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ANALYSIS BY MATHEMATICAL MODEL OF THE ANALOGIES BETWEEN CANCER EPIGENETIC ALTERATIONS INDUCED BY HEAVY METALS AND THOSE BY COVID-19

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Abstract – *Objective:* *Here we propose a retrospective study on 319 subjects already investigated for a correlation between polymorphisms and intoxication by heavy metals, which, in some cases, lead to possible cancer alterations, in order to identify, through mathematical model, the analogies between heavy metals-induced epigenetic alterations and those caused by COVID-19.*

Patients and Methods: *A total of 319 patients, 182 males and 137 females with a median age of 44 for the former and 31 for the latter, have been studied between 2016 and 2019 by performing a hair mineralometry, analyzed with inductively coupled plasma mass spectrometry, and buccal swab followed by DNA extraction. Different polymorphisms have been investigated to find any correlations with heavy metals intoxication.*

Results: *We found a correlation between some of the genotyped polymorphisms and altered levels of heavy metals in 86% of exanimated cases. According to estimates of our mathematical models, the described genetic susceptibilities could raise up the risk of contagion for COVID-19.*

Conclusions: *The carriers of the above polymorphisms are probably more at risk of accumulating toxic metals, thereby, contracting severe oxidative damage. The mathematical model herein applied suggested us COVID-19 could use the same mechanism described for toxic metals. If these preliminary data will be confirmed, we suggest genetic screening as optimum solution to identify both cancer risk subjects and those highly exposed to virus contagion.*

KEYWORDS: *Sars-cov2, Epigenetic, Mathematical model, Heavy metals.*

INTRODUCTION

Epigenetics is the branch of genetics that study genes expression modification which does not involve alteration in the DNA sequence. As the classic DNA changes, also the epigenetic modifications are heritable, but unlike the former, they are reversible[1]. To date, the knowledge of the increasingly various epigenetic markers let us talk about an "Epigenome", a complex molecular network, distinctive for every single cell, but at the same time very plastic and susceptible to environmen-

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tal influences. The signs of the epigenetic machinery are visible since organogenesis[2,3]. A specific "epigenetic pattern" is modeled based on parental DNA information and external stimuli that come from the environment through the mother (fetal programming)[4-6]. From this point of view, the role of the environment is enormous in shaping and programming the formation of our organism in the early stages of our life, with a crucial impact on our physiological and pathological phenotype from birth to later days. Epigenetic modifications are to be considered natural and essential in determining the adaptation and evolution of the species, but the massive presence of chemical pollutants often prevents the physiological function of the epigenetic machinery through the induction of deleterious events with negative effects on human health[7,8].

The main epigenetic modification is represented by methylation, the addition of a methyl group to a cytosine, mainly in the CpG Islands. Nonetheless, in this context, the role of acetylation and other post-translational changes such as phosphorylation, ubiquitination and even aberrant mRNAs also play a leading role[9,10].

Heavy metals can affect normal epigenetics mechanisms[11]. These toxics can damage gametes genome and alter their physiological epigenetic pattern, injuring future generations[12]. Therefore, the study of heavy metals as environmental biomarkers and polymorphisms can have a central role in setting up a cancer prevention program for collective health.

PATIENTS AND METHODS

Patients

A total of 319 patients, 182 males and 137 females with a median age of 44 for the former and 31 for the latter, have been investigated from 01/02/2016 to 30/12/2019 at Nutrigene Institute by Dr Francesco di Tuoro. Informed consent was obtained from all individual participants included in the study. The following genes with some of their polymorphisms have been investigated: MTHFR, MTRR, AHCY, BHMT, IL1, IL6, IL10, SOD1, SOD2, SOD3, CAT, TNF, TLR1, TLR2, TLR9 GSTM1, GSTT1.

