Original Article

Does Periconceptional Fish Consumption by Parents Affect the Incidence of Autism Spectrum Disorder and Intelligence Deficiency? A Case-control Study in Tianjin, China*

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Abstract

Objective This study aimed to explore the association between periconceptional fish consumption by parents and autism spectrum disorder (ASD) and intelligence deficiency (ID).

Methods A case-control study was conducted through a questionnaire with 108 ASD cases, 79 ID cases, and 108 controls. The ASD and ID cases were students from special educational schools in Tianjin from 2012 to 2014. The age- and sex-matched controls were from a high school, three primary schools, and a kindergarten in Tianjin. Multivariate logistic regression was performed.

Results Paternal habit of eating hairtail before fertilization, maternal preference for fruits during pregnancy, and maternal habit of eating grass carp during pregnancy were preventive factors for ASD. Paternal habit of drinking alcohol before fertilization was a risk factor for ID, whereas maternal preference for fruits during pregnancy and maternal habit of eating crucian carp during pregnancy were protective factors for ID.

Conclusion Parental fish consumption is beneficial for the prevention of ASD and ID. Meanwhile, the protective effects of fish consumption on ASD and ID differ. More attention should be paid to the combined effect of other food when eating fish.

Key words: Parental fish consumption; Periconception; Autism spectrum disorder; Intelligence deficiency

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INTRODUCTION

Fish is an important component of a healthy, balanced diet. It provides protein and several key nutrients, including long-chain polyunsaturated fatty acids, iodine, selenium, and vitamin D[1]. However, fish can also be contaminated with mercury, arsenide, dioxin, and polychlorinated biphenyl[2-5] because of environmental pollution and bioconcentration. On the one hand, numerous polyunsaturated fatty acids in fish oil are essential for the development of the brain and central nervous system in the early stages of life; they are involved in boosting synaptogenesis, neurogenesis, inducing antinociception, stimulating gene expression and neuronal activity, and preventing apoptosis and neuroinflammation[6]. Prenatal supplementation with polyunsaturated fatty acids

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such as docosahexaenoic acid and eicosapentaenoic acid are reportedly more effective in improving cognitive function than supplementation provided during infancy[7]. On the other hand, mercury and arsenic are well-known developmental toxins; prenatal exposure is associated with epigenetic variability and immune cell proportion changes[8], which contribute to adverse child health outcomes such as autism spectrum disorder (ASD) and mental retardation[9]. The co-existence of beneficial materials and neurotoxins makes fish consumption during pregnancy a complex issue. According to the Dietary Guidelines for Americans 2010 (Available at http://www.cnpp.usda.gov/dietary-guidelines-2010), the recommendation for seafood consumption in pregnant mothers is no more than 12 ounces per week, and the consumption of tilefish, shark, swordfish, and king mackerel is banned. However, in the Chinese Dietary Guidelines 2007, the corresponding recommendation is increased intake of protein-rich foods such as fish and other seafood[10]. The divergence regarding fish consumption during pregnancy partly results from different perspectives, as well as the differences in socioeconomic status (SES) and dietary structure. The SES can influence brain development through a number of different environmental factors, such as cognitive stimulation, nutrition, and toxin exposure[11].

The combined effects of the nutritional value and neurotoxin content of fish on neurodevelopment remain unclear. A few studies investigated this combined effect on cognitive development in infants and young children, and results revealed different influences on various aspects of neurodevelopment: better language development (social communication) with lower mental development (IQ level)[12]. Therefore, whether maternal fish consumption in pregnancy exerts a different effect on ASD and intelligence deficiency (ID) remains to be studied. ASD is a social disorder, whereas ID is a deficiency of mental development. This study aimed to examine the combined effect of maternal fish consumption on different neurodevelopmental disorders. The population for this study is from Tianjin, which is located on the west bank of the Bohai Sea and is an important center of economy and trade in North China. Tianjin is a large city with well-developed industries and abundant aquatic resources; the residents are therefore used to consuming fish and other forms of seafood. According to the China statistical yearbook of 2014 and the Tianjin statistical yearbook of 2014, the per capita seafood consumption in Tianjin was 11.7 kg, which was higher than the national average (10.4 kg). Thus, conducting the study in such an industrially developed city with high consumption of fish and seafood was appropriate.

