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Maternal PUFA status and offspring allergic diseases up to the age of 18 months

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Abstract

Studies have suggested that maternal PUFA status during pregnancy may influence early childhood allergic diseases, although findings are inconsistent. We examined the relationship between maternal PUFA status and risk of allergic diseases in early childhood in an Asian cohort. Maternal plasma samples from the Growing Up in Singapore Towards Healthy Outcomes mother–offspring cohort were assayed at 26–28 weeks of gestation for relative abundance of PUFA. Offspring (*n* 960) were followed up from 3 weeks to 18 months of age, and clinical outcomes of potential allergic diseases (rhinitis, eczema and wheezing) were assessed by repeated questionnaires. Skin prick testing (SPT) was also performed at the age of 18 months. Any allergic disease with positive SPT was defined as having any one of the clinical outcomes plus a positive SPT. The prevalence of a positive SPT, rhinitis, eczema, wheezing and any allergic disease with positive SPT was 14·1 % (103/728), 26·5 % (214/808), 17·6 % (147/833), 10·9 % (94/859) and 9·4 % (62/657), respectively. After adjustment for confounders, maternal total *n*-3, *n*-6 PUFA status and the *n*-6:*n*-3 PUFA ratio were not significantly associated with offspring rhinitis, eczema, wheezing, a positive SPT and having any allergic disease with positive SPT in the offspring ($P>0·01$ for all). A weak trend of higher maternal *n*-3 PUFA being associated with higher risk of allergic diseases with positive SPT in offspring was observed. These findings do not support the hypothesis that the risk of early childhood allergic diseases is modified by variation in maternal *n*-3 and *n*-6 PUFA status during pregnancy in an Asian population.

Key words: **Allergy: Early childhood: PUFA: Skin prick testing**

Abbreviations: AA, arachidonic acid; SWS, Southampton Women's Survey; ALSPAC, Avon Longitudinal Study of Parents and Children; GUSTO, Growing Up in Singapore Towards healthy Outcomes; SPT, skin prick testing; PC, phosphatidylcholine; FAME, fatty acid methyl ester.

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Allergic diseases are one of the most common group of diseases worldwide, resulting in a significant social and economic burden[1]. In most children, eczema is the earliest clinical manifestation of allergy, starting during the first few months of life[2]. Increasing evidence shows that infants who develop allergy in early life have an altered immune response at birth[3,4], suggesting that allergic diseases may originate *in utero*. Thus, it is now postulated that early life interventions during the antenatal period may confer protective effects on the immune system[5].

Changes in modern lifestyle, including diet, have coincided with the escalating rates of allergic diseases[6,7]. Among dietary factors, patterns of intake of PUFA have drawn great interest. The pro-inflammatory properties of *n*-6 PUFA and anti-inflammatory properties of *n*-3 PUFA are well-established in both human and animal models[8–11]. For example, the *n*-6 PUFA arachidonic acid (AA; 20 : 4*n*-6) produces eicosanoid mediators such as PGE_2, which promotes the production of IgE, and leukotriene B_4, which promotes airway constriction[9]. In contrast, the *n*-3 PUFA EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) act to counter the effects of AA[8]. Consequently, increased intake of *n*-6 PUFA and decreased exposure to *n*-3 PUFA in the antenatal period have been hypothesised to increase the risk of offspring allergic diseases[12].

Fish and fish oil are sources of EPA and DHA. Fish oil supplementation studies in pregnant women[13–15] and observational studies on fish intake during pregnancy[16,17] have suggested protective effects on offspring allergy. However, studies reporting the relationship between maternal plasma PUFA status and childhood allergic diseases have yielded inconsistent results. The Southampton Women's Survey (SWS) study found a weak protective effect of maternal EPA, DHA and total *n*-3 PUFA against non-atopic persistent/late wheezing in offspring aged 6 years[18]. The KOALA Birth Cohort found AA and the ratio of *n*-6:*n*-3 PUFA to be protective against childhood eczema[19]. No such significant associations were found in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort[20], or in another small study containing forty-seven mother–child pairs[21]. Thus, whether higher *n*-3 PUFA status during pregnancy would lower the risk of childhood allergic diseases remains unclear.

In previous publications[18–21], most allergic outcome measurements were reported as performed in Caucasian children aged 4–7 years. No study has been done on an Asian population to investigate allergic diseases at a younger age. In the present study, we investigated the relationship between maternal PUFA status and potential offspring allergic diseases up to the age of 18 months in an Asian multi-ethnic birth cohort.

Methods

Participants

Participants were mother–child pairs in the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort. A detailed study profile has been described elsewhere[22,23]. In brief, the GUSTO study is designed to investigate the role of early life exposures in the development of metabolic and other diseases. Between June 2009 and September 2010, 1162 pregnant women aged 18 years and above were recruited to the main GUSTO. The study was granted ethical approval by the Institutional Review Board of the KK Women's and Children's Hospital and by the National University Hospital. Informed written consent was obtained from each participant.

