

Mineral Imbalance in Children with Autistic Disorders

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Abstract

In order to investigate any role of minerals in autistic disorders, we measured 24 mineral contents in scalp hair samples of 360 autistic children and compared with those of healthy controls. In the male autistic group aged 4-9 years (N=200), the geometric means of many element levels, namely not only essential minerals such as cobalt, chromium, iodine, molybdenum and phosphorus, but also nickel, boron, cadmium and lead were significantly lower than those in the control group ($p < 0.001$). In contrast, selenium level was significantly higher in the autistic group. On the mercury levels, no significant difference was observed between the two groups, though with a tendency of low concentration in the autistic group. Similar mineral imbalance profiles with a global mineral deficiency were observed in the other autistic groups aged 0-3 and 10-15 years (N=85 and 20) and also in the female autistic groups. In addition, high accumulation of a few elements such as iron, manganese, chromium, copper, sodium, aluminum, cadmium, lead, or mercury was observed in some autistic individuals.

These findings indicate that autistic children are suffered from a global mineral deficiency in various trace elements, with some individuals being exposed to a marked accumulation of several elements. Autistic children may be classified to sub-groups, based on their mineral imbalance profile in hair.

Keywords : Autistic children ; Global mineral deficiency ; Mineral imbalance ; Sub-groups.

Introduction

Autism is a complex psychiatric disorder of neuro-developmental origin, characterized by impairments in social interaction and communication associated with repetitive patterns of interest and/or behavior [1-3]. Autistic spectrum disorders (Asperger's syndrome, pervasive developmental disorder and autism) are among the most prevalent developmental disorders with heritability, affecting as many as one in 500-1000 children [1,4-6]. Both genetic and environmental factors have been implicated in the pathogenesis of autism, but its specific etiology remains to be clarified [4-8].

The role of environmental factors is still poorly characterized. Recently, the neurobiology of metal is

receiving growing interest, since it has been linked to major neuro-degenerative diseases [9-11]. It has been suggested that thimerosal, a mercury-containing preservative in vaccines, is a risk factor for the development of autism [8]. In order to investigate the roles of neuro-toxic metals such as mercury, lead, cadmium or aluminum in the etiology of autistic disorder, we have determined 24 minerals contents in hair samples of autistic children and examined relation between mineral balance and autistic disorder.

In this study, we demonstrated that the children with autistic disorders are suffered from a global mineral deficiency in various bio-elements including not only essential minerals but also toxic metals. Furthermore, some autistic individuals were found exposed to a marked accumulation of several elements. These findings indicate that autistic children may be classified to sub-groups, based on their mineral imbalance profile in hair.

Materials and Methods

Hair mineral analysis

The hair samples from total 360 autistic children

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aged 0-15 years (male: 305; female: 55) were collected. The autistic group was comprised of children exhibiting 'autistic features' checked in the mark-seated questionnaire. The data of the children from whose parent informed consent was obtained, were used.

The control group was consisted of healthy children aged 0-15 years with no description of diseases or symptoms in their questionnaires (male: 143; female: 98).

The hair sample of 75 mg was weighed into 50ml plastic tube and washed with acetone and then with 0.01% Triton solution, according to the procedures recommended by the Hair Analysis Standardization Board, as reported previously [12]. The washed hair was mixed in 10 ml of 6.25% tetra methyl ammonium

hydroxide (TMAH, Tama Chemical) with 50 μ l of 0.1% gold solution (SPEX Certi Prep.), and then dissolved at 75 degrees centigrade with shaking for 2 hours. After cooling the solution to room temperature and adjusting its volume gravimetric, the obtained solution was used for mineral analysis. The mineral concentrations were measured with inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500c and 7500i).

Mineral contents in hair were expressed as ng/g hair (ppb) or μ g/g (ppm). The relative level of respective mineral was obtained from the following equation.

Relative level = Geometric mean in autistic children / Geometric mean in control children