Methods

By self-analysis, it was performed a hair mineralometry, analyzed with inductively coupled plasma mass spectrometry, and buccal swab with the aim of collecting saliva and epithelium followed by DNA extraction. The sample was sent for the polymorphism analysis to a clinical analysis laboratory, specialized in molecular genetics. The sample must be closed in a sterile envelope, normally one or more precompiled forms are attached, in which the types of requests are specified.

Statistical analysis

For our statistical analyses we developed a "Script" (software application) which is divided in three section: (i) Data sampling (ii) Selection of the salient features (iii) Training and validation of the machine learning algorithm. The dataset used to train the developed machine learning algorithm contained 90 patients characterized by 27 concentrations of heavy metals and 25 polymorphisms. Based on this information the patients were divided in three risk classes, moderate (0), high (1) and very high (2). (i) First, by applying the SMOTE (Synthetic Minority Over-sampling TEchnique)[13] we have brought ourselves into a situation of similar population in the 3 classes of treated patients to obtain an equally represented dataset. (ii) For the second step we used Boruta, an algorithm to make feature selection[14]. (iii) Finally, the actual classification of patients with respect to the three risk classes 0, 1, 2 was made by Random Forest (RF) a multivariate classifier / regressor consisting of a set of elementary classifiers called decision trees[15]. We evaluated the effectiveness of our model by testing sensitivity and accuracy. The average accuracy obtained is equal to (63.73 ± 4.18) %. The average sensitivity values with respect to the three risk classes were: (64.65 ± 7.14) % 0, (56.44 ± 10.43) % 1, (66.37 ± 4.46) % 2.

RESULTS

The mathematical model analysis (Figure 1) allowed us to identify a correlation between the following polymorphism MTHFR, MTRR, AHCY, BHMT, IL1, IL6, SOD1, SOD2, GSTM1, GSTT1 and the present heavy metals Pb, Hg, Al, As and Cu in the 86% of exanimated cases.

In Table 1 is shown the average concentration of heavy metals in 100 patients. The concentration varies taking into account different toxic metals. The boxplot in Figure 2 denotes heavy metal with higher concentrations. In subjects which exceed normal heavy metal range, we found association with different polymorphisms. About 50% of our case population bared the homozygous "TT' vari-

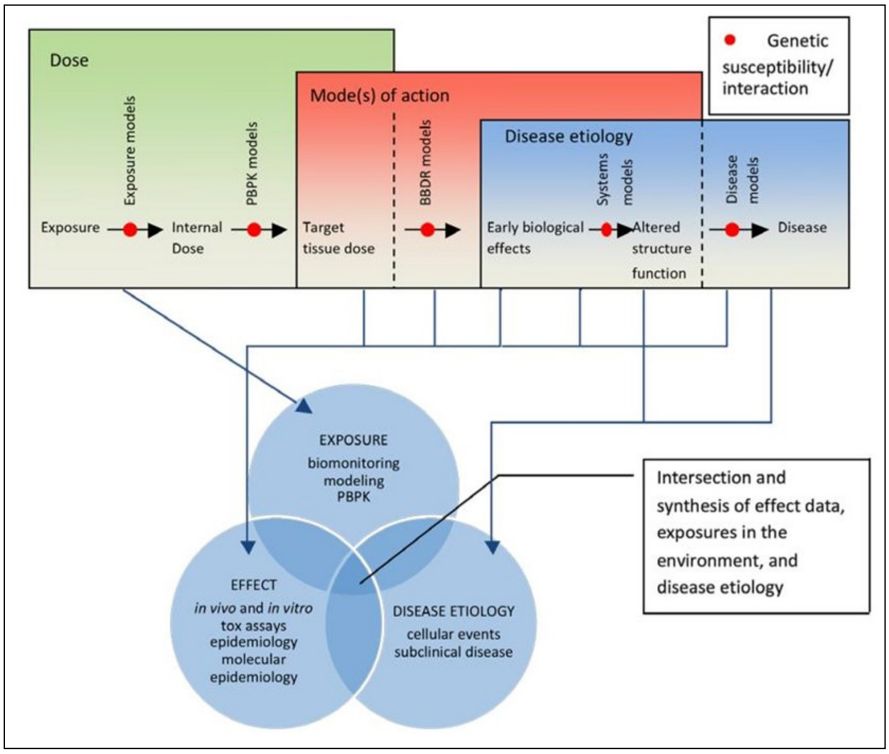

Fig. 1. Refiguration of the applied Mathematical model.