Detailed interviews on maternal characteristics, including demographics, lifestyle, diet and health, were conducted at a recruitment clinic visit and again at 26–28 weeks of gestation. Infant characteristics, such as fetal anthropometry and health outcomes, were collected through examination at home at 3 weeks, 3 months and every 3 months thereafter until 15 months of age. At the age of 18 months, the mothers and infants were invited to the study clinic for detailed clinical assessment including allergic sensitisation (skin prick testing, SPT).

Maternal plasma PUFA

Blood samples were collected into heparinised tubes at 26–28 weeks of gestation. Plasma was prepared and stored at −80°C until analysis. Plasma lipids were extracted with chloroform–methanol (2:1, v/v). Phosphatidylcholine (PC), which contributes about 75 % of plasma phospholipid, was isolated by solid phase extraction. Then, fatty acid methyl esters (FAME) were generated from PC after reaction with methanol containing 2 % (v/v) sulphuric acid. FAME were extracted into hexane and separated by GC. FAME were identified by comparison with retention times of standards run previously, and they were quantified using ChemStation software (Agilent Technologies). Data were expressed as percentage contribution to the total plasma PC fatty acid pool. For all fatty acids within plasma PC, within-assay CV was <3 % and between-assay CV was <6 %. In the present study, we focused on the percentage of total *n*-3 PUFA, total *n*-6 PUFA and *n*-6:*n*-3 PUFA ratio. Additionally, we examined the specific *n*-6 and *n*-3 PUFA, α-linolenic acid (18 : 3*n*-3), EPA, docosapentaenoic acid (22 : 5*n*-3), DHA, EPA+DHA, linoleic acid (18 : 2*n*-6) and AA.

Allergy outcome measurements

Allergic sensitisation – skin prick testing. Allergic sensitisation was assessed by standardised SPT to common inhalant and food allergens. Standardised SPT was conducted by trained doctors during the clinic visit at 18 months of age, using three food allergens (cows' milk, peanut and egg) and three house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis*). Histamine and saline were used as positive and negative controls, respectively. Wheal size ≥ 3 mm was classified as positive. SPT was considered valid only if the positive wheal was ≥ 3 mm in size, and the negative control exhibited no wheal reaction. A positive SPT to at least one allergen was considered indicative of allergic sensitisation.

Early childhood rhinitis, eczema and wheezing. Information on clinical outcomes of potential allergic diseases

(eczema, rhinitis and wheezing) was collected serially at seven time points: 3 weeks; 3 months; every 3 months thereafter till 18 months of age. Standardised questionnaires adapted from the International Study of Asthma and Allergies in Childhood[24] were administered by trained interviewers to mothers or main caregivers. Rhinitis was defined as parents' positive response to the question: 'At any time, has your child had running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has lasted for 2 or more weeks' duration?' A study has shown that cold/flu at this young age rarely goes beyond 2 weeks[25], so the cut-off of 2 weeks could reduce the influence of misclassification with cold symptoms. Doctor-diagnosed eczema was based on a positive answer to the question: 'Has your child ever been diagnosed with eczema?' Wheezing was defined as 'noisy breathing with a high-pitch, whistling sound heard from the chest, not the mouth'. In order to decrease false positive reporting of wheezing, we added another question in which nebuliser/inhaler usage by a doctor was assessed. Wheezing was diagnosed with positive responses to both questions: 'Has your child ever wheezed?' and 'Has your child been prescribed with nebuliser/inhaler treatment since the last visit?' After getting results from the questionnaires, phone calls were made to ask for further details. Presence of doctor-diagnosed eczema, rhinitis or wheezing was indicated by a positive response during any one of the seven follow-up questionnaires in the first 18 months of life.

Any allergic disease with positive SPT was defined as having any one of the above-mentioned clinical outcomes (eczema, rhinitis and wheezing) plus a positive SPT.

Statistical analyses

The statistical analyses were performed using the statistical software package IBM SPSS 20.0 for Windows (SPSS, Inc.). Two-sample t test was used for comparing the means of continuous variables, and χ^2 test was used for comparing the distribution of categorical variables. Binary logistic regression models were used to test the independent associations between the various allergic outcomes (i.e. SPT, rhinitis, eczema, wheezing and any allergic disease with positive SPT in the offspring) and individual maternal PUFA. PUFA of interest were first treated as continuous explanatory variables (continuous model), and then categorised into quartiles within the total cohort in order to test for a possible non-linear relationship and to examine dose–response (categorical model).