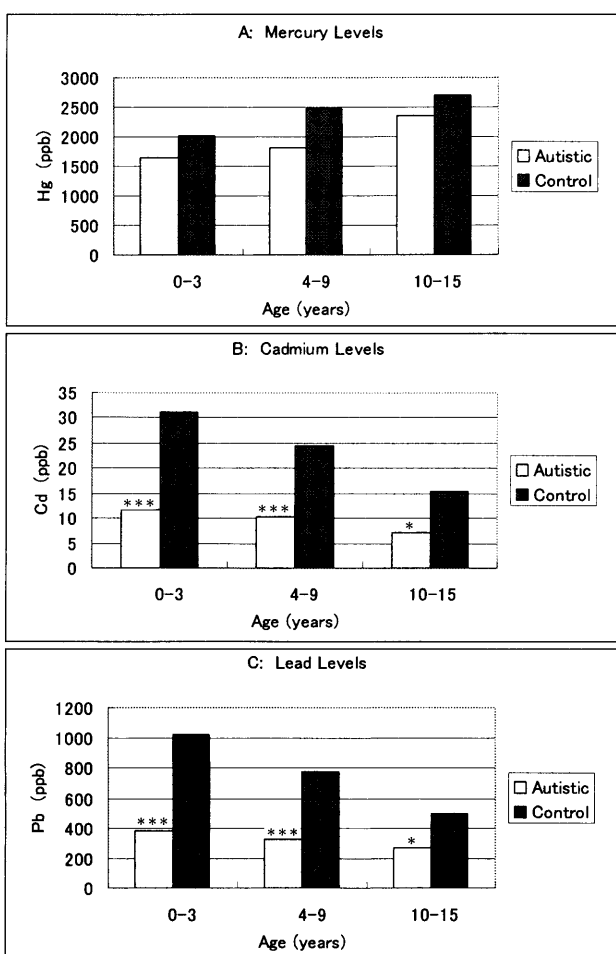


Fig. 1 Hair mercury, cadmium and lead levels in male autistic children. A : Mercury ; B : Cadmium ; C : Lead. Open and closed bars represent the geometric mean values of the hair toxic mineral levels in autistic and control children groups aged 0-3, 4-9 and 10-15 years, respectively. *, **, ***: Significantly different from control group with $p < 0.05, 0.01, 0.001$ respectively.

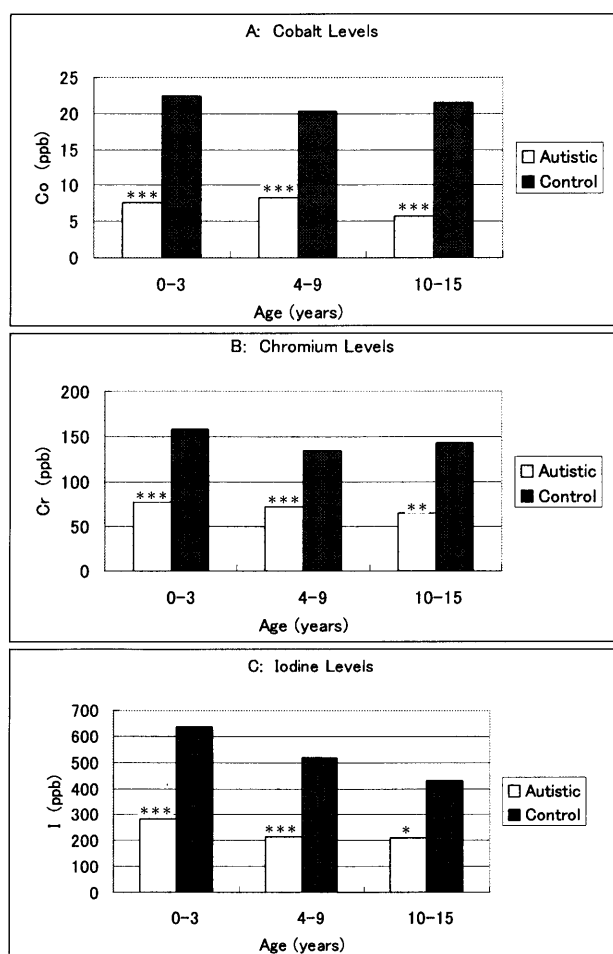


Fig. 2 Hair cobalt, chromium and iodine levels in male autistic children. A : Cobalt ; B : Chromium ; C : Iodine. Open and closed bars represent the geometric mean values of the hair essential mineral levels in autistic and control children groups aged 0-3, 4-9 and 10-15 years, respectively. *, **, ***: Significantly different from control group with $p < 0.05, 0.01, 0.001$ respectively.

Statistical methods

For statistical analysis, the data of the groups containing more than 10 subjects per group were used. That is, the data of female group aged 10-15 years (N=6) were excluded from analysis, and so the data of total 354 autistic children (male: 305; female: 49) were statistically analyzed.

Hair mineral contents were distributed in positive skew, and so all of the values were converted to the logarithm for statistical analysis.

Statistical significance was determined using the Welch's t-test. A p value of less than 0.05 was considered significant.