METHODS

Participants and Recruitment

ASD Group The ASD cases (aged 4-17 years old) were collected from the registry of special educational schools in Tianjin from October 2012 to October 2014. Seven special educational schools are affiliated with the China Disabled Persons' Federation in Tianjin, including four downtown and three in the suburbs. The eligibility criteria for cases were diagnosis by a pediatrician according to the Diagnostic and Statistical Manual of Mental Disorders IV and those with a childhood autism rating scale score ≥ 30. Simultaneously, cases with Rett syndrome, schizophrenia, special language, and other developmental disorders were excluded.

ID Group The ID cases (aged 4-17 years old) were also collected from the abovementioned special educational schools. The children's IQ was assessed by doctors in the children's hospital using Wechsler Young Children Scale of Intelligence or Wechsler Intelligence Scale for Children. The children were eligible if IQ < 70. All the participants in this group were assessed by the Clancy Autism Behavior Scale to exclude cases with autistic symptoms.

Control Group The control group consisted of typical development (TD) children and adolescents who were matched according to age and sex distribution to the case group. These individuals were selected from public kindergartens, elementary schools, junior high schools, or high schools in the same region (Tianjin). The inclusion criteria for controls included normal intelligence, normal development, and no physical and mental illness, with confirmation from the class teacher or school doctor.

Data Collection and Tools

A questionnaire was administered to the parents of all the participants on parental dietary pattern (mainly on the consumption of aquatic products) from 6 months before pregnancy to the

time when their children were born. All research staff completed rigorous professional training with uniform standards before the investigation was initiated. They were not allowed to participate in the investigation unless they passed the examination for the training course. The research staff first explained the purpose and significance of the study to the teachers and parents and then handed out consent forms to every parent to inform them of the study procedures and the policies of confidentiality agreement. A self-reported questionnaire was then administered to all the cases and controls whose parents had signed the consent forms. The parents of both groups were required to fill in the questionnaire with the guidance of trained research staff. The research staff went through the questionnaire with the parents and answered every question asked by the parents to minimize confusion and misunderstanding. All the questionnaires were collected at the investigation sites (in special educational schools for cases or normal schools for controls).

The self-administered questionnaire of this survey was quoted from the project of the National Natural Science Fund, with a title of Environmental risk factors in children with ASD[13-14]. The demographic data (sex, age, occupation, education level, income when child was born, and current income) and the periconceptional dietary status of the parents were investigated by the questionnaires, which originated from the unit of maternal health care of the maternal and child health information system of Tianjin City, including the dietary macronutrient content and drinking status (by food frequency questionnaire) and preference for meats, eggs, fruits, or vegetables, with focus on the species of fish or other aquatic products. Three types of aquatic products were investigated in this survey according to the quantity of sales in the top three local aquatic wholesale markets. The first group was sea fish, such as hairtail (*Trichiurus haumela*), yellow croaker, pomfret, sailfish, and Spanish mackerel; the second group was freshwater fish, such as crucian carp, grass carp, *Cyprinus carpio*, silver carp, and Wuchang Fish; the last group was other aquatic products, such as sea shrimp, shrimps, Mantis Shrimp, sea crab, and freshwater crab. The respondents were allowed to add other aquatic products that were not mentioned in the list. The definition of eating habits of one kind of aquatic product was consumption of that food more than three times per week; for alcohol, the quantity was more than 100 ×*g* per week along with frequency. The local traditional eating habits were also investigated, e.g., paternal alcohol consumption when eating fish or other seafood and couples eating the same food.

Variable Assignment

The dependent variable in the study was the diagnosis (TD = 0, ASD = 1, ID = 2). Data of the independent variable were collected as nominal or ordinal data. For example, binary variables were created to indicate whether the father consumed hairtail (yes = 1, no = 0). For income level, ordinal variables were designed (low = 0; intermediate = 1; high = 2).

Data Analysis

The database was established using EpiData, and IBM SPSS 20.0 was used for statistical analysis. Chi square test or Fisher's exact probability was run to analyze the difference between groups. The Spearman correlation was used to analyze the relationship between different variables, and multivariate logistic regression was used to construct the model of the entire study.

RESULTS

Demographics

A total of 295 participants were recruited in this study: 108 ASD cases, 79 ID cases, and 108 TD controls. No statistically significant difference was found among the three groups regarding age and sex distribution.

Multivariate Logistic Regression Analysis

Every single independent variable was analyzed via multivariate logistic regression. Sixteen significant variables were selected using the likelihood ratio test, such as paternal habit of drinking Chinese alcohol before conception, paternal habit of drinking beer before conception, and paternal habit of eating hairtail before conception (Table 1).

