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Hair Mineral and Trace Element Content in Children with Down's Syndrome

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Abstract
The objective of the present study was to assess the level of minerals and trace elements in 40 children with Down's syndrome and 40 controls aged 1–2 years old. Hair mineral and trace element analysis was performed using inductively coupled plasma mass spectrometry. The obtained data demonstrate that hair levels of Mg, P, I, Cr, Si, Zn, and Pb in Down's syndrome patients exceeded the respective control values by 36, 36, 93, 57, 45, 28, and 54%, whereas hair mercury was more than twofold lower in children with Down's syndrome. The observed difference in the levels of trace elements was age-dependent. In particular, in 1-year-olds, major differences were observed for essential elements (Cr, Si, Zn), whereas in 2-year-olds—for toxic elements (Hg, Pb). At the same time, hair P levels in Down's syndrome patients were 14 and 35% higher at the age of 1 and 2 years in comparison to the respective controls. Multiple regression analysis demonstrated that a model incorporating all elements, being characterized by a significant group difference, accounted for 42.5% of status variability. At the same time, only hair phosphorus was significantly interrelated with Down's syndrome status ($\beta = 0.478$; $p < 0.001$). Principal component analysis (PCA) used As, Ca, Cr, Fe, Hg, I, Mg, P, Pb, Se, Si, Sn, and Zn as predictors, with the resulting $R^2 = 0.559$. The OPLS-DA models also separated between Down's and health control groups. Therefore, 1–2-year-old patients with Down's syndrome are characterized by significant alterations of mineral and trace element status.

Keywords Trisomy 21 · Phosphorus · Mercury · Metals · Hair

Introduction

Down's syndrome (DS) is one of the most common complex genetic disorders associated with trisomy 21 [1]. A 20-year trend demonstrated a significant increase in the prevalence of Down's syndrome and other trisomies from 1990 to 2009 [2]. At the same time, certain studies revealed a decrease in the disease prevalence that may be related to the increased number of terminated pregnancies [3] due to prenatal diagnosis [4].

Medical and social value of Down's syndrome is related not only to neuropsychiatric dysfunction but also to the number of associated diseases and/or comorbidities [5]. In particular, it has been demonstrated that Down's syndrome is associated with immune deficiency [6], cardiovascular abnormalities [7] and diseases [8], and neurodegeneration including Alzheimer's disease [9] and respiratory diseases [10] that may contribute to lower life expectancy.

Down's syndrome is also associated with nutritional and metabolic disorders including obesity [11], celiac disease and thyroid disorders [12], and others. At the same time, certain studies demonstrated inadequate micronutrient intake in Down's syndrome patients [13] that may be less common in the younger patients [14].

However, data on trace element metabolism in DS are scarce. A recent meta-analysis demonstrated that patients with Down syndrome are characterized by reduced blood calcium,

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zinc, and selenium levels, whereas erythrocyte zinc and copper contents were increased [15]. At the same time, only single investigations of hair trace element levels in Down's syndrome exist, being indicative of lower manganese, calcium, and copper [16], as well as zinc [17] levels. At the same time, alteration of trace element metabolism may have a significant impact on cognitive deficiency in Down syndrome [16]. In addition, it has been demonstrated that certain toxic elements may also be associated with metabolic disturbances in Down's syndrome. In particular, excessive arsenic exposure may be associated with higher risk of DS [18]. Serum mercury was also found to be elevated in DS [19]. In addition, altered metal homeostasis in Down's syndrome may play a significant role in mental delay [17].

In contrast to the widely studied blood and urine that are indicative of rapid fluctuations of the levels of essential trace elements, hair analysis may provide an additional tool for assessment of long-term exposure [20]. Taking into account the role of minerals and trace elements in human health and cognitive performance, assessment of trace element status in Down's syndrome may be beneficial. Hypothetically, alteration of trace element status of the patients with Down's syndrome may at least partially contribute to further mental and psychic disorders.