Results

Figure 1 shows the geometric means of hair levels of representative toxic metals, mercury, cadmium and lead, in male autistic groups aged 0-3 (N=85), 4-9 (N=200) and 10-15 years (N=20), in comparison with the respective control group. The mean hair mercury levels in the autistic children were not higher than

those in the control groups, rather tended to be low in the group aged 4-9 years ($p=0.059$). It is more marked that hair cadmium and lead levels were high-significantly lower in the autistic groups aged 0-3 and 4-9 years ($p<0.0000$). In addition, some essential minerals such as cobalt (Co), chromium (Cr), and iodine (I) were also markedly low in the autistic group, being at near about one half of those in the control group (Fig. 2). Furthermore, various minerals such as molybdenum (Mo), copper (Cu), phosphorus (P), boron (B), vanadium (V) and nickel (Ni) were also significantly lower in the autistic children (Table 1). In contrast, hair selenium (Se) and iron (Fe) level was significantly higher in the autistic group ($p<0.0001$ and $p<0.01$, respectively); these minerals are known as "paradox mineral". For visualizing the global alteration of mineral balance in autistic children, the relative levels of 24 minerals in the male autistic group aged 4-9 years are shown (Fig.3).

A similar, mineral imbalance profile exhibiting consistent mineral deficiency was observed in the

Table.1 Mean value and standard deviation of the logarithms of hair mineral levels in autistic children.

Mineral	Autistic (N=200)		Control (N=56)		Welch's t-test P value	
	Mean	S.D.	Mean	S.D.		
Na	3.975	0.424	4.054	0.503	0.2874	
K	4.192	0.306	4.332	0.465	0.0374	*
Mg	4.286	0.290	4.357	0.287	0.1095	
Ca	5.308	0.239	5.273	0.164	0.2103	
Cr	1.856	0.261	2.131	0.177	0.0000	***
Mo	1.585	0.196	1.694	0.192	0.0003	***
Mn	2.302	0.288	2.360	0.280	0.1754	
Fe	4.027	0.187	3.963	0.147	0.0071	**
Cu	4.095	0.205	4.176	0.172	0.0035	**
Zn	5.067	0.112	5.024	0.177	0.0904	
P	5.041	0.087	5.148	0.088	0.0000	***
Se	2.780	0.068	2.706	0.125	0.0001	***
V	1.347	0.506	1.554	0.438	0.0033	**
Co	0.921	0.255	1.307	0.168	0.0000	***
Ni	2.035	0.368	2.361	0.336	0.0000	***
B	2.311	0.379	2.547	0.345	0.0000	***
Ge	2.018	0.099	2.012	0.116	0.7321	
Br	3.561	0.371	3.602	0.348	0.4497	
I	2.330	0.435	2.713	0.412	0.0000	***
Cd	1.009	0.485	1.390	0.373	0.0000	***
Hg	3.258	0.411	3.393	0.483	0.0594	
Al	4.013	0.263	4.064	0.264	0.2066	
Pb	2.517	0.370	2.889	0.344	0.0000	***
As	1.652	0.238	1.680	0.260	0.4673	

The mean value and standard deviation of the logarithms of mineral levels in hair are shown. Statistical significance was determined using the Welch's t-test.

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

other autistic groups of male children aged 0-3 and 10-15 years (Fig.4 and 5) and also in the female groups aged 0-3 and 4-9 years (Fig.6). These results indicate that the autistic children are suffered from universal mineral deficiency.

In addition to this global mineral deficiency, some autistic children were found subjected to high accumulation of several essential and/or toxic minerals. The number and appearance rate of the individuals with

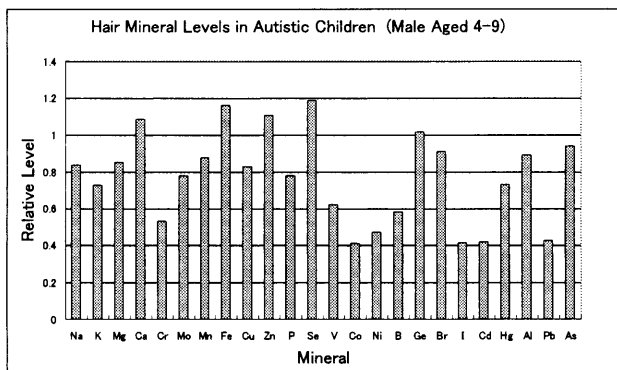


Fig. 3 Hair mineral levels in male autistic children aged 4-9 years. Each bar represents the relative level of the respective mineral in male autistic children group aged 4-9 years (N=200).

