



Study of Polymorphism in the BDNF Gene and its Relationship to Growth Hormone Levels in Children with Autism Spectrum Disorder in Anbar Governorate

1.Haneen Fadhil Jasim

2.Dr. Samir Mishrif Khalaf

3.Hussein Ali Jasim

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- 1- Graduate student in the College of Education for Pure Sciences
- 2- Lecturer in the College of Education for Pure Sciences
- 3- Doctor of Pediatrician in Anbar Health Department

Abstract:

Autism spectrum disorder is a condition associated with brain development that affects how a person recognizes and treats others on a social level, causing problems with interaction and social communication. The disorder also involves limited, repetitive patterns of behavior. The term "spectrum" in the phrase autism spectrum disorder refers to a wide range of symptoms and levels of severity.

The current study was conducted for the purpose of studying the relationship of growth hormone and some physiological factors to polymorphism in the Brain Derived Neurotrophic Factor (BDNF) gene in a sample of 40 children with autism spectrum disorder (ASD) as well as a control sample of 20 children without autism spectrum disorder for the period from 1/10/2021 to 1/12/2021.

The current study showed a significant decrease in $p \leq 0.05$ in the concentration of growth hormone in the serum of the study sample members ($0.345 (\pm 0.32)$) ng/ml compared to its concentration in the serum of the control sample individuals ($6.698 (\pm 5.05)$) ng/ml and the current study showed a significant decrease in $p \leq 0.05$ in the body mass criterion as it was found that the value of the body mass criterion in the subjects of the study sample is ($20.11 (\pm 4.53)$) compared to its value in the control sample individuals ($25.74 (\pm 7.60)$), It was also found that there was an inverse significant correlation $p \leq 0.05$ between BMI and the level of growth hormone in the serum, where the value of the correlation coefficient between the BMI in the study sample members and the level of growth hormone in the blood serum (-0.35989). The current study indicated a significant increase in $p \leq 0.05$ in the level of mercury element in the blood of the study sample members, where its concentration was ($1.13 (\pm 0.69)$) mg/kg compared to its concentration in the serum of the control sample members, where its average concentration was ($0.42 (\pm 0.22)$) mg/kg and also showed a significant correlation $p \leq 0.05$. Inverse between the concentration of growth hormone and the level of mercury in the serum of the study sample subjects where the value of the correlation coefficient (-0.18006). The results of the current study also showed a significant increase in $p \leq 0.05$ in the level of lead element in the blood of the study sample members, where its value was ($11.41 (\pm 4.973)$) mg/kg compared to its value in the blood of the control sample members ($5.227 (\pm 2.808)$) mg/kg. The results of the current study also showed a positive positive $p \leq 0.05$ significant correlation between the concentration of growth hormone and the level of lead element in the blood of the study samples, where the value of the correlation coefficient (0.267159).

Keywords: HGH , Lead , Mercury, Body Mass

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the anterior pituitary gland (Ranabir S. *et al.*,2011),(Greenwood FC. & Landon J. 1966)

The effects of growth hormone deficiency vary depending on the age at which they occur, somodifications in somatomidine can lead to a lack of growth hormone with two known mechanisms; tissue failure to respond to somatomidine, or liver failure to produce somatomidine (Ignatavicius D. & Workman L. , (2015. The main

manifestations of growth hormone deficiency in children are growth failure, short stature and delayed sexual maturity while in adults the change of somatomidine contributes to increased activity of osteochondrosis, leading to weak bones that are more susceptible to pathological fracture and osteoporosis. However, the deficiency is rare in adults, the most common cause is adenoma of the pituitary gland, and Other causes of adults include persistent childhood problem, other structural lesions or trauma, and rarely idiopathic diabetes (Molitch ME). *et al .*,2006). Several studies have been conducted to verify the importance and toxicity of trace metals, showing that metal ions such as aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), lead (Pb), selenium (Se) and zinc (Zn) It has a toxic effect on primary neurons in the hippocampus and most epidemiological studies confirm the increase in the number of cases of autism spectrum disorder associated with the increase in environmental pollution, mostly due to heavy metals, trichloroethylene or vinyl chloride. Baj *et al .*,2020)) Grochowski C. *et al .*, 2019) ,).It was also found that children with autism have elevated levels of heavy metals such as: lead, mercury and cadmium in their hair. A study confirmed the presence of high levels of lead (78%