All 16 variables were entered as independent variables. Income level at childbirth, current income level, paternal education, and maternal education were the covariants (Figure 1). The TD group was set as the control group, and the multivariate logistic regression model was constructed (Table 2). For the ASD group, paternal habit of eating hairtail, maternal

habit of eating fruit, and maternal habit of eating grass carp were selected by the model. For the ID group, paternal habit of drinking Chinese alcohol, maternal habit of eating fruit, and maternal habit of eating grass carp were selected.

Correlation Analysis

Based on the local eating habits, the relationships between SES and parental eating habits,

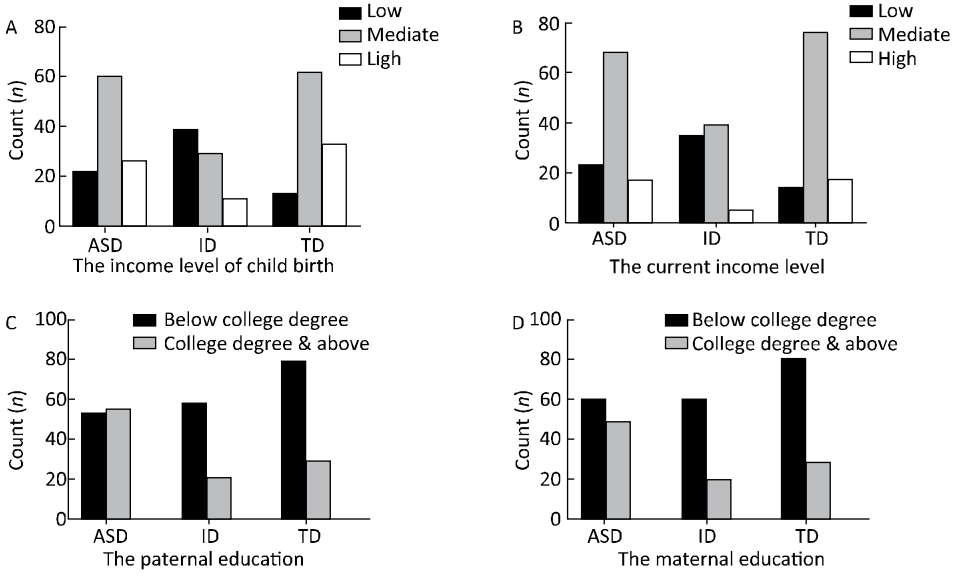

Figure 1. The social economic status of the 3 groups. (A) The economic situation among the three groups when the child was born; (B) The current economic situation among the three groups; (C) The paternal education among the three groups; and (D) The maternal education among the three groups.

Table 1. Results of the Likelihood Ratio Test for Independent Variables

Variables	*N**	Chi-square	*P*
Paternal alcohol consumption	102	14.326	0.001
Paternal beer consumption	130	11.712	0.003
Paternal hairtail consumption	216	14.387	0.001
Paternal yellow croaker consumption	81	8.606	0.014
Paternal grass carp consumption	70	14.612	0.001
Paternal sea shrimp consumption	107	12.349	0.002
Paternal Mantis Shrimp consumption	136	15.733	0.000
Paternal sea crab consumption	77	9.704	0.008
Paternal freshwater crab consumption	88	7.902	0.019
Maternal preference for fruits	109	14.522	0.001
Maternal crucian carp consumption	132	8.663	0.013
Maternal grass carp consumption	49	20.587	0.000
Maternal sea shrimp consumption	80	8.602	0.014
Maternal Mantis Shrimp consumption	93	12.404	0.002
Maternal sea crab consumption	48	9.622	0.008
Maternal intake of folic acid	54	6.060	0.048

Note. N^* = the number who answered yes; a total of 295 participants. Degree of freedom = 2 for all the variables.

between paternal eating habits and maternal eating habits, and between parental eating fish or other seafood and drinking were analyzed using the Spearman correlation test. The correlation between paternal educational level and paternal habit of drinking Chinese alcohol was statistically significant (r= -0.190, P = 0.049).

Several paternal eating habits were correlated with maternal eating habits, including the habit of eating hairtail and grass carp (Table 3).

The correlation between paternal drinking habits and eating habits was also significant, such as that between paternal hairtail consumption and paternal drinking Chinese alcohol, as well as between paternal sea crab consumption and paternal drinking beer (Table 4). The correlation was only observed in fathers, and no correlation was noted between maternal eating and drinking habits.