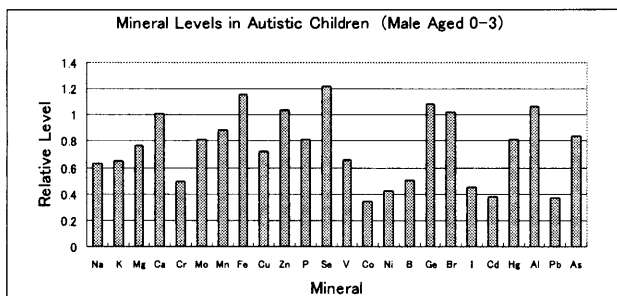


Fig. 4 Hair mineral levels in male autistic children aged 0-3 years. Each bar represents the relative level of the respective mineral in male autistic children group aged 0-3 years (N=85).

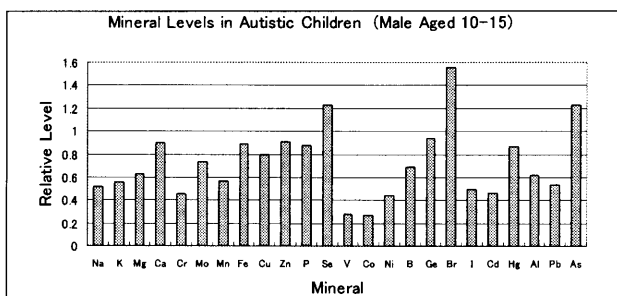


Fig. 5 Hair mineral levels in male autistic children aged 10-15 years. Each bar represents the relative level of the respective mineral in male autistic children group aged 10-15 years (N=20).

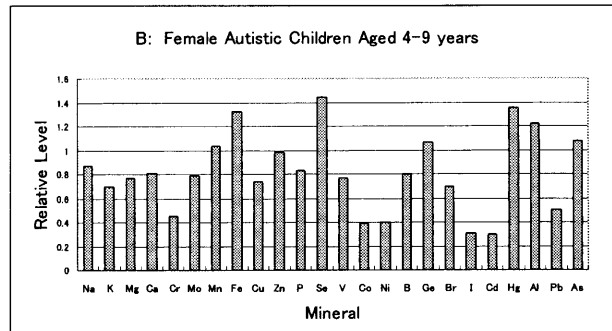
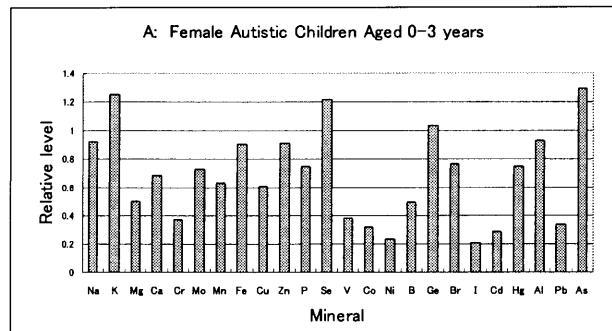


Fig. 6 Hair mineral levels in female autistic children group aged 0-3 and 4-9 years. Each bar represents the relative level of the respective mineral in female autistic children group aged 0-3 and 4-9 years (N=11 and 38, respectively).

excessively high mineral levels are summarized in Table 2. The appearance rate of the individuals with high levels of iron, aluminum, cadmium, manganese, lead, and mercury was 6.4, 2.8, 2.8, 1.9, 1.4 and 0.8 %, respectively. Some of the representative cases of high mineral accumulation are shown in Fig.7-10 The autistic child with high mercury level (Hg : 18.6 ppm vs. 2.0 ppm in control) is shown in Fig.7 The case with a characteristic of high levels of iron (50.6 ppm vs. 9.2 ppm), manganese (1.07 ppm vs. 0.23 ppm) and aluminum (49.3 ppm vs. 11.6 ppm) is shown in Fig.8 The child with high cadmium (963 ppb vs. 25 ppb) and lead (10.9 ppm vs. 0.77 ppm) is shown in Fig.9 The case shown in Fig.10 is characteristic of high sodium and potassium levels (239 ppm vs. 11 ppm and 108 ppm vs. 23 ppm, respectively).

Discussion

Recently, the pathogenic roles of some metals have been interested in various neuro-degenerative diseases [9-11]. Toxic elements such as mercury, lead, aluminum or cadmium were known to cause some fraction of neuro-developmental disabilities [13,14]. A mercury-contained preservative in vaccines, thimerosal, has been considered to be a risk factor for the development of autism [8], although there are some

Table.2 Rate of the individuals with high mineral levels in autistic children.