Therefore, the objective of the present study was to assess the level of minerals (Ca, K, Mg, Na, P), as well as essential (Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, V, Zn) and toxic (Al, As, B, Cd, Hg, Ni, Pb, Sn) trace elements in children with Down's syndrome aged 1–2 years old.

Materials and Methods

The present study was performed in agreement with the ethical standards set in the Declaration of Helsinki (1964) and its later amendments. The protocol of the investigation was approved by the Institutional Ethics Committee (Yaroslavl State University, Yaroslavl, Russia). Informed consent was obtained from the parents, who were informed about the study, its objectives, and methods. All clinical procedures (examination, sampling) were performed in the presence of parents.

Down's syndrome (ICD-10: Q90) was diagnosed based on genetic analysis and neurological investigation. A total of 80 children aged 1–2 years old were enrolled in the present study: 40 children with Down's syndrome and age- and gender-matched 40 neurotypical controls. The number of boys both in the Down's syndrome and control was 27 (67.5%), being in agreement with the earlier reported gender rate (66.7% boys) in patients with Down's syndrome [21]. Considering a tight association between Down's syndrome and obesity, the control group was also body weight- and height-matched. Examination of 1–2-year-old children allowed to avoid several limitations of hair trace element analysis including hair dye, trace element-enriched haircare products, occupational exposure, as well as the impact of lifestyle factors. In turn, it was shown that the use of commercial shampoos does not significantly affect hair trace element content [22]. The following exclusion criteria were used: living in the polluted environment (near industrial sources of metal exposure); the presence of other neuropsychiatric disorders; congenital cardiovascular abnormalities; endocrine disorders (diabetes); acute traumas and inflammatory diseases.

Hair sampling was performed by a nurse using ethanol-precleaned stainless steel scissors. Trace element content of the ethanol used was regularly monitored. Only proximal parts of hair strands (0.5–1 cm) were collected in a quantity of 0.05–0.1 g. The collected samples were stored in paper envelopes till analysis.

Preparation of hair samples to trace element analysis included washing and digestion procedures. In particular, the obtained hair samples were washed with acetone and rinsed three times with double distilled water (18 MΩ·cm) obtained using an electric distiller with combined membrane set DVS-M/1HA-1(2)-L (Mediana-Filter, Russia). It has been demonstrated that the used method allows to remove exogenous contamination [23]. Afterwards the hair samples were dried on air at 60 °C. 0.05 g of washed and air-dried hair samples was introduced into Teflon tubes with concentrated HNO_3 (Sigma-Aldrich, Co., St. Louis, USA) and loaded into Berghof Speedwave 4 microwave digestion system (Berghof Products & Instruments, Germany) for high-temperature (170–180 °C—20 min) microwave digestion. After cooling the system, the obtained solutions were added to a total volume of 15 mL with distilled deionized water. The obtained samples were used for trace element analysis.

Assessment of hair minerals (Ca, K, Mg, Na, P), as well as essential (Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, V, Zn) and toxic (Al, As, B, Cd, Hg, Ni, Pb, Sn) trace elements content was performed using inductively coupled plasma mass spectrometry at NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA). Prior the analysis, the system was calibrated using standard solutions of chemical elements (0.5, 5, 10, and 50 μg/L) prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT 06484, USA). Internal online standardization was performed using 10 μg/L yttrium solution prepared from Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA). The standard was prepared on a matrix containing 8% 1-butanol (Merck KGaA, Darmstadt, Germany), 0.8% Triton X-100 (Sigma-Aldrich, Co.), 0.02% tetramethylammonium hydroxide (Alfa-Aesar, Ward Hill, MA 01835 USA), and 0.02% ethylenediaminetetraacetic acid (Sigma-Aldrich, St Louis, MO, USA). In addition, internal online standardization using yttrium-89 (Y) and rhodium-103 (Rh) Pure Single-Element Standard

(PerkinElmer Inc.) standard solutions (10 μg/L) was also performed.

Briefly, the system characteristics included plasma power, 1500 W; plasma argon flow, 18 L/min; aux argon flow, 1.6 L/min; and nebulizer argon flow, 0.98 L/min. The LoD and BEC values for the studied elements are specified elsewhere [24, 25].