Introduction

Autism spectrum disorder: It is a complex psychological disorder characterized by social and communication deficiencies, language impairment and special or recurrent behaviors and can be diagnosed after the age of three (Gregg *et al.*, 2008). Autism spectrum disorder is prevalent in different countries, and the Centers for Disease Control and Prevention (CDC) in the United States indicates that one in 59 children has autism in the United States of America, and studies have determined that the average prevalence of autism spectrum disorder globally is estimated at 1% (Frieden *et al.*, 2014) . The causes of the autism spectrum are not clear, but studies show that there is a strong relationship between disease and genetic factors including chromosomal abnormalities and point mutations, as the change in genetic bases , interactions between multiple genes and epigenetic factors affects gene expression, leading to the development of the autism spectrum (Rapin *et al.*, 2008), autism spectrum disorder has also been defined as a neurodevelopmental disorder that is usually diagnosed between 2 and 6 years of age and is characterized by poor social communication, repetitive behaviors, or stereotypes, and low interest in environmental stimuli *et al.*, 2013 Abdallah MW), (Pasciuto E. *et al .*,2015 .

Growth hormone (GH) or somatotropin, also known as human growth hormone (HGH) Human Growth Hormone in its human form, is a peptide hormone that stimulates growth, cell proliferation and cell renewal in humans and other animals. A type of metogin specific only to receptors in certain cell types, a protein of 191 single-chain peptides that is manufactured, stored and secreted by somatic cells within the lateral wings of



research and after the approval of all members of the study samples.

Collecting and preserving blood models

Five milliliters of venous blood of the subjects included in the study were withdrawn with a disposable syringe after sterilizing the place of withdrawal with ethanol at a concentration of 70% and then put 2.5 milliliters of the withdrawn blood in a glass tube containing anticoagulant K2_EDTA and then calmly shaken for 5 minutes and then the examination of the complete blood picture was performed, and the rest of the sample was kept in freezing until the extraction of DNA and the rest of the blood sample was placed in tubes It contains a coagulant accelerator (Gel Tube) and then the tubes were placed in the centrifuge at a speed of 3000 rpm for 10 minutes and the serum was withdrawn from other blood components and transferred to the 2 ml pendrov tubes and recorded on them the information of each sample and kept in freezing mode at a temperature of (20-C) until the tests are carried out.

Collecting, preserving and digesting hair models:

- 1- An estimated amount (3 cm) of the hair of the sick child was cut by steel scissors under sterile conditions and placed in (white tube) dedicated to each child.
- 2 - The samples were weighed separately in the sensitive scale device and recorded weights and unified with a weight (200-250 mg).
3. Samples are kept at normal refrigerator temperature until digestion is performed.
- 4- The stored samples were cut into about 0.125 cm and placed in (8ml) of concentrated nitric acid HNO₃ and mixedwell and placed on Hot plate at a temperature of 70 -85 ° C for about half an hour or until the sample is fully

and 16%), mercury (43% and 10%) and cadmium (38% and 8%) in children with autism compared to healthy children respectively At the same time, the statistical results of the research indicate a deficiency of trace elements such as zinc, manganese, molybdenum and selenium in the hair, which is related to Clearly with the occurrence of autism spectrum disorder Tabatadze T. *et al.*, (2015)). Studies suggest a strong association between the imbalance in the stabilization of rare earth metals and autism spectrum disorder Liu S. and Costa M. (2022). Recent results also suggest that the mineral profiles of autism spectrum disorder patients show many changes in for example, a deficiency of ZN, CA, FE, MG, MN and Se has been observed in addition to increasing concentrations of AL , AS , CD and Hg and Pb in hair samples of autism spectrum disorder patients. Toxic metals in patients have shown a relationship with the severity of the autism phenotype (Adams J. B et al., 2013)

Materials and methods:

Sample collection

The study samples were collected from the Prosperity Center for People with Special Needs in Anbar Governorate for a period from 1/10/2021 to 1/12/2021 and the study included two groups:

The first group included (40) samples for children with autism spectrum disorder whose symptoms are very clear to them.

The second group: The control group included (20) samples of children who do not have autism spectrum disorder and do not show any symptoms.

