

Review

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***Metallothionein 2A* gene polymorphisms in relation to diseases and trace element levels in humans**

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Human metallothioneins are a superfamily of low molecular weight intracellular proteins, whose synthesis can be induced by essential elements (primarily Zn and Cu), toxic elements and chemical agents, and stress-producing conditions. Of the four known isoforms in the human body MT2 is the most common. The expression of metallothioneins is encoded by a multigene family of linked genes and can be influenced by single nucleotide polymorphisms (SNPs) in these genes. To date, 24 SNPs in the *MT2A* gene have been identified with the incidence of about 1 % in various population groups, and three of them were shown to affect physiological and pathophysiological processes. This review summarises current knowledge about these three SNPs in the *MT2A* gene and their associations with element concentrations in the body of healthy and diseased persons. The most investigated SNP is rs28366003 (*MT2A* -5 A/G). Reports associate it with longevity, cancer (breast, prostate, laryngeal, and in paranasal sinuses), and chronic renal disease. The second most investigated SNP, rs10636 (*MT2A* +838G/C), is associated with breast cancer, cardiovascular disease, and type 2 diabetes. Both are also associated with several metal/metalloid concentrations in the organism. The third SNP, rs1610216 (*MT2A* -209A/G), has been studied for association with type 2 diabetes, cardiomyopathy, hyperglycaemia, and Zn concentrations. Metallothionein concentrations and *MT2A* polymorphisms have a potential to be used as biomarkers of metal exposure and clinical markers of a number of chronic diseases. This potential needs to be studied and verified in a large number of well-defined groups of participants (several hundreds and thousands) with a focus on particular physiological or pathological condition and taking into consideration other contributing factors, such as environmental exposure and individual genetic and epigenetic makeup.

KEY WORDS: metals and metalloids; rs28366003; rs10636; rs1610216; single nucleotide polymorphism

Pollution has been recognised as a major global health threat. Although exposure to various pollutants, including toxic metals or mixtures of environmental stressors is widespread, the development of diseases caused by direct environmental exposure is, luckily, limited. Whether the disease develops will depend on the causative agent, exposure levels and duration, the period of life when exposure occurs, age, and sex. Other factors that may contribute to the development and progression of a disease include other condition or disease, dietary habits, physical activity, medications taken, and variation in genetic susceptibility (1–3).

In the course of our continuing study of the exposure, health risks, and effects of the main toxic and essential elements lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), zinc (Zn), copper (Cu), iron (Fe), and selenium (Se), we recently came across increasing evidence of a link between the levels of these elements in the body of healthy and diseased persons and specific gene polymorphisms of metallothioneins (MTs). This motivated us to prepare an overview of the relationships between element levels and three most studied single nucleotide polymorphisms (SNPs)

of the *MT2A* gene, namely rs28366003, rs1610216, and rs10636. For the purpose of this review, we searched PubMed database for articles indexed until the end of 2019 using this keyword combination: metallothionein AND polymorphism AND human. The query yielded 113 matches, and the first article on specific *MT2A* polymorphism was from 2005. We excluded 13 review articles, two letters to the editor, and 62 articles dealing with SNPs other than *MT2A*. This review has no intention whatsoever to present these gene polymorphisms as either the main or only contributing factors to element levels in the human body or to the development of chronic diseases, including malignancies, nor does it go into detail of the reported studies. Instead we compare their findings in a series of tables and comment on the relationships between rs28366003 and toxic metal levels in the human body only where we compare them with our own findings (4).

BIOLOGICAL SIGNIFICANCE OF METALLOTHIONEINS

Metallothioneins are a superfamily of cysteine-rich, intracellular, metal-binding proteins present in plants, vertebrates, invertebrates, eukaryotes, and prokaryotes.

