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Cohort study for prevention of atopic dermatitis using hair mineral contents

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ABSTRACT

We undertook a cohort study to determine the association between hair mineral content and the onset of atopic dermatitis (AD) in infants. Eight hundred and thirty-four mother–infant pairs, who donated hair samples during one and ten-month health checkups, had their samples analyzed by proton induced X-ray emission (PIXE) for 32 mineral concentrations, and these mineral concentration data together with their AD family history were statistically examined for any relationships between them. Results indicated that of all minerals, only selenium (Se) and strontium (Sr) showed statistically significant associations for infants, while the same two elements were only marginally significant for mothers. Se deficiency in either infant or mother increased the AD risk. A Sr deficiency in infants increased AD risk, while the same deficiency in mothers decreased the risk. To predict the probability of AD development using this data, we performed logistic regression analysis, which provided a sensitivity of 65.9%, a specificity of 70.5%, a positive predictive value (PPV) of 10.3%, a negative predictive value (NPV) of 97.6% and a relative risk (RR) of 4.2, all far better than any corresponding figures explicitly mentioned in previously published papers.

Introduction

The prevalence of atopic dermatitis (AD) has increased substantially over the past 30 years. A combination of genetic and environmental factors appears to be involved in AD development. Reports on a deficit or excess in certain minerals [1–4] reinforce the involvement of environmental factors. Unfortunately, there are methodological weaknesses in most of the epidemiological studies on mineral exposure and AD. In many cases, the numbers of minerals analyzed were too small to detect any synergistic mineral effect. In other cases, the mineral concentrations in serum or urine samples used for the studies only represented accumulation in the body within the previous 7 days [5], and would not reflect mineral accumulation over long periods. Hair mineral concentrations are now being regarded as ideal biomarkers to measure individual exposures to elements and The Environmental Protection Agency (US EPA) considers scalp hair a suitable biological sample for estimating the body burden of trace elements [6], because hair incorporates elements from the blood at a relatively constant rate and its composition reflects the concentration of elements in blood at the time of formation [5,7]. Therefore, we implemented a study to use hair mineral concentrations measured by proton induced X-ray emissions (PIXE) and comprehensive health questionnaires which included data on AD family history, to develop a model that would be effective for detecting infants with a high risk of developing AD with the ultimate goal of primary prevention.

Methods

Study design

We conducted a cohort study following mother–infant pairs from the national one-month health checkup to the ten-month checkup. The objective was to determine the association between AD and 32 measurable minerals in the hair of infants and mothers, as measured by PIXE, for the development of model that could help in the primary prevention of AD. Fig. 1 displays the process of the study. A previous study by Saunders et al. [8] reported on distributional characteristics and the intra- and inter-individual variabilities of these PIXE hair mineral measurements. That preliminary investigation suggests certain variable transformations of

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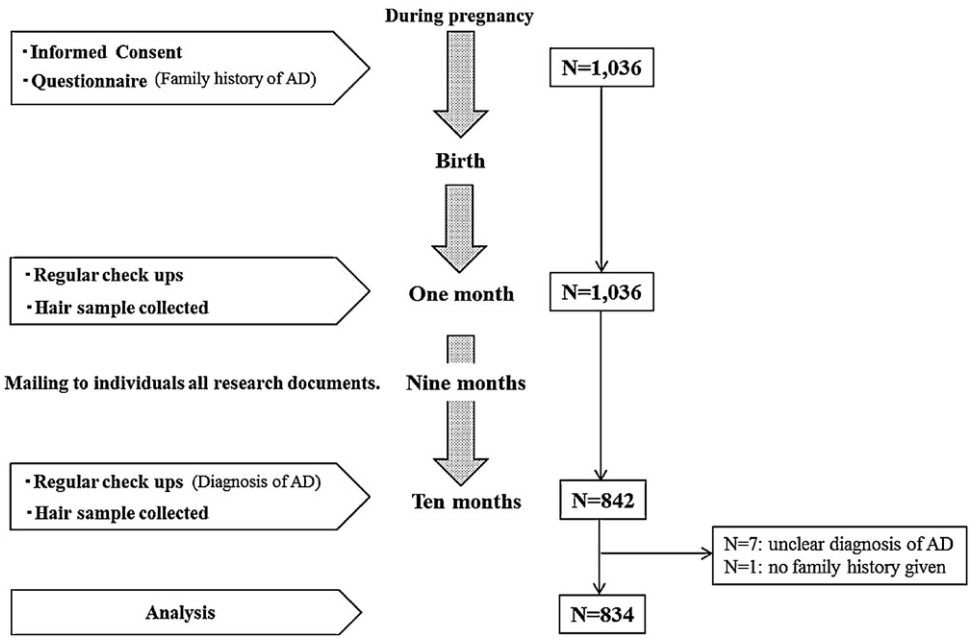