The information was collected for each child through the personal interview with his parents and with the director of the center and recorded in a questionnaire form prepared in advance for this purpose and according to the ethics of scientific



How it works

- 1- 50 µl of both samples and human serum references were added in drills covered with Streptavidin coated microplate antibodies
- 2- 100 µl of the hGH enzyme reagent detector was added for drilling.
- 3- The material was thrust by moving the plate in a circular motion using the Shaker vibrator for 30 seconds.
- 4- It folded the pits with a special adhesive and incubated at a temperature of 25 in the incubator for sixty minutes.
- 5- The contents of the drilling were emptied.
- 6- I washed the drill with the Automatic plate washer three times.
- 7- The drilling was dried using filter paper.
- 8- 100 µl of the Working Substrate Solution was added to the drill and incubated at a temperature of 25 for 15 minutes.
- 9- ML 50 of the Stop Solution was added and mixed on the vibrator for 20 seconds.
- 10- I read the absorption along a wavelength of 450 nm.

Calculations

The rates of photoabsorption values for each group (samples and standard control models) were calculated and then the standard curve was drawn by the absorption rate obtained from each measurement against its focus on the linear drawing sheet, where the absorption values are on the vertical axis or the x-axis and the concentrations on the horizontal axis or the y-axis, then the absorption value of each sample was dropped on the standard curve to know the concentration of the hormone .

digested and the color of the solution becomes sparkling.

5- After the complete digestion of the sample we removed it from the hot plate until it cooled at room temperature, then

we added 1 ml of hydrogen peroxide H₂O₂ at a concentration of 30% to each

sample and then returned it to the hot plate at a temperature of 42 ° C and a little later we raised the temperature to 80 ° C until the solution evaporated and reached the volume to about 2.5 ml.

6- The solution was filtered with filter paper and then transferred to a new glass bottle (Johansen A. *et al.*.,2010).

Measure height and weight and calculate the coefficient of body mass (BMI) Height & Weight Measurement and Calculation of Body Mass Index

The height and weight of patients and healthy people were measured using height measures in units of meters and weight in units of kilograms using an electronic scale, and the coefficient of body mass was calculated according to the following law:

(Bell *et al.*, 2011)

BMI= weight in kilograms/(height in meters*height in meters)

Estimation of the concentration of growth hormone in serum

The hormone in the blood serum was estimated using the ELISA technique according to the method attached to the measurement kit prepared by Monobind Inc.

Preparation of reagents

1. Wash Buffer: Prepared by diluting the concentrated washing solution by adding 1000 milliliters of distilled water.
2. Working Substrate Solution: Prepare this solution by adding equal amounts of both Substrate A and Substrate B.



complete to 200 ml and this series has lower and higher concentrations than the element to be estimated in the sample.

3- The pH solutions of the samples are acidic ranging from 4-(6.5) and this affects the rate of flow of the solution in the

sorrel and therefore the standard solutions must be acidified by adding (5-4) drops of concentrated nitric acid.

4. We start the measurement process with the atomic absorber after performing the steps above.

Calculations:

Theresults and discussion

The results of the current study showed a significant difference $p \leq 0.05$ in the concentration of growth hormone in the serum of the study sample members (0.345 (± 0.32) ng/ml) compared to its concentration in the serum of the control sample individuals (6.698 (± 5.05) ng/ml), and the reason for the significant decrease in the concentration of growth hormone in the blood serum of the study sample members may be attributed to the deficit of dopamine and the decrease in adrenaline levels in autistic patients. People with autism suffered from high levels of stress on a daily basis which leads to adrenaline deficiency because the glands deplete adrenaline and cannot keep up with the production of enough hormones to function normally, autism arises from a dysfunction of the dopamine system in the brain specifically and the imbalance is in two critical pathways in the middle brain, namely The intermediate cortical and dental cortical pathway. They strongly influence the behavioral pathways in autism and this corresponds to what I have reported *et al.*, (2020) (Arzua. While there was a study whose results were inconsistent with the results of the current study, which indicated a significant rise in growth hormone in children with autism compared to normal children (Iwata K. *et al.*, 2011) .