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Historically, the discovery of MTs has closely been related to the study of Cd. The earliest work in this area was reported in 1941 by Maliuga, whereas the data on Cd-binding protein isolated from equine renal cortex, later named *metallothionein*, were first reported by Margoshes and Vallee in 1957 (reviewed in 5–9). Since then, MT has been of great interest in many scientific disciplines, including toxicology, biological and physical chemistry, molecular biology, and various clinical and cancer studies with about 10,000 published papers (7–9). The characteristics of MTs are low molecular mass of 6–7 kDa, high cysteine content (about 30 %), no aromatic amino acid, and high binding affinity for metals, particularly for Zn, Cu, and Cd. The amounts and ratios of metals bound by the thiol (–SH, mercaptide) group will depend on the tissue; human liver MTs mostly contain Zn and small amounts of Cu, while renal cortex MTs mostly contain Cd, then Zn, and then Cu (reviewed in 5, 8–12).

In mammals, including humans, there are four main groups of MTs with different sequences, expression, and characteristics: MT1, MT2, MT3, and MT4. Isoforms MT1 and MT2 are expressed in almost all tissues. MT3 is expressed mainly in the brain, and to a lesser extent in the heart, kidneys, and reproductive organs (reviewed in 8–10, 13–15). MT4 is expressed in the epithelial cells of the skin and mucosa (16). MT molecules are single-chain polypeptides, which contain 61 to 68 amino acids, and 20 of them are cysteine, ordered in the sequences Cys-Cys, Cys-x-Cys, and Cys-x-y-Cys (x and y are amino acid which are not cysteine) (8–10, 17). Cysteine sulphur atoms are responsible for binding divalent metals in two clusters of MTs, connected with a sequence which does not contain cysteine. Amino acids 1 to 30 form a stable α -cluster (C-terminal) with four metal binding sites, whereas amino acids 31 to 68 form a reactive β -cluster (N-terminal) with three binding sites for divalent metals ions. Therefore, each MT molecule can bind up to seven divalent ions of Zn, Cd, Hg, and Pb, 12 of Cu, and 18 of Ag. Four metal ions first fill the α -cluster, and remaining three ions enter the β -cluster. Metals bound to the β -cluster are released more easily than metals bound to the α -cluster (8, 10, 13, 15, 18). The order of metal-binding affinities was tested by *in vitro* studies on rat liver in the 1980s and the reports are not uniform: $Cd > Pb > Cu > Hg > Zn > Ag > Ni = Co$ (19), $Hg > Cu > Cd > Zn > Ni = Co$ (20), and $Hg > Ag > Cu > Cd > Zn$ (21). Many metals have the affinity for MT but only Cu^+ , Cd^{2+} , Pb^{2+} , Ag^+ , Hg^{2+} , and Bi^{2+} can displace Zn^{2+} in MT, which was confirmed in the horse kidney *in vitro* (22) and in the rat liver *in vivo* studies (23). Exchanges between Zn and Cd happen rapidly in the β -cluster, contrary to their slow exchange in the α -cluster. Zinc readily dissociates from MT to make itself available for different biological functions and to stimulate further MT synthesis. Metallothioneins serve as metal ion donors to other ligands or proteins (reviewed in 13, 24, 25). Their degradation depends on metals bound, their distribution in MT

molecules, and the medium. Acidic media are known to speed up metal dissociation from MTs. MTs completely saturated with metals are more resistant to degradation by lysosomal proteases than unsaturated MTs or apothioneins (apo-MTs, that is MTs free of metals). In neutral media, MTs saturated with metals are less resistant to degradation than apo-MTs (25–28).

The synthesis of MTs is induced by numerous factors such as metals and metalloids, various chemical agents, including acetaminophen (paracetamol), cytokines, and many other stress-producing conditions, including oxidative stress, infection and inflammation. The peculiar chemical structure of MTs gives them their molecular stability and specificity and defines their role in various physiological and pathological conditions (8–11, 29–33). Their main biological function is to maintain the homeostasis of essential metals Zn and Cu (reviewed in 12). Studies conducted *in vitro* showed reactivation of apo-enzymes in which Zn or Cu were cofactors (alkaline phosphatase, superoxide dismutase and others) after incubation with Zn-MT or Cu-MT. The mechanism of Zn donation from MT to apo-enzyme is still unknown, but it is assumed that MT binds to an MT-releasing factor, which displaces Zn and makes it available to enzymes (8, 14). It has been shown that MTs participate in Zn regulation by intestinal absorption and excretion. When Zn intake is high, MTs may have a crucial role in restricting its absorption by storing it in the enterocytes and enabling its transfer back to the gut lumen, as confirmed by studies on knockout and transgenic mice (34–39). The induction of intestinal MT by Zn and its interaction with Cu is used in the therapy of patients with Wilson's disease with Zn acetate, a US Food and Drug Administration-approved drug. Wilson's disease is a rare autosomal recessive inherited disorder of Cu metabolism characterised by the accumulation of excessive amounts of Cu in the liver, brain, and eyes. The mechanism of Zn action as an anti-copper agent involves inhibition of both Cu absorption from the gastrointestinal tract and its transfer into the circulation by capturing the Cu-MT complex in the mucosal cell and its ultimate faecal excretion (40, 41).