Fig. 1. Project outline.

the hair minerals were necessary for sound statistical analysis. We apply these transformations in this association study of hair minerals and AD. This study was approved by the Institutional Ethics Review Committee of the Kyushu University on March 30th, 2005.

Subjects

The subjects consisted of 1036 mother–infant pairs who lived in Fukuoka city, birthed naturally, and voluntarily participant in our cohort study by presenting their hair samples at both their one and ten-month national infant checkups, which were performed by 13 obstetricians and 77 pediatricians at 90 hospitals throughout Fukuoka. Of the original 1036 mother–infant pairs recruited by their obstetricians at the first one-month health checkup starting in November 2005, 842 participated in the ten-month pediatrician conducted health checkup (revisit rate: 81.3%). We excluded 7 with an unclear diagnosis of AD and 1 with no family history response resulting in 834 mother–infant pairs for statistical analysis.

Examination items

During the regular infant checkup the mothers answered a questionnaire, which included questions about maternal history such as pregnancy toxemia and premature birth, and questions about childcare and daily habits such as smoking and drinking. The infants were given a detailed medical examination. The medical checks were performed by obstetricians and gynecologists at one-month and by pediatricians at ten months. Of particular interest was any diagnosis of AD at ten months using the following standard pediatric, dermatological criteria: (1) pruritus; (2) typical morphology and distribution of skin lesions; (3) chronic and relapsing course [9].

We added 2 additional items to the standard checkups. One was a questionnaire for mothers about AD family history, and the other was hair sampling from mothers and infants.

The family history questionnaire included questions about parents, grandparents or siblings having AD. This information is a surrogate for the inherited constitution of AD in relation to the spontaneous risk, since AD is generally considered to be a familiarly transmitted disease [10,11]. All the above data were independently input twice at Nagasaki and Kyushu universities, and inconsistencies were checked and corrected.

Hair sample collection, preparation and PIXE analysis

With the advent of the sophisticated measuring devises such as PIXE, we can now quickly obtain data concerning mineral amounts in a diversity of materials ranging from cloth and wine to prehistoric stone implements and aerosols. PIXE provides an excellent method that enables the non-destructive measurement of the over 30 minerals from only a few strands of hair. The samples were collected from the scalp close to the occipital region at a length not exceeding 5 cm using a pair of stainless steel scissors.

For target preparation, the root-side of the hairs were attached to the bottom of a holder with adhesive tape and then fixed on the topside in order to avoid overlapping. After target sample preparation completion, they were sent to Nishina Memorial Cyclotron Center (NMCC), Iwate Medical University, Japan for hair mineral analysis. A small-size cyclotron provides a 2.9 MeV-proton beam on a target after passing through a graphite beam collimator for several minutes. The beam current was 100 nA. X-rays of energy higher than that of K-Kα are detected by a Si (Li) detector (0.0254 mm-thick Be window; 6 mm in active diameter) with a 300 μm-thick Mylar absorber. Another Si (Li) detector (0.008 mm Be; 4 mm in active diameter) is used for the measurement of lower-energy X-rays (<K-Kα). Description of the data acquisition system is reported in [12] and the measuring conditions in [13]. The computer code used for spectrum analysis is "SAPIX" [14,15]. Cases that had no peak (failed to detect) were treated as zero.

Statistical analysis

The primary outcome of interest in this study was whether or not each infant was diagnosed with AD at the ten-month health checkup. The primary risk factors considered were hair mineral amounts in mothers and infants at the one-month checkup as measured by PIXE, and AD family history. After obtaining mineral distributions for infants and mothers it was discovered that some minerals showed extreme skew distributions that could have degraded the association study results. This required us to

Table 1
Study subjects' background and the relation to AD.