Table 2. Results of Multivariate Logistic Regression

Diagnosis	*B*	SE	Wald	*P*	Exp (*B*)	95% *CI*
ASD						
Paternal habit of eating hairtail = 0	1.191	0.475	6.290	0.012	3.291	1.297-8.347
Maternal habit of eating fruits = 0	0.885	0.341	6.750	0.009	2.424	1.243-4.728
Maternal habit of eating grass carp = 0	1.277	0.552	5.350	0.021	3.586	1.215-10.505
Intercept	-2.688	1.038	6.705	0.010		
ID						
Paternal habit of drinking Chinese alcohol = 0	-1.481	0.594	6.219	0.013	0.277	0.071-0.728
Maternal habit of eating fruits = 0	0.890	0.390	5.223	0.022	2.436	1.135-5.277
Maternal habit of eating grass carp = 0	1.312	0.500	6.869	0.009	3.712	1.392-9.900
Intercept	-1.711	1.171	2.133	0.144		

Table 3. Results of Spearman Correlation between Parental Eating Habits

Paternal Eating Habits	Maternal Eating Habits	*r*	*P*
Paternal hairtail consumption	Maternal hairtail consumption	0.425	0.000
Paternal grass carp consumption	Maternal grass carp consumption	0.565	0.000
Paternal yellow croaker consumption	Maternal yellow croaker consumption	0.501	0.000
Paternal sea shrimp consumption	Maternal sea shrimp consumption	0.397	0.000
Paternal Mantis Shrimp consumption	Maternal Mantis Shrimp consumption	0.441	0.000
Paternal sea crab consumption	Maternal sea crab consumption	0.470	0.000
Paternal river crab consumption	Paternal river crab consumption	0.490	0.000
Paternal juice consumption	Maternal fruit consumption	0.195	0.001

Table 4. Results of Spearman Correlation between Drinking and Eating Habits

Eating Habits	Drinking Habits	*r*	*P*
Eating hairtail	Drinking Chinese alcohol	0.227	0.018
Eating sea crab	Drinking Chinese alcohol	0.121	0.038
Eating hairtail	Drinking beer	0.322	0.001
Eating Mantis Shrimp	Drinking beer	0.308	0.001
Eating sea crab	Drinking beer	0.299	0.007
Eating sea shrimp	Drinking beer	0.148	0.011
Eating river crab	Drinking beer	0.150	0.010

Multivariate Logistic Regression Analysis of Combined Variables

To explore the relationship between the correlated variables further, new variables were created for examining the association between the diagnosis and the combined factor of the correlated variables. For example, upon combining paternal hairtail consumption and paternal drinking of Chinese alcohol, new variables were established as follows: paternal drinking of Chinese alcohol without hairtail consumption = 1, non-paternal drinking of Chinese alcohol without hairtail consumption = 2, paternal drinking of Chinese alcohol with hairtail consumption = 3, and paternal hairtail consumption without drinking of Chinese alcohol = 4. On the basis of multivariate logistic regression analysis, the model was significant (Figure 2, Table 5).

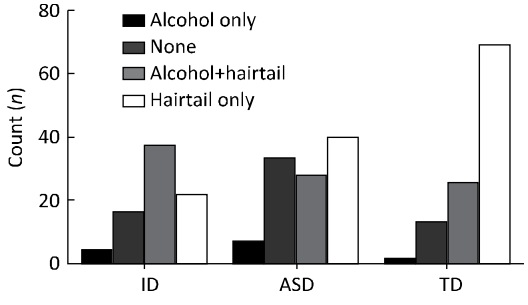

Figure 2. The distribution of the combined factor. 'Alcohol only' means 'paternal drinking of Chinese alcohol without hairtail consumption,' none means 'non-paternal drinking of Chinese alcohol without hairtail consumption,' alcohol + hairtail means 'paternal drinking of Chinese alcohol with hairtail consumption,' and hairtail only means 'paternal hairtail consumption without drinking of Chinese alcohol.'

The rest of the combined factors were created according to Tables 3 and 4, but a statistically significant difference was found between the diagnosis and the combined factors.

DISCUSSION

China has a long history of drinking alcohol at feasts or private dinner parties[15]. In traditional Chinese culture, moderate alcohol consumption was encouraged because it was considered beneficial for health[16]. Drinking habits are also influenced by education. Less educated people are more prone to consume alcohol than individuals with high educational attainment[17-18]. The adverse effects of paternal alcohol exposure on the neurodevelopment of offspring include developmental disabilities, neuropsychiatric impairment, learning difficulties, and speech development deficiency[19-20]. Thus, the childbearing population who has no knowledge of this issue must be informed. Several studies have been performed to explore the effects of alcohol on the absorption of nutrients and on sperm function[21-22].