Metallothioneins have multiple roles. Besides its main function to keep essential elements in balance, they protect the body against free radicals and toxic effects of metal ions (reviewed in 8–10, 13, 24, 42–46). High levels of MTs can be found in foetal and neonatal liver, but these drop to the levels found in adults during the postnatal period. Increased liver MT levels during prenatal period in all mammalian species are believed to protect against potentially toxic Zn and Cu ions before the intestinal control mechanisms develop (46–49). Another important role of MTs is to protect against oxidative stress caused by various environmental stressors, including toxic metals. Experimental studies showed lower acute hepatotoxicity of Cd due to induced MT synthesis and high Cd binding to cytosolic MT, which reduces exposure of target organelles to Cd (reviewed in 32). Studies conducted on knock out

mice showed that those without MT expression were more sensitive to Cd toxicity than control mice. The protective effects of MTs are generally clear against acute metal toxicity and carcinogenicity but not as much against chronic metal toxicity, to be addressed later in the text (8, 50–52). In general, large amounts of –SH groups in MT molecule enable reaction with numerous electrophilic chemicals, as they catch free radicals such as hydroxyl, superoxide or nitric oxide radicals produced during metabolism of xenobiotics (33, 53, 54–57).

Other important roles of MTs involve cell survival, inhibition of apoptosis, angiogenesis and vascular remodelling, and immunomodulation. Studies on human umbilical vascular endothelial cells (HUVECs) have shown that a homeodomain protein HMBOX1, which acts as a transcription factor and is abundantly expressed in the cytoplasm of the endothelial cells, maintains cell survival by promoting autophagy and inhibiting apoptosis by interaction with MT2, which increases intracellular free Zn (58). This role of MTs in vascular remodelling is important in the development of atherosclerosis and malignant tumours. Furthermore, MTs seem to inhibit pro-inflammatory cytokines, such as interleukins IL-6 and IL-12 and tumour necrosis factor TNF- α , and can therefore suppress inflammation (59). Investigations on MT-null mice showed higher susceptibility to the hepatotoxic effects of the anti-inflammatory drug paracetamol (acetaminophen), which points to the protective role of MTs against chemically induced hepatotoxicity (60, 61). The protective antioxidant role of MTs against radiation-mediated immunosuppression and cell damage was confirmed in experiments on MT-null mice (62, 63).

REGULATION OF METALLOTHIONEIN SYNTHESIS AND *MT2A* POLYMORPHISMS

Synthesis of MTs in humans is encoded by a cluster of genes located in the q13 locus of chromosome 16 (16q13). Until now, 17 genes have been identified in this cluster, and at least 11 of them are functional; eight among MT1 isoforms (*MT1A*, *MT1B*, *MT1E*, *MT1F*, *MT1G*, *MT1H*, *MT1I*/*MT1K*, and *MT1X*), and the other three have only one functional gene (*MT2A*, *MT3*, and *MT4*) (reviewed in 8–12, 15, 45, 64–67). The genes consist of two to three exons and one to two introns. Elements that control MT transcription can be divided in basal and inducible. The basal elements of gene sequence are the TATA-box, GC-box, and at least two basal level enhancer (BLE) sequences. The promoter region of the *MT1* and *MT2* genes involve inducible elements that consist of different types of responsive elements: metal response elements (MREs), glucocorticoid response elements (GREs), and antioxidant response elements (AREs). The most investigated mechanism of MT gene transcription by metal ions is via several MREs located in 5' untranslated region (UTR) of

the gene (67–69). Early studies showed that metal transcription factor 1 (MTF-1) binds to MREs in the promoter regions of MT genes via Zn finger transcription (Cys2-His2) factor controlling the expression of the *MT1* and *MT2* genes. Besides Zn, MTF-1 can be activated by reactive oxygen species, tyrosine-specific protein kinase, protein kinase C, and c-Jun N-terminal kinase (68–72).