	All N		Non-AD N		AD N		p-Value[a]
Mother's age	830	30.70 ± 4.35	789	30.70 ± 4.33	41	30.71 ± 4.76	0.921
Miscarriage experience	133	(16.0%)	126	(15.9%)	7	(17.1%)	0.840
Pregnancy condition							
Normal	729	(91.6%)	689	(91.3%)	40	(97.6%)	0.157
Bleeding	27	(3.4%)	26	(3.4%)	1	(2.4%)	0.729
Gestosis	7	(0.9%)	7	(0.9%)	0	(0.0%)	0.536
Diabetes	3	(0.4%)	3	(0.4%)	0	(0.0%)	0.686
Smoking during pregnancy	48	(5.8%)	47	(5.9%)	1	(2.4%)	0.350
Drinking during pregnancy	319	(38.5%)	301	(38.2%)	18	(43.9%)	0.465
Gestation period	831	39.08 ± 1.35	790	39.06 ± 1.35	41	39.44 ± 1.21	0.077
Infant's sex							
Boy	411	(49.3%)	387	(48.8%)	24	(58.5%)	0.224
Girl	423	(50.7%)	406	(51.2%)	17	(41.5%)	
Birthweight	812	3037.73 ± 342.18	771	3038.91 ± 344.94	41	3015.59 ± 288.10	0.774
Feeding							
Breast-feeding	529	(63.5%)	499	(63.0%)	30	(73.2%)	0.389
Bottle-feeding	18	(2.2%)	17	(2.2%)	1	(2.4%)	
Mix	286	(34.3%)	276	(34.9%)	10	(24.4%)	

[a] Adjusted *p*-value by family history of AD.

perform the following mineral transformations. Since 1 ppm is generally regarded as the detection limit of hair mineral measurements by the current PIXE methodology, we substituted 1 ppm for measurements less than 1 ppm, then log transformed them to approximate normality. However, if the detection rate was less than 25%, we categorized according to quartiles: Q_1, Q_2 = Med and Q_3 such that $Q(x) = 0, 1, 2,$ or 3 depending on whether $x < Q_1$, $Q_1 < x < Q_2$, $Q_2 < x < Q_3$ or $Q_3 < x$.

Logistic regression analysis was performed to identify minerals associated with the onset of AD, adjusting for a family history that indicated whether any parents, grandparents or siblings had AD. Considering the results of the logistic analysis, we developed a formula to predict the risk of AD. The formula's performance was assessed by the sensitivity, specificity, positive and negative predictive values and relative risk.

All analyses were done using SAS version 9.1.3.

Results

Background (relationship between onset of AD and items in one-month checkup)

Forty-one infants (5%) were diagnosed with AD at the ten-month checkup and 189 infants (22.7%) had AD relatives (parents, grandparents or siblings). We choose background factors that we felt were likely to affect AD from the one-month checkup sheet, and examined the relation to the onset of AD at ten-month checkup. The results are summarized in Table 1. No other items were shown to be significant factors after adjusting for the family history.

PIXE analysis

We measured 32 minerals for mothers and infants by PIXE. Cadmium (Cd) and barium (Ba) were omitted from statistical analysis, since those minerals were observed in only a few subjects (less than 5%). Sulfur (S) was approximately normally distributed with the coefficient of variation about 0.01 for both infants and mothers. The extremely small coefficient of variation indicates that S concentrations are approximately constant among the samples.

Table 2
Screening of minerals significantly related to AD by logistic model.

N = 834 (AD: 41cases)		
p-Value adjusted by family history of AD	Infant	Mother
<0.05	Sr, Se	
0.05–0.10	Na, Cl	Se, Si, Al
0.10–0.20	Br, Rb, Al, K, Pb	Sr, Cr
0.20–1.00	Mn, As, Co, Cu, Mg, Ca, Mo, Zn, S, Si, V, Fe, Ag, P, I, Ni, Ti, Nb, Hg, Ga, Cr	Br, Mg, Ni, Na, Rb, V, As, Nb, Co, Zn, S, Ca, Mn, Ga, Pb, K, P, Hg, Cu, I, Fe, Ag, Cl, Mo, Ti

Relationship between minerals and onset of AD

After the variable transformations as described in Methods section, we applied the logistic regression model to determine minerals at one month that were significantly associated with the onset of AD adjusting for the family history. The results are shown in Table 2, indicating only infants' Se and Sr were significant, $p = 0.048$ and 0.023 respectively, with mothers' Se and Sr being marginally significant, $p = 0.062$ and 0.115, respectively.