Mineral	High Level	Number #	Rate (%)	Maximum
Fe	> 20 ppm	23	6.39	50.6 ppm
Al	> 30 ppm	10	2.78	76.6 ppm
Cd	> 150 ppb	10	2.78	963 ppb
Mn	> 800 ppb	7	1.94	5728 ppb
Cr	> 300 ppb	5	1.39	1680 ppb
Pb	> 3000 ppb	5	1.39	10910 ppb
As	> 150 ppb	5	1.39	340 ppb
Cu	> 40 ppm	4	1.11	84.8 ppm
Hg	> 15 ppm	3	0.83	23.6 ppm
Na	> 100 ppm	3	0.83	284 ppm

#: Number of the individuals with excessively high mineral level in autistic children (N=360)

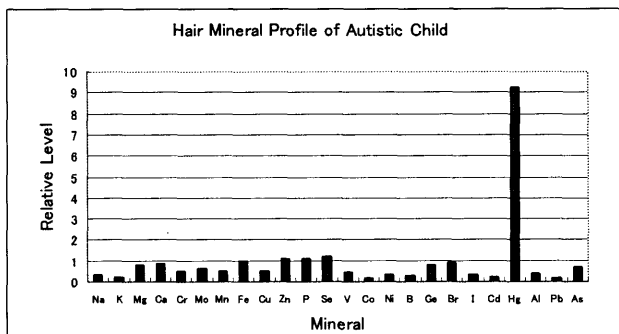


Fig. 7 Hair mineral profile of an autistic child with high mercury level. Each bar represents the relative level of the respective mineral in a male autistic child aged 3 years old.

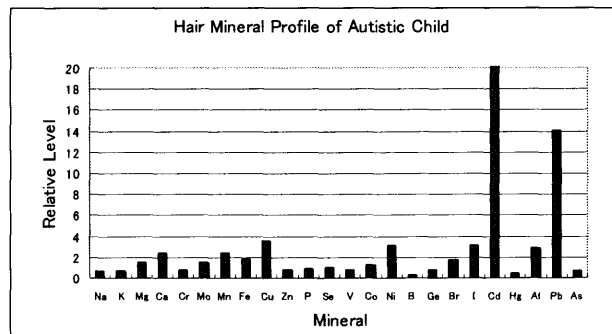


Fig. 9 Hair mineral profile of an autistic child with high cadmium and lead levels. Each bar represents the relative level of the respective mineral in a male autistic child aged 4 years old.

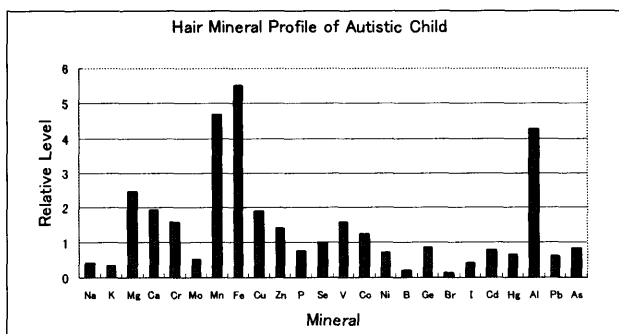


Fig. 8 Hair mineral profile of an autistic child with high iron, manganese and aluminum levels. Each bar represents the relative level of the respective mineral in a male autistic child aged 5 years old.

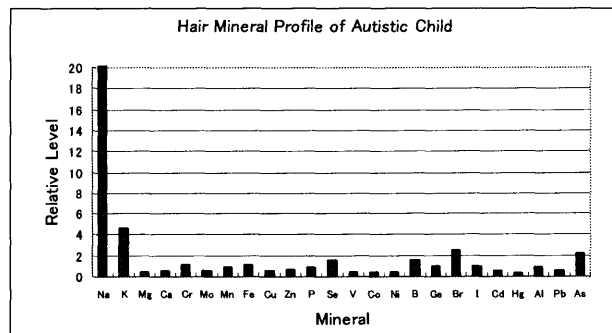


Fig. 10 Hair mineral profile of an autistic child with high sodium and potassium levels. Each bar represents the relative level of the respective mineral in a male autistic child aged 3 years old.

controversial reports on the role of mercury in autism [15-17]. On lead and cadmium, several study teams reported the exposure to these toxic metals in the children with autism or learning disorder [18-21]. Whereas, Shearer et al. [22] and Wecker et al. [23] reported that the hair cadmium levels in autistic children were lower than those in the control children.