In the models, we adjusted for maternal characteristics including maternal age, ethnicity, gravidity, education level and energy intake. The same was done for infant characteristics including sex, birth weight, gestational age, duration of breast-feeding, family history of allergic diseases (which includes allergic rhinitis, eczema and asthma in first-degree relatives of the children (i.e. father, mother and/or sibling), exposure to environmental tobacco smoking, child day care attendance and having a cat or dog at home up to 18 months of age. Subgroup analysis was also done for the group of children with no family history of allergic diseases in order to rule out the possibility of genetic susceptibility as a confounding factor.

To control type 1 error due to the performance of multiple analyses, an adjusted P value $<0{\cdot}01$ ($P = 0{\cdot}05$ divided by five allergy outcomes) was used to indicate statistical significance. Results are presented as adjusted OR with corresponding 95% CI.

Results

Maternal PUFA status and rates of allergy outcomes

Of the 1162 women enrolled in the main GUSTO birth cohort, 998 mothers with singleton live births had blood samples available for the measurement of plasma PC fatty acids. The median percentages for total n-3 and n-6 PUFA were

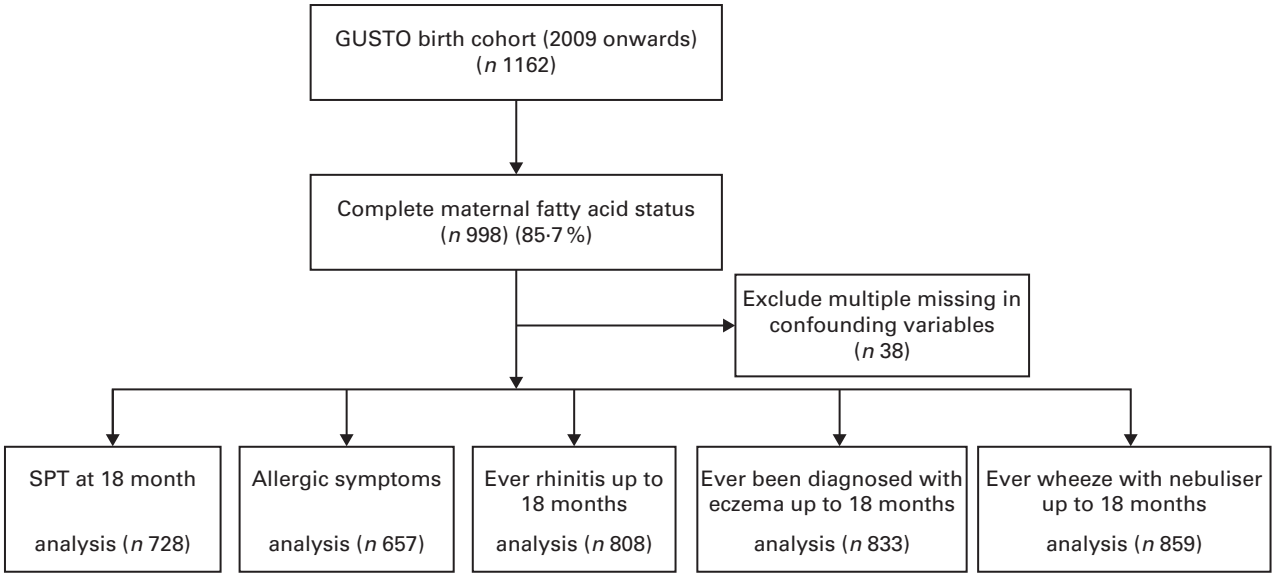

Fig. 1. Flow chart of the participants in the present study. GUSTO, Growing Up in Singapore Towards healthy Outcomes; SPT, skin prick testing.

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6·18 (range 2·22–13·97) and 34·22 (range 10·77–51·29), respectively. Median values with their 25th and 75th percentiles of the other fatty acids can be found in online supplementary Table S1. Similar to previous findings, the predominant *n*-3 PUFA in maternal plasma PC was DHA, and the major *n*-6 PUFA were linoleic acid and AA[20].

After excluding those with multiple missing confounders, only 960 mothers were included in the final analyses. Sample sizes varied for the individual outcomes due to different response rates (Fig. 1). SPT at 18 months was performed in 728 children, among whom 103 (14·1 %) showed positive results. Of the 808 children who had data on parent-reported rhinitis up to 18 months of life, 214 (26·5 %) had rhinitis. Of the 833 children with data on parent-reported, doctor-diagnosed eczema up to 18 months of life, 147 (17·6 %) were diagnosed with eczema. Of the 859 children with data on wheezing symptoms (parent-reported and use of nebuliser or inhaler), ninety-four (10·9 %) had wheezing. Of the 657 children with data on SPT and the occurrence of any allergic disease, sixty-two (9·4 %) showed positive results. Characteristics of mothers who agreed to have SPT performed on their children (*n* 728) and of those who did not (*n* 232) were broadly similar (see online supplementary Table S2), except that those who agreed tended to be slightly older, and were more likely to have more than one child.

