may play a role in modifying the relation between dietary factors and body weight.[5–7] For example, we previously found that high intakes of fish and long-chain n-3 PUFAs are associated with an attenuation of the genetic association with long-term weight gain based on results from three prospective cohorts of Caucasians.[8]

A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs.[9] The strong signals locate in a cluster of fatty acid desaturases genes (*FADS*) that determine PUFAs levels.[9] People living in the Arctic region have been found to be genetically prone to develop obesity[10][11]

INTRODUCTION

Diets rich in fish and marine fatty acids, especially long chain n-3 polyunsaturated fatty

as survival strength for energy storage.[12 13] Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in the Inuit population.[9] Of note, due to long-standing selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various populations such as Europeans and Asians,[14] in coincidence with varying levels of fish/marine fatty acids consumption, and adiposity patterns in these populations.[15] We therefore hypothesised that the genetic signatures might interact with fish and marine PUFAs intakes on body weight.[14]

The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene cluster, genetic signatures of adaptation to fish-rich and n-3 PUFAs-rich diet, in relation to long-term changes in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts the Women's Health Initiative (WHI, US) and the Singapore Chinese Health Study (SCHS).

METHODS
Discovery cohorts
Nurses' health study
The NHS began in 1976, when 121 700 female registered nurses aged 30–55 y residing in 11 states were recruited to complete a baseline questionnaire about their lifestyle and medical history.[16] The current analysis baseline was set in 1990 for the NHS. We included 11 323 women of European ancestry. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, single-nucleotide polymorphism (SNPs) genotyping and imputation when performed have been described in detail elsewhere.[17–23] All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data and genotyping data available based on previous GWASs were included.[17–22] The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

Health professionals follow-up study
The HPFS was initiated in 1986, and was composed of 51 529 male dentists, pharmacists, veterinarians, optometrists, osteopathic physicians and podiatrists, aged 40–75 years at baseline. The male participants returned a baseline questionnaire about detailed medical history, lifestyle and usual diet.[24] In the current analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our analysis included 6833 men whose genotype data were available. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere.[17–23] All participants with baseline long chain n-3 PUFAs and fish consumptions

and covariates data, baseline and endpoint BMI data and genotyping data available based on previous GWASs were included.[17–22] The study protocol was also approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

Replication cohorts
WHI
The Women's Health Initiative (WHI) is a large, multi-ethnic, 40-centre study funded by the National Heart, Lung and Blood Institute that focuses on strategies for preventing heart disease, breast and colorectal cancer and osteoporotic fractures in postmenopausal women. A full description of the WHI study is presented elsewhere.[25 26] For the analyses, all participants with baseline long chain n-3 PUFAs and fish consumption and covariate data, baseline and endpoint BMI data and genotyping data available based on previous GWASs were included. Finally, we included 6 254 Caucasians women who participated in the WHI clinical trial studies at baseline (1994–1998) and at sixth-year follow-up and for whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array V.6.0 (Affymetrix, Inc Santa Clara, California, USA) was used for genome wide SNP genotyping. Human subjects review committees at each participating institution reviewed and approved the study, and all women gave written informed consent.

SCHS cohort
The design of SCHS has been previously described in detail.[27] Briefly, between 1993 and 1998, 63 257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study.[28] Two follow-up interviews were conducted via telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update information on body weight, selected lifestyle factors and medical history. All participants have given informed consent. The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data available were included. Among these participants, genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome Institute of the Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation.[29] Genome-wide genotyping for 717 incident myocardial infarction cases and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip.[30] Among these two case–control studies nested within the cohort, 5264 subjects with

genotyping data had weight reported at both baseline and follow-up two interviews, and were included in this analysis.

Assessment of measures of BMI

Height and body weight were assessed by questionnaire at baseline, and weight information was requested on follow-up questionnaire in all four cohorts. Self-reported weights were highly correlated with directly measured values (r=0.97 in HPFS and NHS) in a validation study.[31] BMI was calculated as body weight (kg)/height (m^2). As defined previously,[8] the long-term changes in BMI was calculated as changes in BMI from 1990 to 2000 in the NHS and HPFS cohorts,[32] and from baseline (1993) to sixth-year follow-up in the WHI,[25 26] and from baseline (1998) to second follow-up (2004) in the SCHS.