In this study, we measured the 24 mineral levels of the hair samples from more than 3 hundreds of chil-

dren with autistic disorders, and demonstrated that they are suffered from mineral imbalance with global mineral deficiency. That is, in the autistic groups, the hair levels of various minerals including essential and toxic metals were markedly lower than those in the control children. Especially their cobalt, chromium, molybdenum, iodine and phosphorus levels were high significantly lower than those of the control children.

Patients with autism appear to have a defect in

serotonin metabolism. If the defect is due to a decrease in tryptophan hydroxylase activity (a tetrahydrobiopterin [BH4] dependent Fe-enzyme), supplementation with folic acid, ascorbic acid and vitamin B12 may increase the activity of tryptophan hydroxylase by increasing BH4 [24]. Cobalt is the central element of vitamin B12 that is an essential vitamin required for nucleotide synthesis and DNA duplication. Thus, the deficiency of B12 induces not only anemia, but also decreases in cell growth and cellular activity [23,25], causing peripheral neuropathy, mental distraction, depression and even dementia [26-28].

Chromium is a component of glucose tolerance factor and plays a key role in cellular sensitivity to insulin, and so its deficiency induces the disorder of glucose metabolism, leading to cellular energy deficiency and disorder of cell activity [29]. It is suggested that chromium may prevent diabetes and heart disease.

Iodine is a key element of thyroid hormone that plays a crucial role in brain development [30,31]. In the children of hypothyroid mothers, behavioral abnormalities including hyperactivity, learning deficits and increased prevalence of depression are reported [32].

In addition to the marked-decreased essential minerals above, molybdenum, copper, phosphate, and some trace elements such as vanadium, nickel, boron and toxic metals such as cadmium and lead were also significantly decreased in the autistic groups. These results indicate the possibility that the autistic children are suffered from global mineral deficiency in their body and also in their brain tissue. Thus, global deficiency of multi essential minerals may be related to the pathogenesis of autistic symptoms.

The present findings indicate the possibility that autistic children have a common disorder in their body mineral balance, with a marked deficiency in some essential minerals. The mineral deficiency or imbalance seems to lead to the disorder of mineral-dependent metabolic pathways regulating brain energy metabolism and neurotransmitter metabolism. Thus, metabolic abnormalities due to mineral imbalance may play a role in the etiology of autism.

Arnold et al. [33] reported that children with autism appear more likely to have deficiency or lower plasma levels in essential amino acids, suggesting the possibility that some children with autism are also

deficient in the main nutrient protein. Our preliminary study also showed that autistic children have significantly low frequencies of protein intake, in comparison with the control children [34]. These findings may give some reason why a dramatic reduction in autistic symptoms was brought about by treatment with diet or nutritional supplements [7]. It is known that many autistic children have food dislike. Therefore, there is considered the possibility that the deficiency in minerals and amino acid may be secondary event due to food dislike. Another possible origin of such deficiency is the intestinal lesion of autistic children [6,7]. The relationship between intestinal abnormalities and autistic disorders is considered [35].

The present study suggests that autism is not due to accumulation of neuro-toxic metals such as mercury or lead, rather maybe due to global and severe deficiency in essential minerals such as cobalt, chromium, molybdenum and iodine etc., that leads to the following disorder of brain metabolism. In addition to this global mineral deficiency, high accumulation of several essential and/or toxic minerals was observed in some autistic individuals. Namely, multiple accumulations of iron and manganese, iron and aluminum, cadmium and lead, cadmium and copper, sodium and potassium or mercury etc. were observed (Fig.7-10). This variation in multiple accumulations may make up some sub-groups of autism with different symptoms. These findings suggest the possibility that the autistic children are classified to some groups, based on their mineral accumulation profile in hair.

In conclusion, we have demonstrated that the children with autistic disorder have a global deficiency in various bio-elements including essential minerals, which probably leads to the disorder of mineral-dependent metabolic pathways in whole body and also brain. These findings may provide some evidence for any molecular and cellular interaction between the mineral disorder and the autistic features. The exact mechanism remains to be established how the mineral imbalance has a relevance to the pathogenesis of behavior disorders.

Importantly, the present study suggests the possibility that the improvement of mineral imbalance in the autistic children may lead to improve their symptoms/ behavior and quality of life. The profile of hair mineral imbalance may be of use as a diagnostic tool for the classification of children with autism and also for the treatment/follow up of them.

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