Measurement of the proportion of heavy elements

Determination of mercury by cold atomization method with atomic absorption device

Solutions used:

- 1- Preparation of a solution of diluted sulfuric acidosis 1:1 as an oxidizing agent
- 2- Preparation of a 10% solution of tin chloride as a reducing agent
- 3- Preparation of a series of standard solutions (0.001, 0.002, 0.004, 0.005 0.01)

How it works:

1- Add 10 ml of sulfuric acidosis to standard solutions for the purpose of oxidizing mercury in standard solutions for the purpose of making a calibrated curved to complete the volume to 150 ml 3- Take a volume of 10 ml of the sample and add 10 ml of sulfuric acidosis for the purpose of oxidizing mercury in the samples and complete the volume to 150 ml

4- Add 10 ml of tin chloride as a reducing agent for the purpose of reducing mercury and converting it into vapor for standard solutions to measure absorption 5- Add 10 ml of tin chloride as a reducing agent for samples for the purpose of making a calibrated curve to measure the absorption of samples with the atomic absorption device.

- 1- The measurement is done using the mercury system of cold ablation without flame .

Calculations:

Estimation of lead by atomic absorption method

The method included:

- 1 - Install the optimal conditions for the work of the flame atomic absorption device .
- 2 - Prepare a series of standard solutions from the standard solution 1000 mg / l for lead and this is done with a weight of 0.3196 g of lead nitrate $Pb(NO_3)_2$ and dissolve it with water distilled twice and



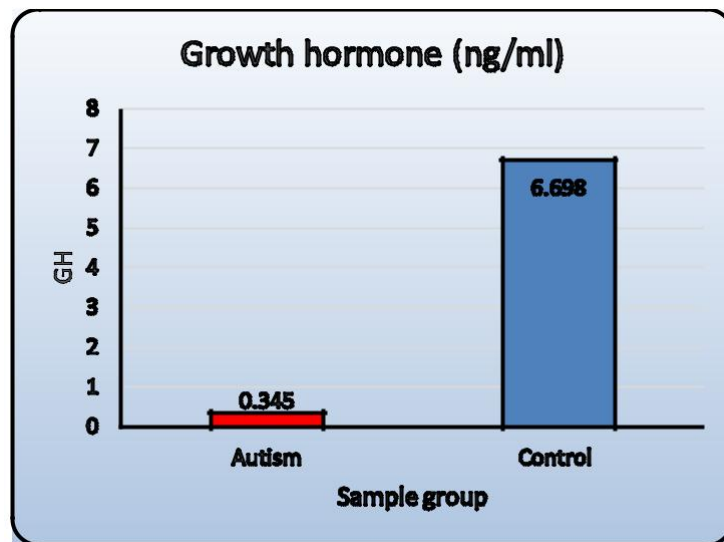


Figure (1-1) Concentration of growth hormone in the blood serum of study subjects

It was noted through the results of the current study that there is a significant decrease of $p \leq 0.05$ in the body mass criterion as it was found that the value of the body mass criterion in the subjects of the study sample is $(20.11 (\pm 4.53))$ compared to its value in the individuals of the control sample $(25.74 (\pm 7.60))$. The significant decrease in the body mass criterion among the subjects of the study sample could be explained as having a relationship with the levels of the hormone gerlene and endodontics, the decrease in their levels is attributed to the low level of growth hormone in children with autism (2002., Caminos JE). *et al*). But another study of autistic patients and normal children indicated that the only significant difference between them was body weight, which was significantly higher in the autism group (2005Matochik J. , ,).

The results also show a table indicating an inverse significant correlation $p \leq 0.05$ between BMI and growth hormone in the serum, where the correlation coefficient is -0.35989 in the study sample members and the correlation coefficient between growth hormone in the blood and BMI is -0.35989 .