Assessment of diets and other covariates

Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all four cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried food intakes and other dietary factors at baseline were assessed by validated food frequency questionnaires (FFQ) in the NHS and HPFS.[33 34] A 165-item validated semiquantitative FFQ was used to collect dietary data and supplemental use of fish oil in the SCHS.[28] Dietary data and supplemental use of fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI.[35] Alternate health eating index was previously calculated in the NHS, HPFS,[36] WHI and SCHS. Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities, and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously in the NHS and HPFS.[37] In the WHI, an estimated metabolic equivalent (MET) level for each type of activity was assigned from a compendium of activities.[38] Physical activity was assessed using eight continuous categories ranging from never to 31 or more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS.[27]

FADS variants selection and genotyping

Three of the six *FADS* SNPs reported in a recent scan of Inuit genomes for signatures of adaptation[9] were derived from genome-wide scans available in the NHS, HPFS. We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication (see online supplementary table 1).

Patient and public involvement

Neither patients nor public were involved.

Statistical analyses

We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity measures and long-term changes in BMI using multiple linear regression model. Interactions between the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake and total or food-sourced long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating index and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if available). We further tested the genetic associations with long-term changes in BMI according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to the *FADS* genotypes using multiple linear regression model after adjustment of potential confounders. Linear trend across categories of long chain n-3 PUFAs and fish intakes was quantified with a Wald test for linear trend by assigning the median value to each category and modelling it as a continuous variable.[39] Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models (p≥0.05 for heterogeneity between studies).[40] The individual participant data from the NHS and HPFS cohorts were pooled to generate the predicted 10-year changes in body weight according to the *FADS* genotypes. Hardy-Weinberg equilibrium was tested using X^2 test. All reported p values are nominal and two sided. Statistical analyses were performed in SAS V.9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

Table 1 shows the baseline characteristics for all participants in the NHS, HPFS, WHI and SCHS cohorts. The present study included 11 323 women with genetic data from the NHS cohort, 6833 men with genetic data from the HPFS cohort, 6254 women from the WHI and 5 264 Chinese from the SCHS. The distribution of the *FADS* genetic variants in the four cohorts is shown in online supplementary table 1. χ^2 test showed that the *FADS* rs174570 is in Hardy-Weinberg equilibrium. We did not observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term changes in BMI in the three US cohorts (p>0.05). However, we found that the *FADS* genotype was significantly associated with baseline BMI in the SCHS (p=0.002) (see online supplementary table 2).

Table 1 Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

	NHS*	HPFS	WHI	SCHS
	n=11 323	n=6833	n=6254	n=5264
Age (year)	57±9	57±11	68±5	56±7
Female (%)	100	0	100	58.7
Body weight (kg)	70.1±14.9	82.8±12.5	73.7±15.0	60.3±9.8
Body mass index (kg/m^2)	26.2±5.1	25.9±3.3	28.3±5.5	23.4±3.3
Alcohol consumption (g/day)	5.14±9.23	10.97±15.05	6.00±11.96	1.97±8.02
Physical activity (MET-h/week)	19.3±22.1	36.9±39.5	11.6±13.1	0.5±1.0†
Television watching (h/week)	17.5±14.8	10.5±8.2	/	2.2±0.8
Current smokers (n, (%))	1557 (13.8)	493 (7.3)	407 (15.0)	1364 (20.0)
Total energy intake (kcal/day)	1766±502	1949±578	1602±654	1606±573
Alternative health eating index score	53.4±10.8	53.8±11.4	53.5±10.6	55.8±8.2
Sugar sweetened beverage intake (servings/day)	0.13±0.39	0.23±0.48	0.39±0.82	0.69±2.40‡
Total fried food (servings/day)	0.12±0.20	0.22±0.28	/	/
Fish intake (servings/day)	0.31±0.29	0.33±0.30	0.23±0.20	0.16±0.07
Food-sourced EPA (g/day)	0.08±0.14	0.12±0.20	0.04±0.04	/
Food-sourced DHA (g/day)	0.17±0.14	0.22±0.19	0.07±0.07	/
Food-sourced EPA+DHA (g/day)	0.23±0.19	0.31±0.25	0.11±0.10	0.33±0.20
Total EPA+DHA (g/day)	0.26±0.27	0.35±0.37	0.38±0.48	/