The results (Table 3) imply that a Se deficiency in either mother or infant increases the risk of AD. On the other hand, while a Sr deficiency in infants increases the risk, a Sr deficiency in mothers decreases the risk. The indications prompted us to obtain combined scores of infants and mothers: Sum_Se = Q(infant's Se) + Q(mother's Se), which is the sum of quadrisected Se of infants and mothers, and D_log Sr = log(infant's Sr) − log(mother's Sr), which is the difference between the logarithm of Sr between infants and mothers. Hereafter, these terms will be designated the combined scores for Se and Sr. Both of the combined scores are strongly associated with the onset of AD, $p = 0.006$ and 0.005, respectively. Fig. 2 shows the individual and combined histograms of those minerals for infants and mothers by AD status. The combined scores of Sr tend to be higher for AD than for non-AD. On the other hand, the combined scores of Se tend to be lower for AD than for non-AD.

Then we applied a logistic model to predict the onset of AD using the family history and the combined scores to obtain the following

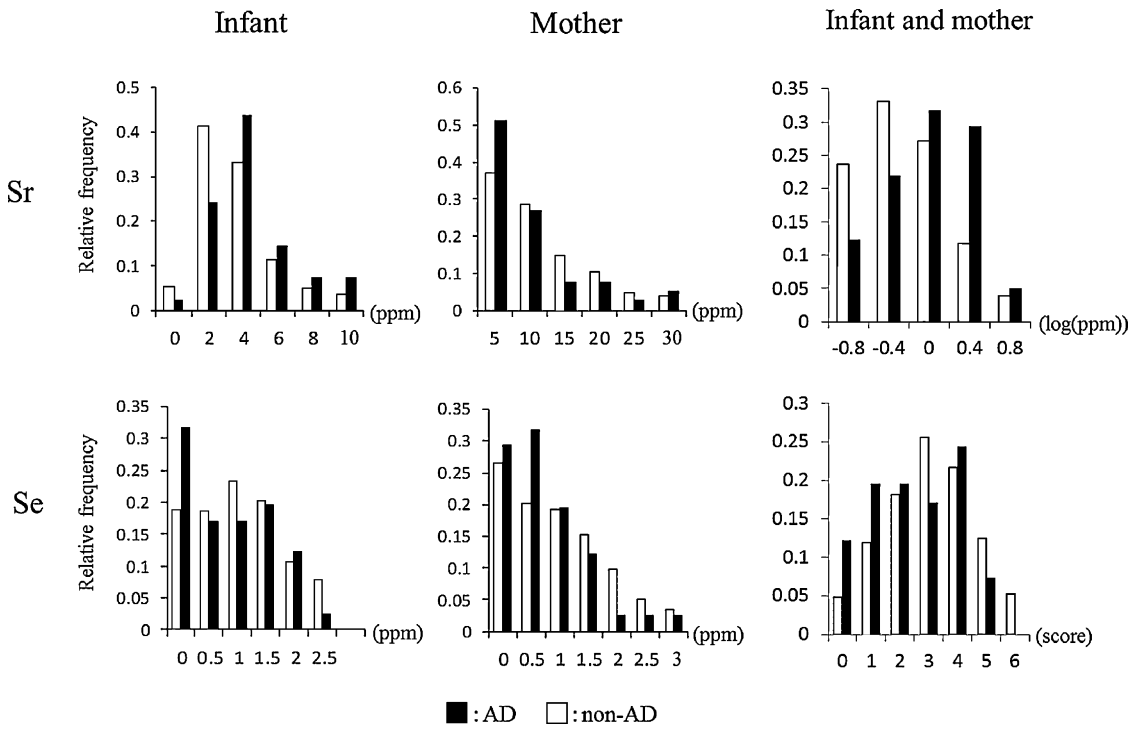

Fig. 2. Histograms of Se (upper) and Sr (lower) for the infants (left), the mothers (center) and both (right) by AD status. The combined log(Infant's Sr)–log(Mother's Sr) and *Q*(Infant's Se)+*Q*(Mother's Se)) scores are shown for infants and mothers together (right).