Laboratory quality control was performed throughout the study (twice a day) using the certified reference material (CRM) of human hair GBW09101 (Shanghai Institute of Nuclear Research, Academia Sinica, Taipei, Taiwan, China). The recovery rates for all studied trace elements were within the limits of 85–110% for all studied elements and corresponded to the certified limits specified by the manufacturer. In addition, the laboratory is also a participant of Occupational and Environmental Laboratory Medicine External Quality Assessment Schemes (OELM EQAS).

Statistical Analysis

The obtained data were processed using Statistica 10.0 (Statsoft, Tulsa, OK, USA) and R language ver. 3.4.4 (2018-03-15) [26]. Packages dplyr 0.7.4 and tidyr 0.8.0 and built-in packaged were used for subsetting and statistics; ropls 1.8.0 for PCA and OPLS-DA and their plots. Data normality was assessed using Anderson-Darling test. As the data were not normally distributed, descriptive statistics of hair elements content included the estimation of median and the respective 25 and 75 percentile boundaries. Mann-Whitney *U* test was used for group comparison. False discovery rate (FRD) adjustment for *p* value was applied due to multiple comparisons. The difference between the group values was considered significant at $p < 0.05$.

Multiple linear regression was used for analysis of the association between Down's syndrome status and hair trace element and mineral levels. The levels of elements, being characterized by a significant group difference, were used as independent predictors, whereas Down's syndrome status (1/0) was the dependent variable.

The orthogonal projections to latent structures discriminant analysis (OPLS-DA) with two orthogonal components based on NIPALS algorithm was applied. PCA clusterization was used as a diagnostic tool for OPLS model to prove results obtained and prevent overfitting.

Results

The results of hair analysis demonstrated that the level of electrolytes was significantly affected in children with Down's syndrome (Table 1). In particular, hair levels of Mg and P in Down's syndrome patients exceeded the control values by 36%. Although there was a 14 and 17% decrease in hair Na and K content, respectively, this difference was not significant.

Table 1 Trace element and mineral content in hair of children with Down's syndrome and healthy controls

Group	Control	Down's syndrome	*p* value
Al	7.916 (5.919–14.75)	7.823 (5.726–11.90)	0.785
As	0.037 (0.026–0.049)	0.040 (0.031–0.064)	0.142
B	2.080 (1.323–2.939)	2.432 (1.595–3.112)	0.500
Ca	278.6 (180.5–344.4)	284.5 (209.7–424.9)	0.095
Cd	0.022 (0.016–0.051)	0.026 (0.020–0.043)	0.384
Co	0.010 (0.007–0.015)	0.008 (0.006–0.011)	0.361
Cr	0.156 (0.121–0.233)	0.245 (0.155–0.339)	0.019*
Cu	9.620 (8.804–13.59)	9.537 (8.505–12.23)	0.852
Fe	13.56 (8.865–20.68)	16.90 (10.56–24.10)	0.221
Hg	0.156 (0.066–0.360)	0.070 (0.045–0.160)	0.020*
I	0.611 (0.424–1.508)	1.176 (0.572–2.080)	0.040*
K	1056 (354.7–2194)	873.1 (478.8–1761)	0.912
Li	0.027 (0.016–0.039)	0.028 (0.016–0.046)	0.494
Mg	17.32 (14.48–23.81)	23.52 (18.44–33.70)	0.032*
Mn	0.252 (0.153–0.345)	0.241 (0.189–0.356)	0.494
Na	325.4 (158.0–854.7)	280.7 (163.5–576.5)	0.524
Ni	0.181 (0.134–0.271)	0.171 (0.119–0.238)	0.476
P	141.8 (128.4–168.0)	193.0 (167.1–204.1)	< 0.001*
Pb	0.657 (0.402–1.065)	1.011 (0.587–1.738)	0.010*
Se	0.400 (0.313–0.460)	0.419 (0.353–0.475)	0.229
Si	19.24 (12.52–22.70)	27.94 (15.65–38.42)	0.001*
Sn	0.319 (0.162–0.596)	0.387 (0.254–0.538)	0.162
V	0.022 (0.014–0.047)	0.023 (0.015–0.045)	0.935
Zn	84.44 (52.55–125.5)	116.6 (71.18–189.0)	0.010*