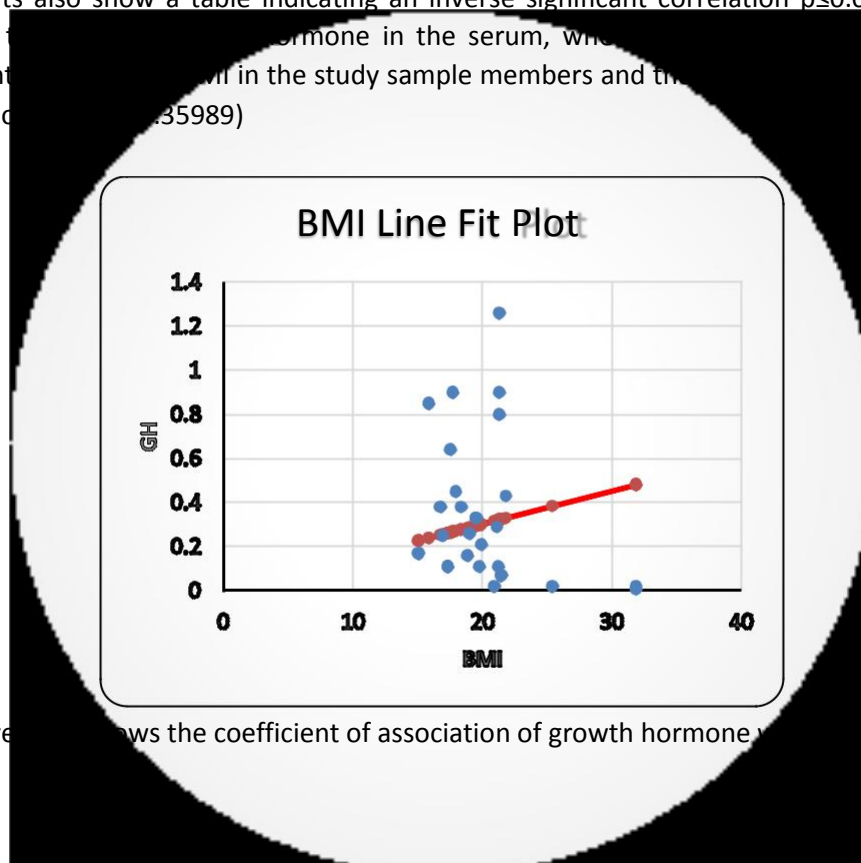


Figure 1-2 shows the coefficient of association of growth hormone and body mass

The results of the current study table (4-1) Figure (4-12) proved the presence of a significant increase in $p \leq 0.05$ in the level of mercury element in the blood of the study sample members, where its concentration reached $(1.13 (\pm 0.69) \text{ mg/kg})$ compared to its concentration in the serum of the control sample individuals, where its average concentration was $(0.42 (\pm 0.22) \text{ mg/kg})$. The presence of a significant rise in the level of mercury in the study sample can be explained by the strong relationship between toxic heavy elements such as mercury and chronic diseases such as autism (Ijomone *et al.*, 2020), the accumulation of which leads to an increase in the activity of free radicals in the body, which in turn affects the structure of the nervous system, It is noteworthy that detoxification and elimination of heavy metals involve their association with glutathione, whose concentrations are much lower in people with autism spectrum disorder. Filon *et al.*, 2020) Some studies have reported that there is no relationship between the high body load of toxic metals and the diagnosis of autism spectrum disorder and it has also been found that the patterns of secretion of heavy metals from hair among children with autism have decreased significantly compared to control and this is inconsistent with the results of our study (Kern *et al.*, 2020)

The results of the current study also showed a significant correlation of $p \leq 0.05$ inverse between the concentration of growth hormone and the level of mercury element in the blood serum of the study sample members where the value of the correlation coefficient (-0.18006)