Population characteristics

Tables 1 and 2 show the main characteristics of the study population and bivariate associations with the various clinical allergic outcomes. There was a higher tendency for infants with eczema, wheeze and any allergic disease with positive SPT to be breast-fed for longer than 4 months. The prevalence of rhinitis and eczema was highest in infants with both parents having allergic disease compared with those with one parent having allergic disease, and was lowest in those with no family history of allergic disease. There was a higher prevalence of rhinitis and wheeze in infants who attended childcare during infancy. Additionally, the prevalence of eczema was higher in children of first-time pregnancies and in those whose mothers had higher educational qualifications, while the prevalence of wheeze was higher in male infants and in infants with shorter gestational age. For all of the clinical allergic outcomes, Malay infants had the highest prevalence, followed by Chinese infants, and Indian infants had the lowest prevalence. This was in agreement with the prevalence of infants having a family history of allergic diseases among the ethnic groups. In addition, Chinese mothers tended to have the highest plasma PC *n*-3 PUFA levels, lowest plasma PC *n*-6 PUFA levels and *n*-6:*n*-3 PUFA ratio, while Malay mothers had the highest *n*-6:*n*-3 PUFA ratio (see online supplementary Table S3).

Association between maternal PUFA status and early childhood allergy outcomes

In bivariate analyses using quartiles of PUFA (Table 3), weak trends of higher maternal plasma PC *n*-3 PUFA, being

Table 1. Maternal characteristics of the study participants and bivariate associations with clinical allergic outcomes (Mean values and standard deviations)

	SPT					Any allergic diseases with SPT*					Ever rhinitis					Ever eczema					Ever wheeze				
	No (*n* 625)		Yes (*n* 103)			No (*n* 595)		Yes (*n* 62)			No (*n* 594)		Yes (*n* 214)			No (*n* 686)		Yes (*n* 147)			No (*n* 765)		Yes (*n* 94)		
Maternal characteristics	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†
Age (years)	30·8	5·2	31·5	5·3	0·21	31	5·2	32	5·2	0·15	31·0	5·1	30·6	5·6	0·41	30·8	5·1	31·3	5·2	0·25	30·8	5·1	30·1	5·3	0·22
Gravidity >1																									
No	248	86·4	39	13·6	0·75	239	91·9	21	8·1	0·41	249	72·8	93	27·2	0·70	278	79·2	73	20·8	0·04	328	91·1	32	8·9	0·12
Yes	377	85·5	64	14·5		356	89·7	41	10·3		345	74	121	26		408	84·6	74	15·4		437	87·6	62	12·4	
Educational status																									
Primary/secondary	189	85·9	31	14·1	0·68	176	91·2	17	8·8	0·75	170	73·3	62	26·7	0·99	211	86·8	32	13·2	0·01	226	90·8	23	9·2	0·40
Post-secondary	230	87·1	34	12·9		217	91·2	21	8·8		208	73·5	75	26·5		250	83·6	49	16·4		267	87·3	39	12·7	
University	206	84·4	38	15·6		202	89·4	24	10·6		216	73·7	77	26·3		225	77·3	66	22·7		272	89·5	32	10·5	
Ethnicity																									
Chinese	340	86·1	55	13·9	0·05	333	92	29	8	0·01	348	76	110	24	<0·005	375	80·6	90	19·4	0·02	432	89·6	50	10·4	<0·005
Malay	161	81·7	36	18·3		150	85·2	26	14·8		128	64·3	71	35·7		178	80·5	43	19·5		182	83·1	37	16·9	
Indian	124	91·2	12	8·8		112	94·1	7	5·9		118	78·1	33	21·9		133	90·5	14	9·5		151	95·6	7	4·4	
Total energy intake (kJ)	7845	2414	8171	2380	0·20	7928	2469	8247	2117	0·33	7858	2469	8021	2381	0·40	7849	2481	8176	2222	0·14	7853	2423	7858	2481	0·99
Total *n*-3 PUFA‡ (%)	6·4	1·9	6·5	1·8	0·50	6·4	1·9	6·8	1·8	0·21	6·4	1·9	6·6	1·8	0·15	6·4	1·9	6·5	1·9	0·50	6·4	1·9	6·6	2·0	0·22
Total *n*-6 PUFA‡ (%)	34·3	3·3	33·8	3·5	0·18	34·2	3·4	34	2·8	0·66	34·2	3·5	34·1	2·9	0·87	34·1	3·5	34·1	2·9	0·99	34·3	3·4	33·4	2·9	0·02
n-6:*n*-3 PUFA ratio	5·9	2·0	5·6	1·9	0·28	5·8	2·0	5·5	1·9	0·20	5·9	2·1	5·6	1·6	0·01	5·8	2·0	5·7	1·9	0·57	5·9	2·0	5·5	1·8	0·11

SPT, skin prick testing.
*Any allergic diseases with SPT were defined as having any one of the clinical outcomes with a positive SPT.
†*P* values were obtained by two-sample *t* test for continuous variables and χ^2 tests for categorical variables; $P \leq 0{\cdot}05$ is considered significant.
‡Fatty acids were presented as percentage of total plasma fatty acids.