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994–1998) and the SCHS (1993–1998), respectively. Television watching assessed in 1992 for NHS and in 1990 for HPFS.
EPA: 20:5 n-3; DHA: 22:6 n-3; MET denotes metabolic equivalents. Total EPA+DHA includes food-sourced and supplemental EPA+DHA.
*Plus-minus values are means±SD.
†Hours per week of moderate activity in the SCHS.
‡Glasses per week of soda intake in the SCHS.
 DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HPFS, Health Professionals Follow-up Study; MET, metabolic equivalent; NHS, Nurses' Health Study; PUFA, polyunsaturated fatty acids; SCHS, Singapore Chinese Health Study; WHI, Women's Health Initiative.

Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes

We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes of variously sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed significant interaction with long chain (LC) n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (eicosapentaenoic acid (EPA)+docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with long-term changes in BMI (P for interaction=0.02 in NHS, 0.05 in HPFS and 0.007 in combined cohorts) (figure 1). We successfully replicated our results in the WHI cohort (P for interaction=0.04) and the SCHS cohort (P for interaction=0.02).

The pooled analyses of the 3 US (Caucasian) samples or all four cohorts showed that high intakes of food-sourced n-3 PUFAs intake (P for interaction=0.008 and 0.009, respectively) significantly accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (figure 2). No significant heterogeneity in the interaction effect was observed among these cohorts. Differences in long-term changes of BMI per T allele were

−0.105 (SE 0.067), 0.027 (SE 0.064) and 0.120 (SE 0.067) kg/m^2 across three tertiles of food-sourced n-3 PUFAs in pooled results from all the four cohorts.

Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for interaction=0.003) showed similar interaction patterns; and the interactions remained significant when supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (figure 2).

In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS* genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results (pooled P for interaction=0.006), and the differences in BMI changes per T allele were −0.096 (SE 0.071), 0.041 (SE 0.052) and 0.251 (SE 0.151) kg/m^2 across three categories (≤1 serving/week, 1~6 servings/weekand ≥1 serving/day) of fish intake in combined results from all the four cohorts.