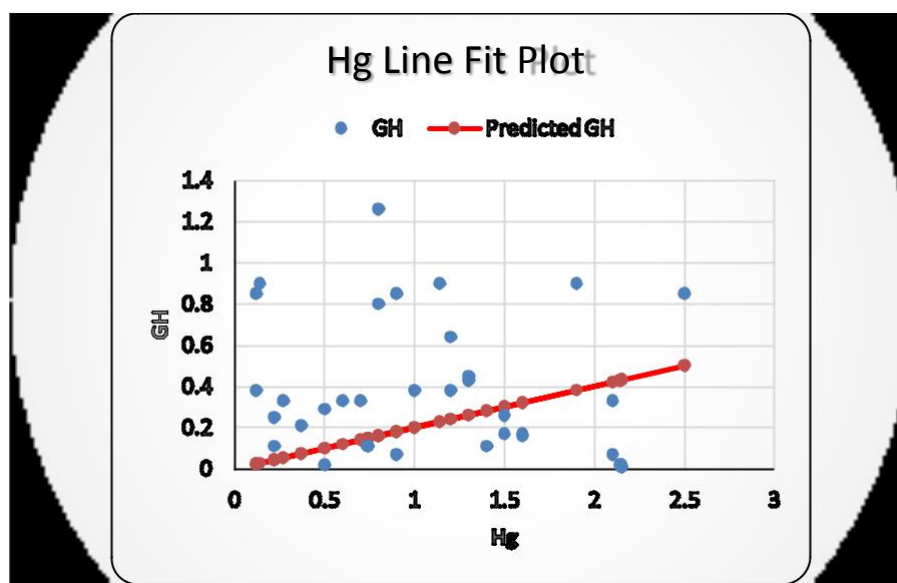


Figure (1-3) shows the coefficient of association of growth hormone with the concentration of mercury element

The results of the current study showed a significant increase in $p \leq 0.05$ in the level of lead element in the blood of the study sample individuals, where its value was $(11.41 (\pm 4.973) \text{ mg/kg})$ compared to its value in the blood of the control sample individuals $(5.227 (\pm 2.808) \text{ mg/kg})$

The presence of a significant rise in the level of lead element in the study sample can be explained by the existence of a positive relationship between lead levels in the hair and autism spectrum disorder (Goodlad *et al.*, 2013). Because lead has a strong effect on the



functions of both the central and peripheral nervous system as well as on the senses and that excessive exposure to lead to convulsions, changes in brain function, changes in the EEG as well as severe encephalopathy and other brain disorders It is highly toxic, has the ability to easily cross biological barriers and accumulates in internal organs (2013Yasuda H. *et al .*), While some studies have reported that there is no correlation between the high body

load of toxic metals including lead and the diagnosis of autism spectrum disorder , it was ⁵³⁹¹ found that patterns of heavy metal secretion from hair among children with autism were significantly reduced compared to control (Kern J.K. *et al .*, 2020. The results of the current study showed a positive $p \leq 0.05$ significant correlation between the concentration of growth hormone and the level of lead element in the blood of the study samples, where the value of the correlation coefficient was (0.267159).

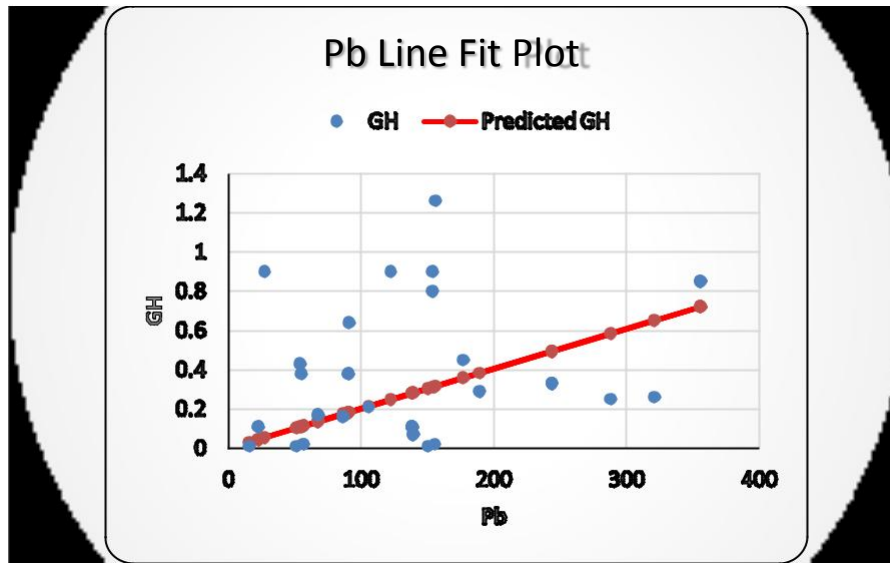


Figure (1-4) shows the coefficient of association of growth hormone with lead ratio concentration

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Conclusions

- 1- HGH has an important role to play in assessing the condition of people with autism spectrum
- 2- The autism spectrum or its negative repercussions of social isolation cause a decrease in the body mass standard.
- 3- People with autism spectrum have high concentrations of heavy elements that have a large role in the development of the condition or spectrum in the sufferer.

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