Data presented as median (25–75 percentiles); *significant group difference at $p < 0.05$ (Mann-Whitney *U* test)

Of toxic hair trace element levels, only hair mercury was more than twofold lower in children with Down's syndrome as compared to the control values. At the same time, the patients were characterized by a significant 54% elevation of hair lead levels. Despite a 21% increase in hair tin levels, the observed difference was not significant.

It has been also demonstrated that hair levels of essential elements were altered in Down's syndrome. In particular, the examined patients were characterized by a 93, 57, 45, and 28% increase in hair I, Cr, Si, and Zn levels, as compared to the control values, respectively. The observed 25% increase in hair iron levels in children with Down's syndrome was not statistically significant.

Adjustment for age (Table 2) indicated that the observed difference in the levels of trace elements was age-dependent. In 1-year-old examinees, major alterations were observed for essential elements. In particular, the level of hair Cr, Si, and Zn was increased in Down's syndrome patients by 32% ($p =$

Table 2 Age-dependent difference in hair trace element content in children with Down's syndrome and neurotypical controls

Group	Control		Down's syndrome	
Age	1 year old	2 years old	1 year old	2 years old
Al	7.870 (6.125–16.04)	7.962 (4.386–12.70)	9.146 (6.629–15.10)	7.471 (5.329–10.28)
As	0.037 (0.026–0.049)	0.037 (0.023–0.049)	0.036 (0.030–0.058)	0.048 (0.037–0.068)
B	2.080 (1.718–2.991)	1.965 (1.037–2.883)	2.381 (1.310–3.814)	2.436 (1.709–2.772)
Ca	275.5 (185.7–347.1)	281.8 (170.2–316.6)	279.9 (207.8–428.3)	285.9 (211.4–421.1)
Cd	0.019 (0.016–0.040)	0.026 (0.017–0.053)	0.025 (0.015–0.041)	0.030 (0.022–0.063)
Co	0.011 (0.007–0.017)	0.009 (0.005–0.013)	0.008 (0.006–0.011)	0.009 (0.006–0.011)
Cr	0.154 (0.128–0.215)	0.196 (0.114–0.244)	0.257 (0.182–0.393)*	0.181 (0.131–0.285)
Cu	9.567 (8.935–14.02)	9.674 (8.739–11.69)	9.136 (8.486–10.61)	11.31 (9.503–13.56)
Fe	13.73 (9.162–21.28)	13.39 (8.963–17.46)	17.99 (12.85–26.06)	12.29 (8.933–19.34)
Hg	0.088 (0.054–0.329)	0.216 (0.100–0.336)	0.078 (0.052–0.189)	0.052 (0.039–0.098)
I	0.983 (0.401–1.568)	0.542 (0.428–0.900)	1.168 (0.753–2.276)	1.195 (0.493–1.696)†
K	1379 (727.2–2344)	428.0 (142.2–2056)	805.3 (455.8–1679)	873.6 (637.2–1778)
Li	0.025 (0.016–0.038)	0.028 (0.016–0.038)	0.022 (0.016–0.043)	0.033 (0.020–0.052)
Mg	17.47 (14.66–22.91)	16.51 (14.04–32.09)	21.31 (17.90–30.81)	26.35 (19.12–37.03)
Mn	0.253 (0.145–0.359)	0.225 (0.154–0.341)	0.261 (0.198–0.372)	0.231 (0.178–0.318)
Na	420.1 (245.5–857.7)	205.1 (111.3–383.3)	240.3 (162.3–507.8)	329.5 (177.6–633.8)
Ni	0.249 (0.150–0.349)	0.160 (0.123–0.185)	0.190 (0.148–0.276)	0.147 (0.114–0.191)
P	141.8 (134.4–170.9)	142.9 (122.4–162.8)	192.9 (163.3–202.0)*	193.1 (178.6–225.5)†
Pb	0.688 (0.533–1.125)	0.460 (0.365–1.050)	0.892 (0.583–1.553)	1.079 (0.662–1.723)†
Se	0.371 (0.270–0.455)	0.411 (0.323–0.503)	0.405 (0.320–0.480)	0.449 (0.392–0.464)
Si	18.91 (12.60–22.50)	19.38 (11.03–25.25)	28.92 (16.56–38.03)*	26.23 (15.76–38.51)
Sn	0.347 (0.174–0.616)	0.308 (0.169–0.458)	0.350 (0.306–0.713)	0.462 (0.239–0.485)
V	0.022 (0.015–0.046)	0.020 (0.015–0.047)	0.024 (0.018–0.047)	0.023 (0.015–0.042)
Zn	57.12 (39.90–113.3)	93.52 (63.83–125.1)	137.5 (76.32–191.7)*	99.89 (56.45–172.3)