Table 2. Infant characteristics and bivariate associations with clinical allergic outcomes
(Mean values and standard deviations)

	SPT					Any allergic diseases with SPT*					Ever rhinitis					Ever eczema					Ever wheeze				
	No (*n* 625)		Yes (*n* 103)			No (*n* 595)		Yes (*n* 62)			No (*n* 594)		Yes (*n* 214)			No (*n* 686)		Yes (*n* 147)			No (*n* 765)		Yes (*n* 94)		
Infant characteristics	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†
Gestational age (weeks)	38·6	1·39	38·8	1·41	0·41	38·7	1·35	38·6	1·56	0·51	38·7	1·38	38·6	1·21	0·30	38·7	1·32	38·7	1·76	0·91	38·8	1·3	38·3	1·66	<0·005
Sex					0·11					0·35					0·13					0·20					0·05
Male	310	83·8	60	16·2		297	89·5	35	10·5		296	71·2	120	28·8		346	80·7	83	19·3		389	87	58	13	
Female	315	88	43	12		298	91·7	27	8·3		298	76	94	24		340	84·2	64	15·8		376	91·3	36	8·7	
Months of breast-feeding					0·46					0·06					0·70					0·02					0·06
None	47	88·7	6	11·3		46	95·8	2	4·2		52	77·6	15	22·4		57	86·4	9	13·6		67	95·7	3	4·3	
< 4	279	87·7	39	12·3		266	93	20	7		255	74·1	89	25·9		309	86·6	48	13·4		333	90	37	10	
≥ 4	252	83·7	49	16·3		245	88·1	33	11·9		250	72·9	93	27·1		273	78·2	76	21·8		323	88	44	12	
Unknown	47	83·9	9	16·1		38	84·4	7	15·6		37	68·5	17	31·5		47	77	14	23		42	80·8	10	19·2	
Birth weight (g)	3107	444	3176	413	0·14	3118	434	3157	414	0·66	3117	447	3135	417	0·61	3100	433	3159	468	0·14	3125	446	3061	407	0·19
Family history of allergic diseases					0·75					0·27					<0·005					<0·005					0·11
No	361	86·6	56	13·4		334	92·5	27	7·5		318	80·1	79	19·9		370	87·1	55	12·9		399	91·3	38	8·7	
One parent	178	84·0	34	16·0		173	87·4	25	12·6		143	66·8	71	33·2		169	75·8	54	24·2		198	88·8	25	11·2	
Two parents	52	85·2	9	14·8		50	87·7	7	12·3		35	55·6	28	44·0		47	74·6	16	25·4		53	82·8	11	17·2	
Sibling only	34	89·5	4	10·5		32	91·4	3	8·6		25	67·6	12	32·4		31	81·6	7	18·4		32	82·1	7	17·9	
Unknown											73	75·3	24	24·7		69	82·1	15	17·9		83	86·5	13	13·5	
Environmental smoking during infancy					0·23					0·36					0·54					0·82					0·35
No	312	83·9	60	16·1		309	89·6	36	10·4		307	75·1	102	24·9		339	81·7	76	18·3		391	90·1	43	9·9	
Yes	208	88·9	26	11·1		197	92·9	15	7·1		184	72·7	69	27·3		226	82·5	48	17·5		244	86·8	37	13·2	
Unknown	105	86·1	17	13·9		89	89	11	11		103	70·5	43	29·5		121	84	23	16		130	90·3	14	9·7	
Childcare attendance during infancy					0·96					0·68					<0·005					0·18					<0·005
No	472	85·7	79	14·3		460	91·1	45	8·9		453	76·1	142	23·9		516	82·8	107	17·2		586	90·6	61	9·4	
Yes	46	86·8	7	13·2		44	88	6	12		35	55·6	28	44·4		45	73·8	16	26·2		44	69·8	19	30·2	
Unknown	107	86·3	17	13·7		91	89·2	11	10·8		106	70·7	44	29·3		125	83·9	24	16·1		135	90·6	14	9·4	
Having a cat/dog at home during infancy					0·99					0·34					0·67					0·33					0·91
No	464	85·8	77	14·2		456	91·4	43	8·6		439	74·3	152	25·7		506	82·5	107	17·5		566	89	70	11	
Yes	54	85·7	9	14·3		48	85·7	8	14·3		49	73·1	18	26·9		55	76·4	17	23·6		66	88	9	12	
Unknown	107	86·3	17	13·7		91	89·2	11	10·8		106	70·7	44	29·3		125	84·5	23	15·5		133	89·9	15	10·1	

SPT, skin prick testing.
* Any allergic diseases with SPT were defined as having any one of the clinical outcomes with a positive SPT.
† *P* values were obtained by two-sample *t* test for continuous variables and χ^2 tests for categorical variables; $P \leq 0{\cdot}05$ is considered significant.