ant for MTHFR C677T polymorphisms or the homozygous "CC" variant for A1298C polymorphisms in the same gene. Moreover, we found association with other polymorphisms. About half of the examined patients bared the TNFα 308 G/A. Polymorphisms in different interleukins (IL-6, IL-10, IL-1alfa, IL-1Beta) have been identified. More than 80% of our cohort also bared a variation in the high polymorphic GSTM1(Glutathione S-transferase Mu 1) gene (Figure 3, Table 2).

DISCUSSION

Discussion on the most representative polymorphisms

The 25% of studied populations is a carrier of the wild-type homozygous "CC" MTHFR polymorphism (and at least others 11 variant who act concurrently with one or more of them). MTHFR gene bares different polymorphisms, the main represented by C677T and A1298C. Both are known to reduce the activity of the Methylenetetrahydrofolatereductase (MTHFR) and represent an emerging risk factor for various cardiovascular diseases and other relevant clinical problem[16,17]. C677T polymorphism is broadly represented in different geographic areas (especially in Mediterranean ethnic groups). In the Caucasian population the frequency of homozygous is estimated at 4-16%, while in African and Afro-American

TABLE 1. Heavy metal average concentration.

Heavy metals	*Average concentration (ug/g)*	*Standard deviation*
Al	8.963	11.04
As	0.079	0.401
Ba	1.044	1.027
Cd	0.161	0.241
Hg	1.181	1.214
Pb	0.911	1.229
Ti	1.198	0.939
Ni	0.433	0.493
Be	0.003	0.032
Bi	0.002	0.013
Pt	0.003	0.025
Tl	0.012	0.154
Ca	733.4	465.4
Mg	102.8	117.3
Na	132.9	84.02
Zn	134.8	73.42
Cu	19.39	10.99
Se	6.454	5.914
Cr	0.241	0.137
Mn	0.248	0.244
V	0.017	0.059
Mo	0.022	0.108
B	1.067	1.025
Li	0.010	0.019
P	106.7	49.36
Co	0.073	0.146
Fe	22.55	11.89