Data presented as median (25–75 percentiles); *significant group difference as compared to 1 year old controls at $p < 0.05$ (Mann-Whitney U test); † significant difference in comparison to 2 years old controls at $p < 0.05$ (Mann-Whitney U test)

0.011), 33% ($p = 0.004$), and 46% ($p = 0.006$) as compared to the respective control values. At the same time, no significant difference in toxic element levels was observed. Oppositely, in 2-year-olds, significant difference was observed for hair toxic elements. Particularly, hair levels of mercury were nearly fourfold ($p = 0.007$) lower in children with Down's syndrome, whereas hair Pb content was more than twofold ($p = 0.008$) higher in comparison to the control values, respectively.

It is notable that the increase in hair P content was also age-dependent, being significant in both studied groups. In particular, hair P levels in Down's syndrome patients were 14% ($p < 0.001$) and 35% ($p < 0.001$) higher at the age of 1 and 2 years in comparison to the respective control values.

Multiple regression analysis (Table 3) demonstrated that a model incorporating all minerals and trace elements, being characterized by a significant group difference, accounted for 42.5% of status variability. At the same time, only hair phosphorus was significantly interrelated with Down's syndrome status.

Table 3 Regression analysis of the association between Down's syndrome and hair mineral and trace element levels in children

Element	β	PC	p
Cr	0.158	0.206	0.080
Hg	− 0.125	− 0.165	0.163
I	0.037	0.051	0.667
Mg	0.123	0.163	0.168
P	0.478	0.527	< 0.001*
Pb	0.174	0.227	0.054
Si	0.149	0.191	0.106
Zn	0.043	0.053	0.656
R^2	0.695		
Adjusted R^2	0.425		
P for a model	< 0.001		

Down's syndrome status is used as dependent variable, whereas the level of trace elements and minerals, being significantly different between the groups, was used as predictors; PC, partial correlation; *significant at $p < 0.05$

A principal component analysis (PCA) was first performed to show a trend of intergroup separation on the score plot (Fig. 1a), in which Down's and healthy patients were visibly separated from each other. The initial model was generated using all the elements as predictors, and PCA clusterization failed. Based on x-loading graph, some predictors were excluded as generating the noise and being non-informative. The resulting clusterization uses as predictors following elements: As, Ca, Cr, Fe, Hg, I, Mg, P, Pb, Se, Si, Sn, Zn (Fig. 1a). This PCA revealed little structure within the data with the first two components accounting for 55.9% of variability.