Table 3. Infant allergy outcomes according to quartiles (Q) of maternal total plasma phosphatidylcholine *n*-3 PUFA, *n*-6 PUFA status and *n*-6:*n*-3 PUFA ratio

(Percentages for categorical variables and ranges)

Allergy outcomes	Q1	Q2	Q3	Q4	*P**
Total *n*-3 PUFA†					
Range	2·2–5·0	5·1–6·1	6·2–7·5	7·6–14·0	
n	240	240	240	240	
SPT (%)	13·2	13·1	11·7	18·3	0·22
Ever rhinitis (%)	20·7	28·6	30·2	25·9	0·25
Ever eczema (%)	17·6	18·5	14·5	19·9	0·79
Ever wheeze (%)	10·2	10·0	11·3	12·2	0·43
Any allergic diseases with SPT‡ (%)	6·6	9·4	7·8	13·3	0·07
Total *n*-6 PUFA†					
Range	10·8–32·3	32·4–34·2	34·3–36·1	36·2–51·3	
n	240	240	240	240	
SPT (%)	17·5	9·9	13·2	16·0	0·92
Ever rhinitis (%)	27·1	24·9	29·6	24·5	0·45
Ever eczema (%)	15·7	18·3	17·6	18·9	0·45
Ever wheeze (%)	13·6	11·6	11·0	7·6	0·06
Any allergic diseases with SPT‡ (%)	9·6	7·9	11·7	8·7	0·93
n-6:*n*-3 PUFA ratio†					
Range	1·9–4·5	4·6–5·4	5·5–6·9	7·0–16·6	
n	240	240	240	240	
SPT (%)	18·8	11·5	13·4	12·5	0·13
Ever rhinitis (%)	26·6	27·9	32·0	18·9	0·21
Ever eczema (%)	20·6	11·4	19·7	18·8	0·88
Ever wheeze (%)	14·3	10·6	10·0	8·5	0·06
Any allergic diseases with SPT‡ (%)	12·6	8·8	9·4	6·4	0·07

SPT, skin prick testing.

* *P* values were obtained by χ^2 tests for categorical variables.

† Fatty acids were presented as percentage of total plasma fatty acids.

‡ Any allergic diseases with SPT were defined as having any one of the clinical outcomes with a positive SPT.

associated with any allergic diseases with positive SPT in infants ($P=0·07$); lower maternal plasma PC *n*-6 PUFA, being associated with wheeze in infants ($P=0·06$); and lower maternal *n*-6:*n*-3 PUFA ratio, being associated with wheeze and any allergic diseases with positive SPT in infants ($P=0·06$; $P=0·07$), were observed. These trends were not clearly observed in the group of infants without a family history of allergic diseases ($P>0·1$ for all) (see online supplementary Table S4).

Upon adjustment for potential confounders (Table 4), no statistically significant linear relationships were observed between the individual maternal PUFA as continuous variables and any of the various allergic outcomes. From quartile analyses, a weak positive trend persisted between maternal plasma PC *n*-3 PUFA and any allergic diseases with positive SPT in infants. The OR of any allergic diseases with positive SPT was highest (OR 2·09) in the highest quartile of *n*-3 PUFA when compared with the lowest quartile (reference), although this was not statistically significant. The same was also observed in the group of infants without a family history of allergic diseases (see online supplementary Table S5). No clear associations were observed with maternal plasma PC total *n*-6 PUFA status and the risk of having any allergic disease with SPT up to 18 months of age. Correspondingly, a negative trend was observed between maternal plasma PC *n*-6:*n*-3 PUFA ratio and the risk of having any allergic disease with SPT up to 18 months of age in the whole cohort only, although this did not reach statistical significance.

Although the OR for wheezing in infants appear to be lower with increasing quartiles of maternal plasma PC *n*-6 PUFA and *n*-6:*n*-3 PUFA ratios, and the OR for eczema 'ever' in infants appear to be higher with increasing quartiles of maternal plasma PC *n*-6 PUFA in both the whole cohort and in the group of infants without a family history of allergic diseases, these associations were not statistically significant. The OR for rhinitis 'ever' in infants appear to be higher with increasing quartiles of maternal plasma PC *n*-3 PUFA and *n*-6:*n*-3 PUFA ratios, but only up to the third quartile, in both the whole cohort and in the group of infants without a family history of allergic diseases (Table 4 and online supplementary Table S5). However, these associations were not statistically significant.