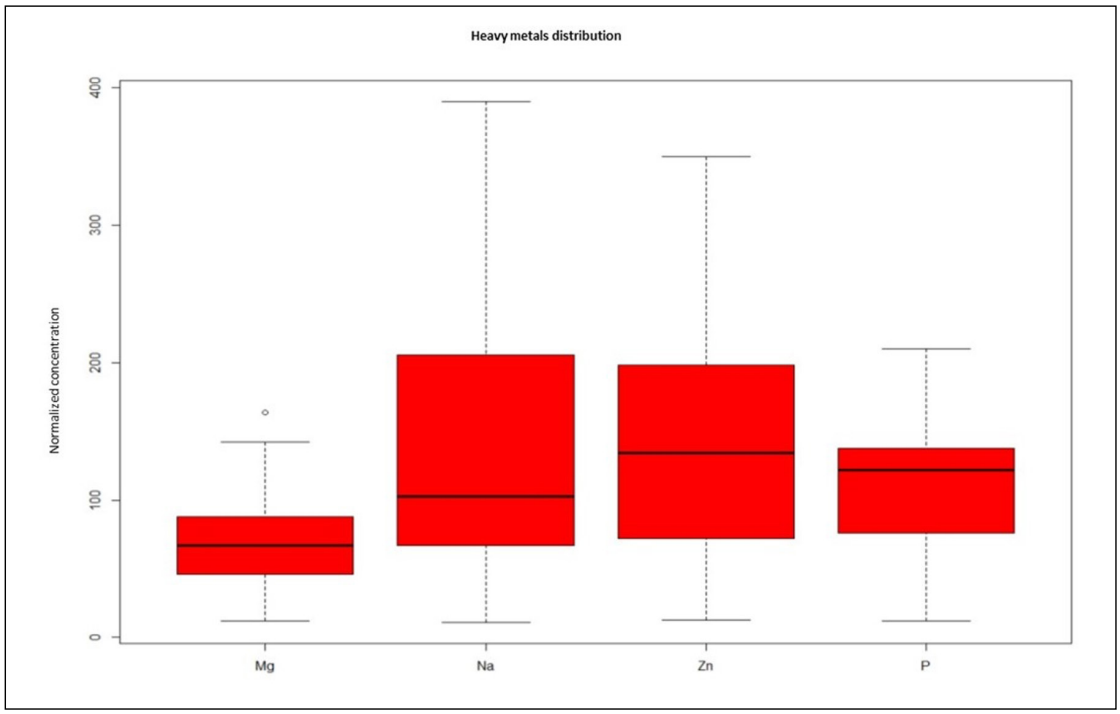

Fig. 2. Distribution of heavy metals beyond the normal range.

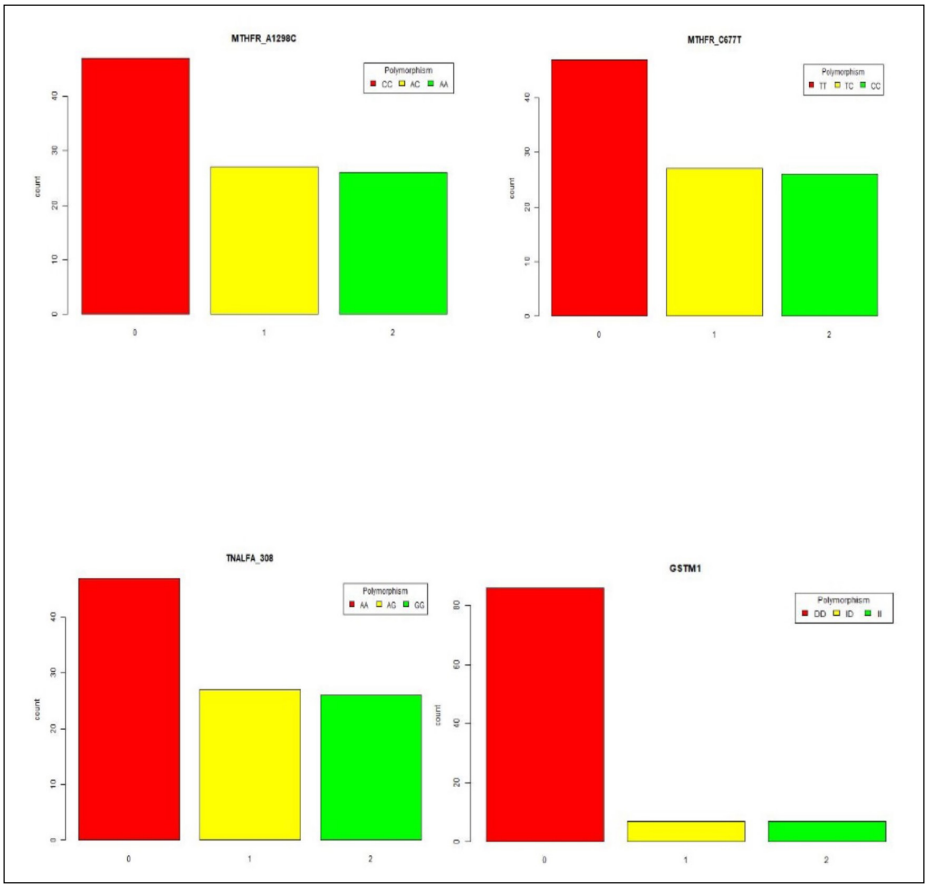

Fig. 3. Distribution of some of the polymorphisms found in patients.