In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI in the
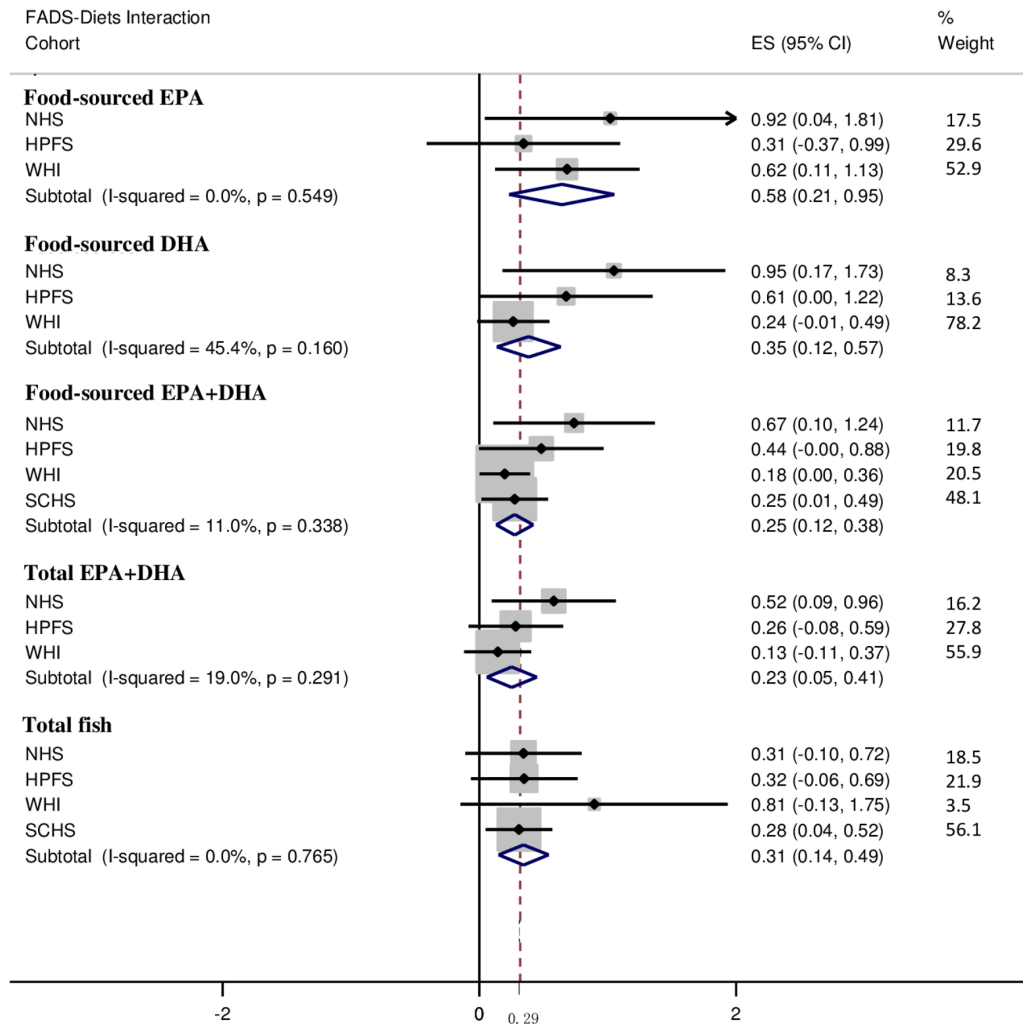
Figure 1 Genetic variant of *FADS* rs174570, long chain n-3 PUFAs and fish intakes and long-term BMI changes ES (95% CI) values are β coefficients for interaction between the *FADS* variant rs174570 (additive model) and diets from results of the NHS, HPFS, WHI and SCHS cohorts. Data on BMI, long chain n-3 PUFAs (food sourced EPA+DHA and total EPA+DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994–1998) and the SCHS (1993–1998), respectively. Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively. Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively. The multiple linear regression model was used to test the *FADS* variant-diets interaction by including a multiplicative interaction term in the models after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses ($p \geq 0.05$ for heterogeneity between studies). BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ES, effect size; *FADS*, fatty acid desaturases gene; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; SCHS, Singapore Chinese Health Study; WHI, Women's Health Initiative.

NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed (see online supplementary table 3 and 4).

Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype

We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had significantly greater increase of BMI (mean±SE = 0.74±0.06, kg/m^2) than did those who consumed the lowest (T1) (mean±SE=0.39±0.07, kg/m^2) among the T allele carriers, whereas the corresponding BMI changes were 0.68±0.03 kg/m^2 and 0.49±0.03 kg/m^2, respectively,

among the non-T carriers in four cohorts combined (table 2 and see online supplementary table 5). Similarly, we observed different associations between fish intake and BMI changes among the T allele carriers ($p=1.5\times10^{-6}$) and non-carriers ($p=0.01$) in the pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed among the cohorts.