OPLS-DA was further applied. The elements used as predictors were the same as in the resulting PCA model. Predictive performance of the model was 0.42 being indicative that trace elements alone allow to predict whether the child had Down's syndrome or not with 42% accuracy. The OPLS-DA resulting plot indicates visible separations between Down's (red) and health control (blue) groups (Fig. 1b). The loading plot (Fig. 1c) shows contributions (loadings) of each element into resulting components (axes). Location of each predictor denotes coefficient of contribution, which vary from 0 to 1, into corresponding component. It was demonstrated that P makes a greatest contribution (0.57) into separation between groups (x-axes). It is also notable that there are also two visible clusters between predictors. The upper cluster (Fe, Sn, I, Ca, Mg, Pb, Cr) contributes into separation between Down's and healthy group with coefficient from 0.1 to 0.3, which is good for the model, but at the same time, it gives from 0.3 to 0.5 into first orthogonal component, which is high for model. In total, it appears that upper cluster predictors are insignificant and so they may be excluded from the predictive model with no effect. Therefore, predictive performance calculated for the dropped model is 0.43 using only six predictors: As, Hg, Se, Si, P, Zn.

Discussion

Generally, the obtained data demonstrate that early-age children with Down syndrome are characterized by a significant increase in hair essential element and lead levels, whereas hair mercury was decreased as compared to the healthy controls. It is notable that the first study of hair trace element content in Down's syndrome patients demonstrated significantly lower levels of toxic metals as well as increased hair content of essential elements and minerals [27]. The observed disorders in trace element metabolism in Down's patients may be associated with altered metal handling. In particular, it has been demonstrated that children with Down's syndrome are characterized by altered ceruloplasmin, haptoglobin [28], as well as transferrin [29] levels. The latter as well as metal-transferrin binding was shown to be associated with metal accumulation in the brain [30] and development of dementia in DS patients [31]. Taking into account the role of hair as a minor excretory mechanism, we propose that elevated hair levels of the studied elements may be indicative of their increased excretion that may ultimately lead to deficiency. The latter may significantly contribute to cognitive (dementia) and metabolic disorders associated with DS.

The most significant associations with Down's syndrome were observed for hair phosphorus. Earlier study demonstrated a significant increase in salivary phosphorus in DS [32]. Bone pathology may provide a link between altered hair phosphorus levels and Down's syndrome. In particular, it has been demonstrated that DS is associated with reduced bone mineral density [33] and bone turnover [34], associated with trisomy 21 [35]. D hypovitaminosis being frequently observed in DS patients may also result in altered bone metabolism [36]. Taking into account the interaction between bone physiology, calcitriol, and phosphorus metabolism [37], these changes may at least partially mediate the observed alteration of hair P levels. At the same time, it has been demonstrated that both elevated serum [38] and hair [39] phosphorus levels are associated with dementia.

Zinc is essential for brain development and functioning [40, 41]. Marques et al. [42] and Lima et al. [43] revealed a significant increase in erythrocyte zinc levels, although its concentrations in plasma and urine were reduced as compared to the controls. Taken together, these data are indicative of zinc redistribution in Down syndrome. Plasma Zn levels were also found to be lower in DS patients with celiac disease [44]. At the same time, Magenis revealed higher zinc intake in DS patients [45]. However, the existing data on hair zinc levels in DS are also contradictory, being indicative of both increase [46] and decrease [17]. Koc et al. (2015) observed a significant increase in hair Zn levels in Alzheimer's disease patients that is pathogenetically related to DS [47]. It has been also demonstrated that altered Zn status may have a significant impact on immunity in DS [48]. In this regard, certain studies aimed at assessment of beneficial effects of Zn supplementation. It has been demonstrated that Zn supplementation may increase DNA reparation in DS patients [49]. However, certain indications of the lack of association between altered Zn metabolism and DS comorbidities exist [50].

The observed alteration of hair iodine is generally in agreement with the indications of high frequency of thyroid pathology in Down's syndrome [51], including hyperthyrotropinemia, hypothyroidism, iodine deficiency, and iodine overload [52]. Thyroid pathology was also shown to be associated with reduced cognitive performance in these patients [53]. In addition, hypothyroidism in Down's syndrome was also shown to be associated with oxidative stress and osteoporosis [54] that may also contribute to the observed alteration in phosphorus metabolism.