population, such as in Brazil and in the USA, the frequency drops below 2% for the "TT" variant. The prevalence increases in the Mediterranean and Hispanic population, for the latter is about 21%. Finally, the prevalence is around 18% and 12% respectively for northern China and Japan. MTHFR C677T polymorphism influences the metabolic rate of the methylenetetrahydrofolate reductase and in our group its prevalence is about 50% in homozygosity and 23% in heterozygosity. Even more, also a polymorphism on Glutathione (GSH), specifically in GSTM1 gene, reported in literature as associated to a multitude of disease, was found to be associated in more than 80% of the subjects with the above-mentioned metals beyond the normal range. We also described TNFα 308 G/A, which has previously been reported in association with cardiovascular disease and Celiac Disease[18]. Furthermore, in rheumatoid arthritis it seems to predict a poor response in patients under treatment with TNF-α inhibitors[19]. Finally, we also found and shown polymorphisms in interleukins such as IL-6, IL-10, IL-1alfa, IL-1Beta, related to different pathologies such as cerebrovascular events, obesity, cancer[20-22](data not shown).

Considerations for hypothesizing the synergistic damage of metals and COVID-19 infection

In support of our hypothesis according to which COVID-19 infection is prompted by previous damage due to heavy metals, we should mention:

1. the biochemical mechanisms related to the most frequent polymorphisms a) MTHFR (D-Dimer -> Hyperhomocysteine-> Folate-> MTHFR polymorphism b) IL6 / IL10 (Viral Infections -> inflammatory pathologies -> Interleukin-6 -> genetic polymorphism G634C, G174C c) TNF associated polymorphisms, Leiden Factor V (F5), Prothrombin (Factor II), the same Methylenetetrahydrofolatereductase (MTHFR).
2. Viruses are among the most important epigenetic "starters" (already documented for EBV, CMV, HHV6, ZOSTER etc.).

TABLE 2. The table shows heavy metals with an anomalous concentration and polymorphisms found in a sample of 90 patients. The two datasets were analyzed by mathematical model to find any correlation. *I/D= Insertion/deletion.

Heavy metals beyond the normal threshold	*Gene*	*Variation/ polymorphism name*	*rs*
Al, As, Ba, Cd, Hg, Pb, Ti, Ni, Sb, Be, Bi, Pt, Tl, Th	0	CBS	C699T
	T1080C	rs234706	rs1801181
	MTR	A2756G	rs1805087
	BHMT	G742A	rs3733890
	VDR	Taq1	rs731236
	CYP1B1	Asn453Ser	rs1800440
	ACE	I/D*	rs4646994
	IL1A	G/T	rs17561
	IL1B	-511 C-T	rs16944
	IL1R	1970C/T	rs2234650
	IL6	G-634C	
	G-174C	rs1800796	rs1800795
	IL6R	Asp358Ala	rs2228145
	IL10	-1082G/A	rs1800896
	IL-4R	1902A > G	rs1801275
	MnSOD	-C28T	
	SOD2	Ala16Val	rs4880
	SOD3	C760G	rs1799895
	IFNG	874 T-A	rs2430561
	TNF-α	-308 G-A	rs1800629
	MTHFR	677T	
	A1298C	rs 1801133	rs 1801131
	AHCY	257A>G	rs773162208
	CAT	-262 C-T	rs1001179
	GSTM1	I/D	rs366631
	BHMT2		rs682985

Abbreviations: FHC = family history of cancer.

3. In virus-metal synergy, the ultrafine particulate acts as a carrier[23,24]. These indications suggest that Pm2.5-units virus bind to the epithelial surface and are subsequently internalized by cells. Once inside the cytoplasm the fine particles alter the oxidative balance, disrupt the structural organization of the cytoskeleton inducing changes in the actin and tubulin filaments, thus releasing the undisturbed virus RNA filament to replicate quickly[25].