Figure 3 presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts consistently showed that the associations of food-sourced n-3
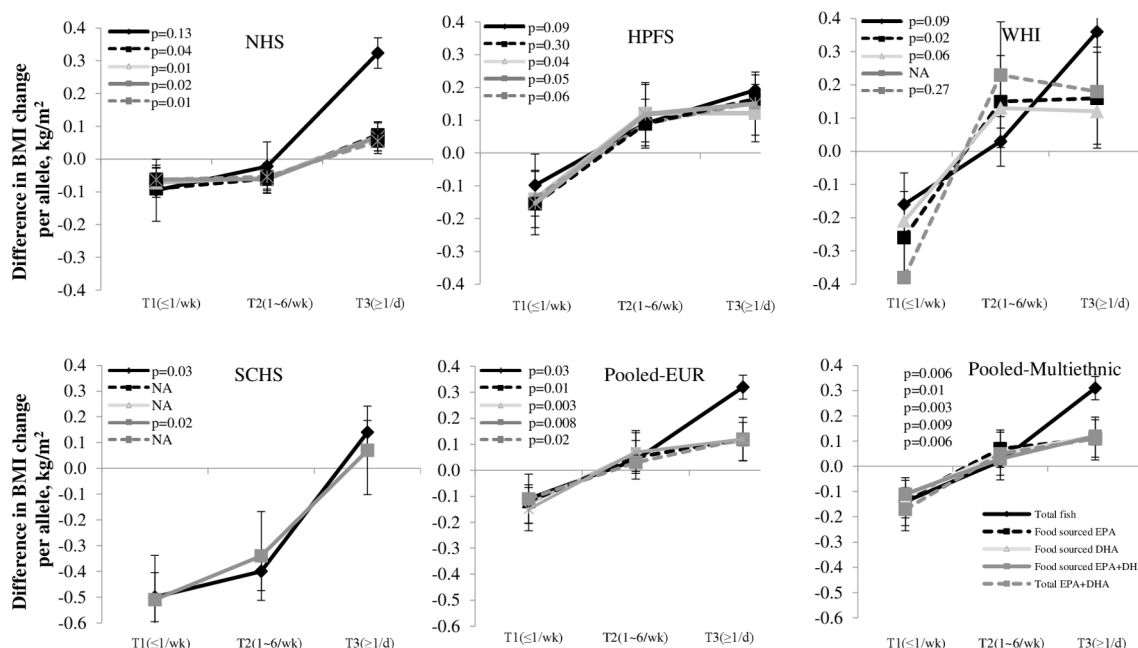
Figure 2 Genetic association of *FADS* variant rs174570 with long-term BMI change according to long chain n-3 PUFAs and fish intakes Pooled-EUR: data from NHS, HPFS and WHI were pooled. Pooled Multiethnic: data from NHS, HPFS, WHI and SCHS were pooled. Data are β coefficients±SE Numbers of participants across three categories (≤1/wk/1~6/wk/≥1/d) of fish intake in the NHS, HPFS, WHI and SCHS are 1618/8465/1239, 977/5108/748, 894/4675/684 and 752/3935/576, respectively. Frequency of fish intake: ≤1 serving per week, 1~6 servings per week and 1 serving per day data on BMI, long chain n-3 PUFAs (food sourced EPA+DHA and total EPA+DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994–1998) and the SCHS (1993–1998), respectively. Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively. The multiple linear regression model was used to test the genetic association of the *FADS* variant (additive model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses (p≥0.05 for heterogeneity between studies). BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; *FADS*, fatty acid desaturases gene; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; SCHS, Singapore Chinese Health Study; WHI, Women's Health Initiative.

PUFAs and fish intakes with long-term changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled results, the beta±SE for associations of food-sourced n-3 PUFAs (figure 3) and fish intake (figure 4) with long-term changes in BMI were $0.79\pm0.19\,\mathrm{kg/m^2}$ per g (p=0.000003) and $0.64\pm0.16\,\mathrm{kg/m^2}$ per serving (p=0.00002) among the T carriers, and whereas the corresponding beta±SE were $0.16\pm0.10\,\mathrm{kg/m^2}$ per g (p=0.08) and $0.18\pm0.08\,\mathrm{kg/m^2}$ per serving (p=0.01) among the non-T carriers.

DISCUSSION

In four large prospective cohorts of the US and Chinese populations, our hypothesis-driven analyses showed reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS* rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.

Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight and obesity

risk have generated conflicting results.[3][4] In addition, several randomised controlled trials supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss,[41–43] but the benefit was not evident in other trials.[44–46] The results from the current study lend support to our hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.