equation:

$$\log \frac{P(X)}{1-P(X)} = -2.17 + 1.19 \cdot \text{Family\_History} + 0.438 \cdot \text{D\_log Sr} - 0.303 \cdot \text{Sum\_Se} \quad (1)$$

The receiver operating characteristic (ROC) curve using Eq. (1) is shown in Fig. 3. Three classification matrices are shown in Table 4: (4.1) using family history only, (4.2) using this equation with a cutoff point that results in the same sensitivity as (4.1), and (4.3) using this equation with a new cutoff point that resulted in a higher sensitivity and relative risk.

Family history alone produces a sensitivity of 41.5%, a specificity of 78.1%, a PPV of 8.9%, a NPV of 96.3% and a RR of 2.4 (Table 4.1). However, the use of Eq. (1) results in a sensitivity of 65.9%, a specificity of 70.5%, a PPV of 10.3%, a NPV of 97.6% and a RR of 4.2 (Table 4.3).

Table 3
Significant factors relating AD to mineral amounts by logical regression analysis.

N = 834 (AD: 41cases)				
Variable	Crude	Adjusted crude for family history of AD		
	p-Value	Estimate	SE	*p*-Value
Family history of AD				
Mother	0.009**			
Father	0.138			
Sibling	0.002**			
Grandparents	0.391			
log(infant's Sr)	0.032**	0.52	0.23	0.023**
log(mother's Sr)	0.110	−0.27	0.17	0.115
D_log Sr	0.006**	0.42	0.15	0.005**
Q(infant's Se)	0.054*	−0.31	0.16	0.048**
Q(mother's Se)	0.102	−0.27	0.15	0.062*
Sum_Se	0.011**	−0.30	0.11	0.006**

* *p*-value < 0.1.
** *p*-value < 0.05.

Discussion

Nurmatov et al. [4] reviewed 62 studies that dealt with the primary prevention of asthma and atopic disorders of children selected from 12,863 papers published between January 1988 and May 2009, and graded them according to the reliability of their designs. Besides vitamins, only Se and Zn were treated in these reports. Their conclusion was that the body of evidence in relation to Se was methodologically weak and unsupportive for an association between childhood asthma and allergy and Se status during pregnancy or childhood, although some studies assessed associations between Se concentrations in maternal blood, umbilical cord, and early childhood, and related these to childhood wheezing, asthma, or atopic sensitization.

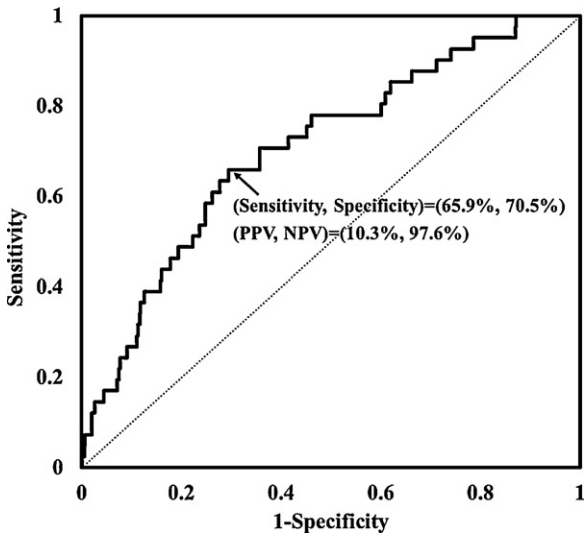

Fig. 3. Receiver operating characteristic curve.

Table 4
The classification matrices.