While examining the individual PUFA (α-linolenic acid, EPA, docosapentaenoic acid, DHA, EPA+DHA, linoleic acid and AA), it appears that docosapentaenoic acid and EPA were the key *n*-3 PUFA driving the association with higher risk of any allergic diseases with positive SPT, whereas DHA was the key *n*-3 PUFA driving the association with higher risk of rhinitis. For the two *n*-6 PUFA examined (linoleic acid, AA), there was no clear association with higher risk of wheeze and eczema (see online supplementary Table S6). Analyses were also conducted using PUFA concentrations, rather than percentages, with allergic outcomes, and results were not different from those described above (data not shown).

Discussion

In the present Asian birth cohort study, we did not find any significant protective effects of higher percentages of *n*-3 PUFA or lower percentages of *n*-6 PUFA on maternal plasma PC against offspring allergic diseases in early childhood.

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Table 4. Association between maternal plasma phosphatidylcholine PUFA status at 26–28 weeks of pregnancy and early childhood allergic diseases (Ranges, adjusted odds ratios* and 95 % confidence intervals)

Quartiles of plasma fatty acids	Range (wt%)	SPT (*n* 728†‡)		Any allergic diseases with SPT§ (*n* 657†‡)		Ever rhinitis (*n* 808†‡)		Ever eczema (*n* 833†‡)		Ever wheeze (*n* 859†‡)	
		OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Total *n*-3 PUFA											
Continuous model		1·03	0·91, 1·16	1·08	0·93, 1·25	1·07	0·98, 1·17	0·99	0·89, 1·09	1·06	0·93, 1·20
Categorical model											
1	≤5·00	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
2	5·01–6·18	0·91	0·48, 1·72	1·38	0·58, 3·28	1·56	0·96, 2·54	1·02	0·60, 1·74	0·90	0·45, 1·78
3	6·19–7·49	0·83	0·43, 1·58	1·10	0·45, 2·71	1·67	1·03, 2·70	0·67	0·38, 1·18	1·09	0·56, 2·13
4	≥7·50	1·41	0·77, 2·58	2·09	0·91, 4·78	1·34	0·81, 2·21	0·93	0·55, 1·60	1·12	0·57, 2·2
Total *n*-6 PUFA											
Continuous model		0·97	0·90, 1·03	1·00	0·92, 1·09	1·01	0·96, 1·06	1·03	0·98, 1·10	0·94	0·88, 1·01
Categorical model											
1	≤32·38	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
2	32·39–34·22	0·48	0·25, 0·90	0·75	0·34, 1·68	0·92	0·58, 1·46	1·29	0·76, 2·20	0·79	0·43, 1·46
3	34·23–36·17	0·71	0·39, 1·28	1·26	0·59, 2·65	1·17	0·74, 1·84	1·37	0·80, 2·35	0·76	0·40, 1·43
4	≥36·18	0·98	0·55, 1·74	1·02	0·47, 2·23	1·00	0·63, 1·60	1·56	0·91, 2·68	0·67	0·34, 1·34
n-6:*n*-3 PUFA ratio											
Continuous model		0·96	0·85, 1·08	0·93	0·79, 1·09	0·91	0·83, 1·00	1·03	0·93, 1·14	0·93	0·82, 1·07
Categorical model											
1	≤4·52	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
2	4·53–5·49	0·60	0·33, 1·10	0·70	0·34, 1·46	1·11	0·7, 1·75	0·55	0·32, 0·97	0·70	0·38, 1·30
3	5·50–6·94	0·67	0·37, 1·21	0·75	0·36, 1·56	1·34	0·85, 2·11	1·19	0·72, 1·97	0·64	0·34, 1·23
4	≥6·95	0·66	0·36, 1·22	0·52	0·23, 1·21	0·66	0·40, 1·10	1·21	0·71, 2·06	0·63	0·32, 1·25

SPT, skin prick testing.

* OR for the independent association between maternal total *n*-3, total *n*-6 PUFA status and *n*-6:*n*-3 PUFA ratio in plasma phosphatidylcholine at 26–28 weeks of pregnancy and various childhood allergic outcomes. Binary logistic regressions were performed using PUFA as continuous variables (continuous model) and then as categorical variables (divided into quartiles in the categorical model), respectively.

† Number of cases: SPT at 18 months of age in 103 out of 728 children, any allergic diseases with SPT in 62 out of 657 children, ever rhinitis at 0–18 months of age in 214 out of 808 children, ever diagnosed eczema in 147 out of 833 children and ever wheezing with nebuliser in 94 out of 859 children.

‡ Adjusted for maternal age, education level, energy intake, infant ethnicity, sex, gravidity, birth weight, gestational age, length of breast-feeding, family history of allergic diseases, exposure to environmental tobacco smoking, child day care attendance, having a cat/dog at home during infancy.

§ Any allergic diseases with SPT were defined as having any one of the clinical outcomes plus a positive SPT.