We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle, the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit, who had high fish/n-3 PUFAs intakes.[9] The high frequency of the T allele in Inuit reflects genetic adaptation to the special fish-rich and n-3 PUFA-rich diet. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that the signature allele (T) was related diffrently to weight

Table 2   Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes

	FADS genotypes	Three categories of long chain n-3 PUFAs and fish intakes			P for trend	P for interaction*
Total fish, serving/day		≤1/wk	1~6/wk	≥1/d		
NHS	Non-T carriers	0.82±0.06	0.98±0.04	1.15±0.13	0.006	0.03
	T carriers	0.73±0.11	0.95±0.08	1.55±0.25	0.0007	
HPFS	Non-T carriers	0.43±0.05	0.52±0.04	0.59±0.12	0.73	0.03
	T carriers	0.21±0.11	0.52±0.07	0.79±0.22	0.02	
WHI	Non-T carriers	0.11±0.08	0.28±0.06	0.28±0.34	0.04	0.09
	T carriers	0.02±0.15	0.29±0.11	0.94±0.67	0.01	
SCHS	Non-T carriers	−3.08±0.19	−3.00±0.17	−3.35±0.18	0.32	0.01
	T carriers	−3.61±0.17	−3.10±0.15	−3.25±0.17	0.13	
Pooled*	Non-T carriers	0.50±0.03	0.67±0.03	0.81±0.08	0.01	0.0007
	T carriers	0.38±0.07	0.63±0.05	1.11±0.16	2×10^{-4}	
Food-sourced EPA+DHA, g/day		Tertile1	Tertile2	Tertile3		
NHS	Non-T carriers	0.79±0.06	0.92±0.05	1.11±0.06	0.01	0.005
	T carriers	0.71±0.10	0.84±0.11	1.19±0.11	0.0001	
HPFS	Non-T carriers	0.46±0.05	0.53±0.05	0.49±0.05	0.79	0.02
	T carriers	0.23±0.11	0.48±0.10	0.58±0.09	0.02	
WHI	Non-T carriers	0.02±0.08	0.21±0.08	0.41±0.08	0.06	0.04
	T carriers	−0.03±0.15	0.29±0.14	0.35±0.15	0.004	
SCHS	Non-T carriers	−3.32±0.17	−3.15±0.18	−2.99±0.17	0.16	0.035
	T carriers	−3.55±0.16	−3.34±0.16	−3.05±0.16	0.02	
Pooled*	Non-T carriers	0.49±0.03	0.64±0.03	0.68±0.03	0.01	0.0003
	T carriers	0.39±0.07	0.57±0.06	0.74±0.06	1.5×10^{-6}	
Total EPA+DHA, g/day		Tertile1	Tertile2	Tertile3		
NHS	Non-T carriers	0.79±0.06	0.94±0.05	1.08±0.06	0.8	0.01
	T carriers	0.72±0.11	0.87±0.10	1.16±0.11	0.02	
HPFS	Non-T carriers	0.47±0.05	0.53±0.05	0.49±0.05	0.88	0.13
	T carriers	0.23±0.10	0.50±0.09	0.57±0.10	0.16	
WHI	Non-T carriers	0.39±0.10	0.04±0.08	0.23±0.10	0.42	0.27
	T carriers	0.04±0.18	0.28±0.15	0.28±0.16	0.84	
Pooled*	Non-T carriers	0.57±0.04	0.62±0.03	0.67±0.04	0.65	0.005
	T carriers	0.39±0.07	0.60±0.06	0.74±0.07	0.01	

Data are means±SE for long term changes in BMI.
Total EPA+DHA include food-sourced and supplemental EPA+DHA.
Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.
Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994–1998) and the SCHS (1993–1998), respectively.
Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.
Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.
The multiple linear regression model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.
The results were pooled by means of fixed effects meta-analyses (p≥0.05 for heterogeneity between studies).
*P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).
BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ES, effect size; *FADS*, fatty acid desaturases gene; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; PUFA, polyunsaturated fatty acid; SCHS, Singapore Chinese Health Study; WHI, Women's Health Initiative.

changes (decrease or increase), depending on the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body weight when they are exposed to a diet low in fish and n-3 PUFAs.