The clinical significance of altered chromium metabolism in patients with Down's syndrome is unclear due to a lack of the respective data. It has been also demonstrated that obesity

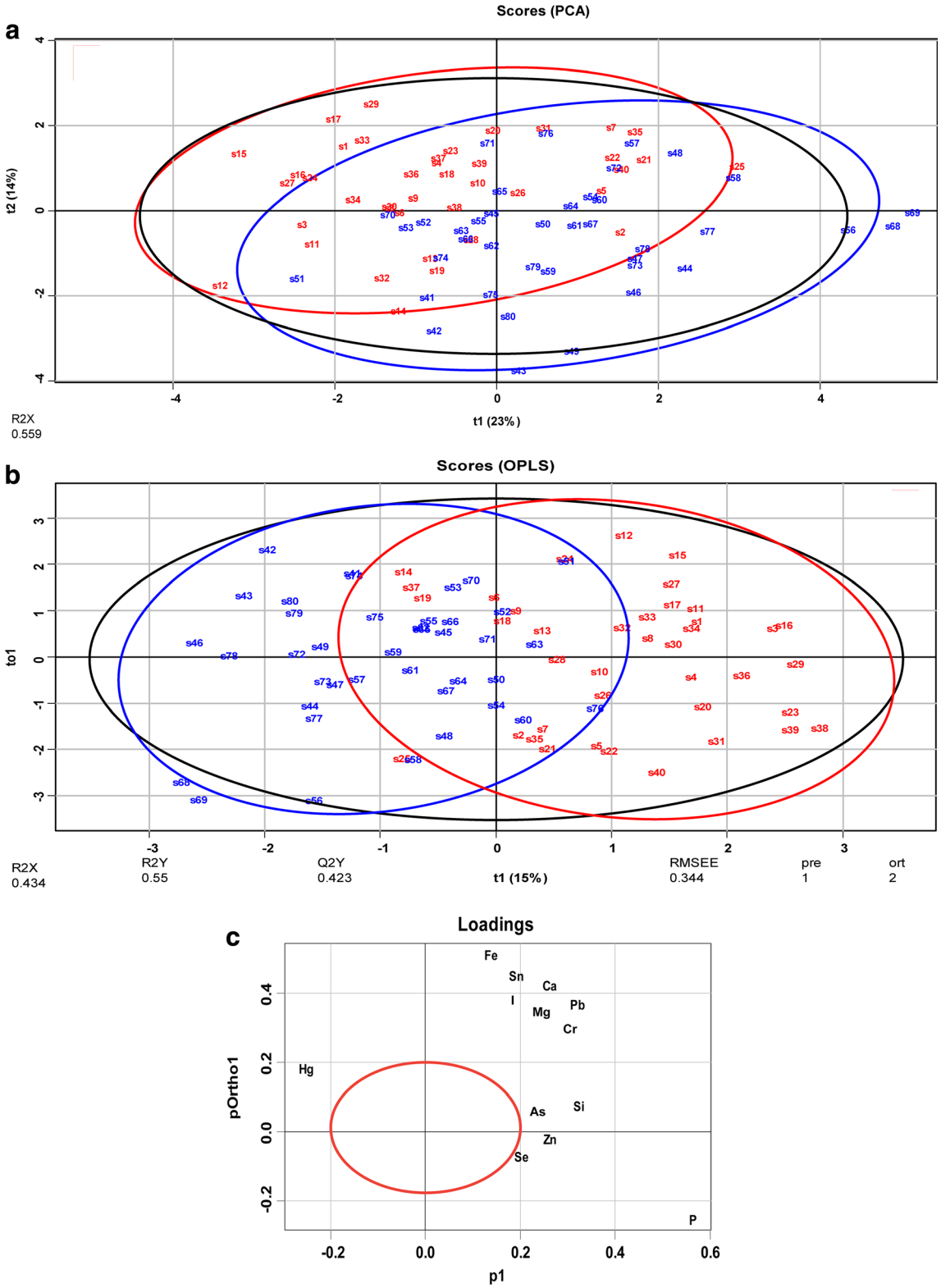

Fig. 1 PCA model and OPLS-DA models with corresponding values of R2X, R2Y, and Q2. **a** PCA score plot of healthy controls (blue) vs Down's syndrome patients (red). **b** OPLS-DA score plot of healthy controls (blue) vs Down's syndrome patients (red). **c** Loadings (contributions) of each element into the resulting components

is associated with reduced chromium status [52]. Therefore, one can propose that altered Cr metabolism in patients with DS may at least partially contribute to obesity in DS similarly to Cr deficiency-associated hyperglycemia in AD [55]. It has been also demonstrated that chromium supplementation may significantly improve cognitive function in adults with early cognitive impairment and Alzheimer's disease [56].