Metal ions other than Zn can induce MT synthesis by mechanisms different than the one described above. Toxic metals cannot activate MTF-1 and, due to high binding affinity for MT, they replace Zn ions in MT molecules and thus increase intracellular Zn levels (reviewed in 13–15, 24). Free Zn then stimulates further synthesis of MT by binding to MTF-1, which then binds to MRE and ultimately has impact on metal toxicity. In other words, under conditions of acute exposure to high doses of toxic metals such as Cd or Hg, higher MT expression may reduce their toxicity. However, in chronic exposure to either of the toxic metals (Cd or Hg), increased MT synthesis leads to prolonged retention of that metal in the body, which increases the risk of toxic effects. In addition, increased MT may capture essential elements in internal organs, primarily Zn in the liver, making them less available for their physiological roles such as transfer to the developing foetus through placenta during pregnancy (reviewed in 8, 73).

Metallothionein expression can be induced by oxidative stress when generated hydrogen peroxide (H₂O₂) radicals oxidise MT, and Zn is released, which then activates MTF-1 (64). Glucocorticoids also regulate MT transcription by binding to their response elements (GREs) in the promoter region of the MT genes (74). MT expression can be also induced also by tissue hypoxia (75), catecholamines (76), or hypothermia (77).

Single nucleotide polymorphisms (SNPs) are genetic variations characterised by the replacement of one nucleotide with another in a certain stretch of DNA, which occurs in at least 1 % of the population and differs between population groups. Given their location, SNPs can either be in the coding or non-coding gene region. Those in the coding region may affect amino acid arrangement or influence protein kinetics, mRNA structure, and stability, while SNPs in the promoter region or other regulatory gene regions affect protein production (reviewed in 67, 78). According to the National Center for Biotechnology Information (NCBI) database on polymorphism, dbSNP (79), 24 polymorphisms in the *MT2A* gene have been identified in humans, three of which may affect physiological and pathophysiological processes. The most studied SNP in *MT2A* was rs28366003 (–5A/G), followed by rs10636 (+838G/C), whereas rs1610216 (–209A/G) has been the least investigated. Only Starska et al. (80, 81) and Krześlak et al. (82) studied all three *MT2A* SNPs and their associations with several malignant tumours in a Polish population.

Below we describe these and other information about *MT2A* polymorphisms with the focus on reported relationships with trace elements. We set up three sets of

interrelated tables, in which we present the following groups of data for each SNP: 1) literature data on the related genotype frequencies; 2) reported associations with human diseases; and 3) reported associations with element concentrations in humans. Of the 36 selected references, 21 deal with relationships with elements (in healthy and/diseased persons), 13 with diseases only, and two with genotype frequencies only.

MT2A polymorphism rs28366003

The rs28366003 (*MT2A* -5A/G) SNP is an A/G substitution that occurs in the core promoter region of the *MT2A* gene between the TATA box and the site where the transcription begins. As it occurs near 5'UTR, it can affect MT transcription through reduced MTF-1 binding on MRE (reviewed in 11, 67). A study on human embryonic kidney cells 293 (HEK 293) showed that substitution of the A allele with the G allele near 5'UTR reduced Cd-induced transcription. Reduced MT transcription can therefore affect element concentrations in the body and adversely affect health (46, 83).