We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction patterns in
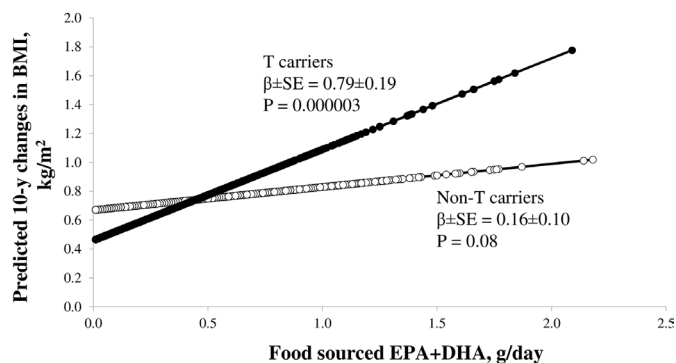
Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS* genotypes numbers of T carriers/Non-T carriers in the NHS and HPFS are 1698/9625 and 1025/5808, respectively. Black circles for T allele carriers and open circle for non-T-carriers. The multiple linear regression model was used to test the associations of long chain n-3 PUFAs intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The individual participant data from the NHS and HPFS cohorts were pooled. BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; *FADS*, fatty acid desaturases gene; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; PUFA, polyunsaturated fatty acid; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study.

Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes numbers of T carriers/ Non-T carriers in the NHS and HPFS are 1698/9625 and 1025/5808, respectively. Black circles for T allele carriers and open circle for non-T-carriers. The multiple linear regression model was used to test the associations of and fish intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The individual participant data from the NHS and HPFS cohorts were pooled. BMI, body mass index; *FADS*, fatty acid desaturases gene; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study.

relation to long-term changes in BMI; and the interactions also remained significant when supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is robust for fatty acids from various sources.

The mechanisms underlying the observed gene-diet interactions remain unclear. However, such interactions are biologically plausible. It has long been known that the *FADS* genes such as *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases, respectively, which are the important rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic acid (n-6) and α-linolenic acid (n-3).[47] The selected allele of *FADS* rs174570 is significantly associated with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway.[47] Further, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function.[48] In addition, storage of energy and body fat is very important for the Arctic population, who are regularly exposed to extremely low temperatures and fishes rich in n-3 PUFAs.[12 13] Under natural selection, these people are genetically prone to high fish intake to keep body fat.[10 11] Therefore, it's not surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying selective *FADS*
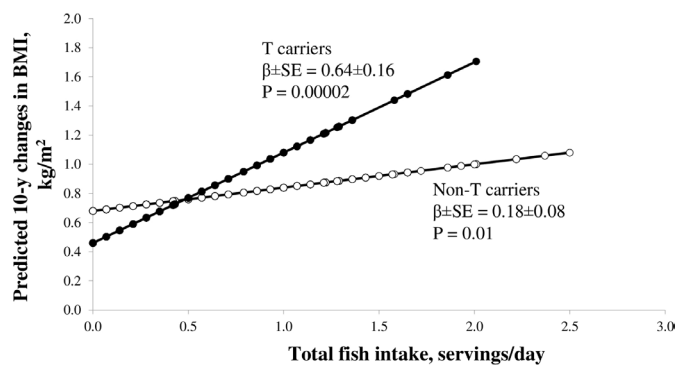
signature.[49 50] Our findings support the view that extra n-3 PUFAs may not have much benefit for Europeans with selective *FADS* signature.[9 14]

Strengths

Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results from four well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings. Other major strengths include the prospective design, the large sample size, use of long-term change of BMI and replication of the results. Although we prospectively analysed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change from the baseline, which by default builds in the starting point (ie, the cross sectional association).

Limitations

However, several limitations need to be acknowledged. First, dietary fatty acids, fish and adiposity measures were self-reported, measurement errors in these variables are inevitable. Despite this, the food frequency questionnaires and adiposity measures data have been well validated.[28 31 33–35] Second, confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish consumption, and adiposity cannot be inferred from an observational study.

Fourth, all subjects with genetic data were selected in each cohort. The source of genotyping data was diverse (eg, subcohort, case–control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses. Finally, the participants included in our study were middle aged and older adults of Caucasians in the USA and Chinese in Singapore, and it is unknown whether our findings could be generalised to other demographic or ethnic groups.

CONCLUSIONS

In summary, our data provide reproducible evidence from four multiethnic cohorts that high long chain n-3 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings emphasise the importance of considering precision nutritional interventions on prevention and treatment of obesity. We acknowledge that these results are hypothesis-generating and need to be confirmed in additional cohorts.

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