4.1. Prediction of onset of AD by family history only						
		Onset of AD				
		Yes	No	Total		
Family history	Yes	17	173	190	PPV	8.90%
	No	24	620	644	NPV	96.30%
	Total	41	793	834		
		Sensitivity	Specificity		RR = 2.4	
		41.50%	78.20%			
4.2. Prediction by family history and hair minerals content						
		Onset of AD				
		Yes	No	Total		
Family history + Sr + Se	Yes	17	125	142	PPV	12.00%
	No	24	668	692	NPV	96.50%
	Total	41	793	834		
		Sensitivity	Specificity		RR = 3.4	
		41.50%	84.20%			
4.3. Prediction by family history and hair minerals using the new cutoff point						
		Onset of AD				
		Yes	No	Total		
Family history + Sr + Se	Yes	17	234	142	PPV	10.30%
	No	24	559	573	NPV	97.60%
	Total	41	793	834		
		Sensitivity	Specificity		RR = 4.2	
		65.90%	70.50%			

The design of this study distinguishes itself in 3 ways from these previously published reports.

First, ours is a prospective cohort study and subjects were regarded as a random sample from healthy infants born in Fukuoka city, since obstetricians invited mothers, who had normal births, to donate hairs for studying AD and almost all of them volunteered samples, because they were concerned about AD and were very open to the idea of free hair mineral analysis and any possibilities of improving their children's health. This nearly perfect participation makes the study subjects a virtual random sample; therefore PPV and PNV as well as the sensitivity and specificity are regarded as approximately unbiased.

Second, concerned with a valid AD diagnosis, Chamlin et al. [10] reviewed prospective studies that determined the risk of AD for children and were particularly concerned with the diagnosis criteria of AD. They highlight inconsistencies in the diagnostic criteria for the risk of AD, but concluded that family history is easy to obtain and may be a practical way to identify risk, even if the PPV of the family history on the development of AD is unclear. For instance, one study defined AD as the presence of a positive IgE test against at least one allergen while another used positive skin test results only, treating IgE levels as reference [10]. Bergman and Edenharter [16] used only the parental AD history to predict the development of AD in the first 24 months and found a 17% sensitivity. They conclude that a validated and practical screening tool to identify high-risk infants is essential. In our study, the diagnosis of AD was performed based on the same criteria for each infant at the same age by attending pediatricians making for a reliable diagnosis.

Third, we feel the most distinguishing feature of this study is the use of 32 hair mineral concentration measurements taken from mothers and infants at one month after birth. There are some studies that examined whether a single mineral might be associated with AD, however, for any large combination of minerals little research has been done, and there is almost no evidence describing their combined effects regarding any association with AD [4]. We felt that analyzing as many minerals as possible would provide a better understanding of the interdynamic relationship that they may have on one another. This could be accomplished most effectively with PIXE. Other methods such as inductively coupled plasma (ICP) method were not applicable because the hairs on some one-month infants were too few to fit the sample volume requirement.

The results indicate the combined scores of Se and Sr measured at one month after birth are jointly, significantly associated with the onset of AD diagnosed at ten months. Se deficiency was repeatedly suggested as a possible cause of AD [17–19], but Sr was rarely discussed.

The 41.5% sensitivity shown in Table 4.1 is far better than 17% found by Bergman and Edenharter [16] although both use only family history of AD. The cause of this discrepancy may be due to differences in the diagnosis of AD, the follow-up period, and the definition of family history. PPV is improved from 8.9% in Table 4.1 to 12.0% in Table 4.2 by taking into account Se and Sr in addition to family history. The 41.5% sensitivity, 96.3% NPV and 2.4 RR in Table 4.1 improved to 65.9%, 97.6% and 4.2, respectively, in Table 4.3 using Eq. (1). The high sensitivity with an only 2.4% false negative suggests the screening for AD at one month using Eq. (1) warrants further studies of Se and Sr for establishing the primary prevention of AD.

Although AD is generally accepted as a familial transmitted disease, Chamlin et al. [10] found 16 distinct ways of summarizing AD family history, due to the lack of general agreement on the genetic mechanism. Our results clearly indicate that when a mother had AD, her child had a higher risk of AD. Such an association fails to exist with the father. This result is in accord with Leung and Bieber [11], who suggest the existence of genes specific to AD with strong maternal influence. This may support the fact that only the mother's mitochondria is inherited by the child and this mitochondria can produce a protein for itself in response to certain stimuli [20]. It is also widely accepted that if a child lives in an insalubrious environment with more stimulus from miscellaneous foreign substances, which can result in a stronger immunological system with the ability to resist foreign substances, there is a decreased

risk of AD [11]. These considerations suggest that immunological system abnormalities associated with AD could partly originate in the mitochondria.

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