CONCLUSIONS

The carriers of the above homozygous or heterozygous polymorphisms are probably more at risk of accumulating toxic metals and electrophilic stress damage and they also lack the ability of removing these metals from their body[26]. These subjects have a continuous oxidative storm that affect their cells (Figure 4).

The mathematical model herein applied suggested us COVID-19 could use the same mechanism described for toxic metals with a difference in quantitative terms, due to the rapid intracellular replication of the virus[27]. These could be at the base of the clinical picture that characterize the most advanced forms.

Particular attention is given to MTHFR polymorphisms (indicated by our model as the most frequent) notoriously related to a higher risk of developing venous thromboembolism.

Many studies have shown a correlation between heavy metals and venous and arterial thromboembolism. In a widespread and generalized form, it could modify the evolution of a basic pathological picture, such as an inflammation of the airways, with transformation into a systemic multi-organ pathology.

We believe that it is essential to investigate this aspect since, if confirmed, it could lead to the use of drugs which act on thromboembolic phenomena (primarily low molecular weight heparin).

We are studying possible models of analysis with the help of artificial intelligence to identify exposure scenarios and define the relevant biomarkers for the exposure-disease paradigm.

Genetic screening could be an aid, if the hypothetical data will be confirmed on a widespread range of cases, to screen polymorphisms carriers and help them understanding their risks and taking into account a series of valid options for preventive actions towards them.

Informed Consent:
The authors declare that they have no conflict of interest to disclose

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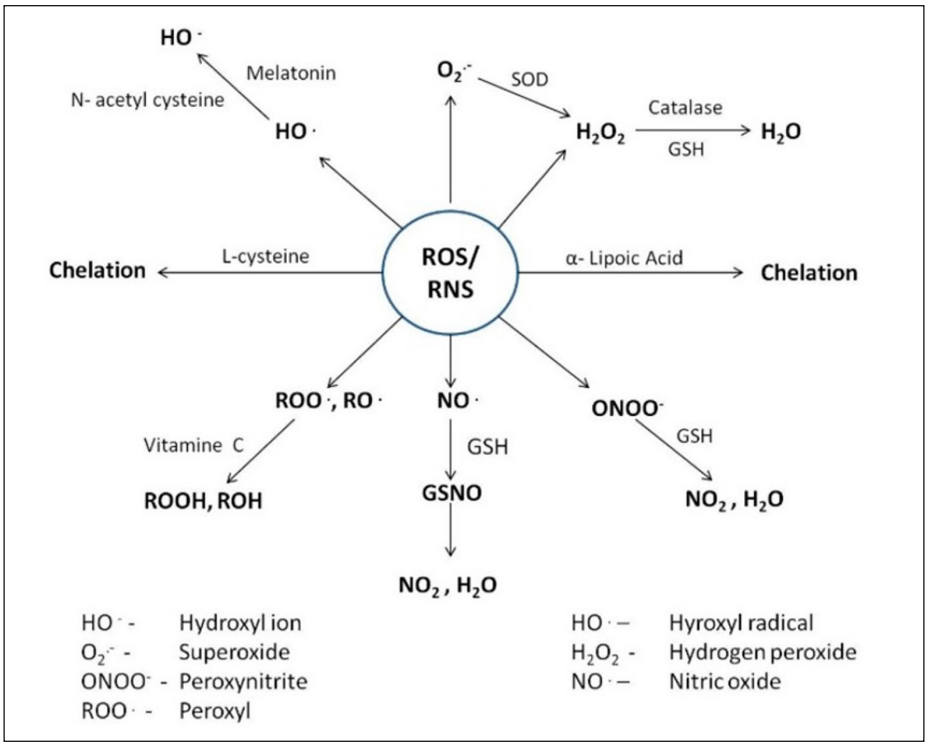

Fig. 4. Representation of the ROS (reactive oxygen species) cascade.

Ethical Committee:
Considering the retrospective nature of the study the Ethical Committee approval is not required.

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