These results are in line with the large ALSPAC cohort[20] that showed no significant relationship between maternal red cell PUFA and offspring wheezing and eczema before 4 years of age, and a small study by Yu & Björkstén[21] found no significant association between maternal serum PUFA and offspring asthma, eczema, allergic rhinoconjunctivitis and SPT up to 6 years of age among forty-seven mother–child pairs. The levels of *n*-3 PUFA in the two studies cited above appear to be lower than those of the present study (DHA+EPA median level for ALSPAC study 2·62 %; mean level in Yu *et al.*'s study 2·72 %). This most likely reflects the different fractions reported to have different PUFA contents.

Despite lower levels of maternal plasma PC total *n*-3 PUFA (median = 5·01 %) than those in the present study, the SWS study[18] has reported a modest protective effect of DHA, EPA and total *n*-3 PUFA against non-atopic persistent wheezing up to 6 years of age, but not on other phenotypes of wheezing. In contrast, we found a weak trend of higher total *n*-6 PUFA and lower likelihood of wheeze 'ever' in the present study cohort. A possible explanation for the differences in the present results could be the specific wheezing patterns that SWS used, which were not captured in the present study. Another possible explanation is the younger age of offspring in the present study group, as respiratory allergy usually occurs at an older age (from pre-school age)[26]. Interestingly, the KOALA Birth Cohort[19] unexpectedly reported a protective effect of maternal AA against eczema in the first 7 months of life, and of the ratio of *n*-6:*n*-3 PUFA against eczema in 6- to 7-year-old children. This is against the widely held notion that excessive AA and a high ratio of *n*-6:*n*-3 PUFA might increase the risk of allergic disease[6,27]. In contrast, we found a weak trend of increased total *n*-6 PUFA and increased likelihood of eczema 'ever'.

The inconsistent results emerging from the above-mentioned observational studies are in contrast to the results from some interventional studies using fish oil supplementation. Fish oil supplementation during late pregnancy appears to protect against developing a positive SPT, food allergy, and IgE-associated eczema and asthma in the offspring[13–15]. In contrast, the present study suggested a trend of higher maternal *n*-3 fatty acids being associated with higher likelihood of any allergic diseases with SPT and rhinitis in the offspring, but found no associations between maternal *n*-3 PUFA and risk of the other clinical allergic outcomes.

Another possible explanation for the lack of such associations in the present study is that children aged 18 months may be too young for allergic evaluation, as many symptoms of wheezing, rhinitis and eczema are not yet associated with obvious allergy (i.e. positive SPT)[26]. Further follow-up studies are necessary, as although the prevalence of allergic

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diseases increases with age, it has not been elucidated whether maternal PUFA status during pregnancy has a long-term effect, and influences allergy development in children beyond the age of 18 months.

The present study has some methodological strengths. Recall bias of the allergic clinical outcomes was reduced by the repeated questionnaires with relatively short time intervals, and phone call confirmation after interviews, and data on confounding variables were collected prospectively. Blood samples were used to measure PC PUFA concentrations, which would be a more reliable nutrient biomarker than dietary recalls of PUFA intakes, which can be subjected to recall bias and under-reporting.

Some limitations of the present study merit consideration. First, maternal plasma PC PUFA levels in our analysis were based on a single measurement at 26–28 weeks of pregnancy, which only reflects recent fatty acid intake in the preceding few weeks, rather than long-term intake[28–31]. Therefore, the analysis did not reflect the levels of maternal PUFA throughout the whole pregnancy. It is also known that PUFA levels in plasma phospholipids change throughout pregnancy[32]. Second, we did not consider the influence of postnatal fatty acid exposure of the children, which has also been reported to be associated with childhood allergic diseases[33]. Third, we could not rule out the possibility of misclassification as some of the exposure and outcome measurements (e.g. maternal allergy and allergic diseases in infants) were based on self- or parent-reported information, rather than clinical diagnosis by a medical doctor, or through objective measures such as IgE analyses. Furthermore, subjects who did not report a positive answer at any time point but had missing data at more than two time points were classified as 'missing', while those with missing data at only one time point or two were included as controls. It is acknowledged that this may lead to an overestimation of the prevalence of clinical outcomes. Moreover, the information obtained by the questionnaires did not assess in detail the severity of the outcomes and different phenotypes of clinical outcomes. Finally, as with any observational studies, we cannot rule out the possibility of residual confounding by unknown factors, even though we controlled for major known confounders.

Conclusion

Findings of the present study provide no certain support to the hypothesis that the risk of early childhood allergic diseases in an Asian population is modified by variation in maternal exposure to *n*-3 and *n*-6 PUFA during pregnancy. Overall, results from observational studies examining the relationship between maternal PUFA and offspring early allergic outcomes are still inconclusive. Well-conducted and sufficiently powered dietary or supplementation trials to examine dose–response would be warranted to further investigate and validate this hypothesis.

Supplementary material

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