Although certain studies demonstrated the protective role of magnesium in brain diseases [57], no significant alteration of Mg status in patients with DS was observed in saliva [58] and serum [59]. However, significant decrease in erythrocyte and thrombocyte Mg levels was observed [60]. It can be also proposed that the increased hair Mg levels in DS patients may be indicative of increased mineral excretion, although further studies are required to justify this hypothesis.

To our knowledge, not indications of altered silicon levels in DS exist to date. Taking into account a pathogenetic association between AD and DS [9], as well as increased Al absorption in DS [61], one can propose that the interplay between aluminum and silicon may significantly contribute to DS neurology, although the hair levels of Al were unaffected in the present study. In particular, silicon administration significantly reduces aluminum absorption and retention [62]. In addition, drinking silicon-rich water resulted in a significant aluminum excretion in Alzheimer patients [63]. Aluminum was proposed to be the one of the key etiological factors in Alzheimer's disease [64], although certain studies demonstrate that hair Al levels may be normal [65] or reduced in AD. In addition, higher hair silicon levels were detected in autistic children [66].

The observed data on the level of toxic metals in hair of children with DS are of great interest. In particular, we have revealed significantly reduced levels of mercury and elevation of hair lead content. Earlier studies demonstrate that serum mercury was also found to be elevated in DS, being associated with oxidative DNA damage [19]. However, no data on hair Hg levels in DS exist to date. Earlier study by Kern et al. (2007) demonstrated that reduced hair mercury levels may be associated with reduced mercury excretion and its sequestration in brain resulting in neurotoxicity and brain disorders [67]. Mercury was also shown to be involved in pathogenesis of autism [68] that is detected in up to 42% of all patients with Down's syndrome [69]. It is also proposed that mercury plays a significant role in AD development [70], whereas patients with AD were characterized by reduced hair mercury levels [71].

Similarly, no data on the markers of lead exposure in DS exist to date. Soil lead levels were shown to be associated with intellectual disability [72]. In addition, early-life Pb exposure is associated with impaired DNA methylation and increased risk of AD [73]. In addition, perinatal lead exposure causes tau-hyperphosphorylation and cytoskeletal impairments [14] that are also implicated in Down's syndrome-associated mental disorders [74].

At the same time, the present study has certain limitations. First, nutritional intake of trace elements and minerals in the studied children was not assessed to investigate, whether the present findings could be related to dietary intake or altered handling. Second, analysis of the maternal trace element and mineral status would be beneficial to study the perinatal causes of the observed differences in the case of 1–2-year-old children. Finally, serum trace element and mineral analysis could provide an additional insight into the mechanisms and severity of the observed disorders.

Hypothetically, altered trace element and mineral status in patients with Down's syndrome may at least partially contribute to aggravation of DS-associated neurological dysfunction as well as metabolic disturbances including obesity, osteoporosis, AD, and autism. However, the causes as well as the mechanisms of altered trace element and mineral status in patients with Down's syndrome are unclear. These suggestions may be confirmed using blood and urine analysis to assess the balance of the studied elements.

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Compliance with Ethical Standards

The present study was performed in agreement with the ethical standards set in the Declaration of Helsinki (1964) and its later amendments. The protocol of the investigation was approved by the Institutional Ethics Committee (Yaroslavl State University, Yaroslavl, Russia). Informed consent was obtained from the parents, who were informed about the study, its objectives, and methods. All clinical procedures (examination, sampling) were performed in the presence of parents.

Conflict of Interest The authors declare that they have no conflict of interest.

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