Hairtail is one of the most popular fish; it has high nutritional value and is widely distributed in the eastern waters of China[23-24]. Reports on the association between paternal hairtail consumption and ASD are few, because researchers have been more interested in the effects of methylmercury in hairtail[25]. However, not all studies support the view that marine fish contaminated with methylmercury have an adverse effect on ASD. Some researchers argue that modifying factors other than fish consumption, such as SES, play an influential role on fetal development[26]. Several studies reported that adequate selenium in marine fish neutralizes the harmful effects of methylmercury[27-28], and polyunsaturated fatty acids in fish oil benefit sperm

Table 5. Results of Multivariate Logistic Regression on Alcohol + Hairtail Consumption

Diagnosis	*B*	SE	Wald	*P*	Exp (*B*)	95% *CI*
ASD						
No hairtail with alcohol	2.491	1.087	5.249	0.022	12.075	1.433-17.130
No hairtail without alcohol	1.477	0.383	14.864	0.000	4.379	2.067-9.277
Hairtail with alcohol	0.659	0.339	3.764	0.052	1.932	0.993-3.758
Hairtail without alcohol	0[b]					
ID						
No hairtail with alcohol	2.529	1.145	4.884	0.013	12.545	1.331-18.226
No hairtail without alcohol	1.351	0.447	9.151	0.022	3.860	1.609-9.261
Hairtail with alcohol	1.535	0.356	18.559	0.009	4.642	2.309-9.333
Hairtail without alcohol	0[b]					

quality[29]. According to the Chinese food composition table (second edition) compiled by the Institute of Nutrition and Food Safety of Chinese Center for Disease Control and Prevention (CDC), the fish oil content of hairtail was the highest (4.9 ×*g* / 100 ×*g*) among the marine fishes investigated in this study. Therefore, hairtail is more important among the fish investigated in this study from the perspective of paternal reproductive health.

In this study, the maternal habit of eating fruits was found to be a protective factor for ASD and ID. Fruits provide several nutrients such as vitamins, minerals, plant chemicals, bioactive compounds, and dietary fiber[30]. Childbearing women are encouraged to eat plenty of fruits by many health education and health promotion programs for pregnant women[31-32]. In the Dietary Guidelines for Americans 2010, consumption of fruits is strongly recommended as they provide folic acid, magnesium, potassium, dietary fiber, and vitamins A, C, and K, as well as being low in calories.

Another finding of this study was that the maternal habit of eating grass carp and crucian carp were protective factors for ASD and ID, respectively. In addition to polyunsaturated fatty acids and trace elements, a protein hydrolysate in grass carp has neuroprotective and antioxidant properties[33]. This protein hydrolysate is easily dissolved and absorbed in the gastrointestinal tract, and it is used as a natural antioxidant[34]. According to the Cultured Aquatic Species Information Program, crucian carp is not studied as a food in Western countries because of its small body and fish bones in meat. In China, however, it is usually made into carp soup with ingredients such as Chinese yam and bean curd. Crucian carp soup is used to reduce weakness of the spleen and stomach and enable lactation according to traditional Chinese medicine[35-36].

The main strengths of this study include (1) a model of effects of periconceptional parental fish consumption on ASD and ID; (2) exploration of Chinese eating habits, such as fish consumption with alcohol and couples eating the same food; and (3) exploration of the interaction between fish and alcohol on ASD and ID. Finally, our results suggest that fertilization with paternal hairtail consumption exerted a preventive effect on ASD, and maternal freshwater fish and fruit consumption during pregnancy helped prevent ASD and ID. Moreover, drinking alcohol with fish consumption reduced the benefits of fish consumption. Freshwater food affected fetal neurodevelopment differently compared with marine food, and this phenomenon needs further investigation.

A major weakness of this study is recall bias, which is inherent because of the case-control nature of the study; however, the dietary pattern of adults hardly changes over time[37]. In the future, we aim to establish effective community cohorts to explore the connection between parental fish consumption and ASD and ID by performing prospective studies.

CONCLUSION

A case-control study using questionnaire surveys was conducted to examine the effect of periconception parental fish consumption on ASD and ID in Tianjin, China. By performing multivariate logistic regression, paternal consumption of hairtail and maternal consumption of fruit and grass carp were found to be associated with a low risk of ASD, and maternal consumption of fruit and crucian carp were associated with a low risk of ID. Moreover, paternal consumption of Chinese alcohol was associated with a high risk of ID. The traditional habit of drinking alcohol when eating fish reduced the benefits of hairtail consumption.

CONFLICT OF INTERESTS

On behalf of all authors, the corresponding author declares no conflict of interest.

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