Studies of the rs28366003 SNP were mostly conducted in Turkish and Polish populations, but several were also done in Japan and the United States, China, Thailand, Spain, and Croatia. Table 1 shows the frequencies of AA, AG, and GG genotypes reported in these studies. According to the literature data, the frequency of the AA genotype ranges from 84.0 % to 95.5 % in healthy Polish (80–82, 84–88), 86.0 % to 90.4 % in Turkish (89–94), and about 82 % in Japanese population (95, 96). The highest frequencies were found in a healthy Spanish population (97.9 %) (97), US black women (97.9 %) (98), Croatian women (93–94.0 %) (4, 99), and a healthy Chinese population (92.5 %) (100). The lowest frequency of 57.8 % and 53.4 % was found in healthy Iranian and Columbian populations, respectively (101, 102). The frequency of the AG ranged from 2.1 % in healthy Spanish population (97) and black US women (98) to 37.8 % and 43.6 % in Iranian and Columbian population, respectively (101, 102). We conducted the first study of that kind in Croatia and found that nearly 6 % of the healthy postpartum women were G allele carriers (4). Several authors reported higher percentages of G allele carriers in case study groups than controls (80, 84, 97, 100), and others reported no differences (88, 96). Higher frequency of AG genotype was reported among white (12.8 %) than black (2.1 %) women in the USA (98).

Table 2 summarises associations between the rs28366003 SNP and various clinical entities reported in literature. The associations were found for different types of cancers in the breast, prostate, paranasal sinus, larynx and stomach (31, 80–82, 84–87, 100, 101) and chronic diseases, such as type 2 diabetes mellitus, chronic kidney disease (95), and neovascular and dry forms of age-related macular degeneration (97). Several studies reported no association between rs28366003 SNP and prostate cancer

(88), type 2 diabetes mellitus (103), or sporadic amyotrophic lateral sclerosis (96).

Table 3 summarises association between rs28366003 SNP and element levels in various healthy population groups or subjects with defined disease. These findings are controversial, as a number of studies found correlations with element concentrations in the human organism (84–86, 89–91, 104) and others did not (95, 99, 102, 105, 106).

In our recent study in healthy Croatian postpartum women (4) we found no significant association between rs28366003 and either Cd or Pb concentrations in the placenta and maternal and cord blood, although stepwise multiple regression analysis showed marginal contribution of this SNP to higher placental Cd and Pb, maternal Pb, and cord blood Cd concentrations. We did find lower placental Fe in non-smoking G allele carriers (persons with AG and GG genotype) than non-smoking persons with the wild AA genotype, which surprised us at first, as Fe-MT binding has mostly been underestimated in literature (reviewed in 107). This result can be at least partly explained by the links between MT and Fe. Conditions of an acidic lysosomal-like environment created *in vitro* can stimulate MT release of Zn or Cu, which increases MT expression and facilitates Fe-MT binding (108). Through this mechanism, MT may protect from lysosomal destabilisation due to Fe overload-induced oxidative stress (reviewed in 109). In contrast to our study in Croatian population (n=268), studies in Turkish population (89, 90) reported higher Cd (n=95) and Pb (n=91) concentrations in maternal blood, higher Fe in the umbilical cord blood, lower Cd in the placenta, and no difference in placental Fe concentrations in non-smoking G allele carriers vs. persons with the wild AA genotype. However, those studies report an odd discrepancy between high blood and low placental Cd levels. Blood Cd was much higher than reported earlier in non-smoking Turkish population (110) and placental Cd was at the lower end of the scale of the overall placental Cd levels ever measured and reported in literature between 1976 and 2011 (111). These findings point to an unidentified source of Cd and/or analytical error, which may have blurred the association between rs28366003 and metal concentrations. Since studies on the association between this SNP and toxic and essential elements in mother-newborn pairs are inconsistent, further research is needed with a large number of subjects (after either spontaneous delivery or Caesarean section) with defined sources of exposure to toxic metals, including cigarette smoking and dietary habits, as they all may overcome the influence on element levels of this or the other two discussed *MT2A* SNPs, which, as a rule, have low genotype frequency at the population level. Table 3 shows that population studies of the association between rs28366003 and element levels to date have included between 100 and 700 participants. The only exception is the Japanese study (95), which included >2700 participants. More such studies with large population samples are needed.

Table 1 Genotype frequencies of the rs28366003 (*MT2A* –5A/G) single nucleotide polymorphism in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Genotype frequencies (%)		
				AA	AG	GG
Stajanko et al., 2019 (99)	Croatian	136	Pregnant women	93.0 [#]	7.0 ^s	
	Slovenian	176	Non-pregnant women	95.0 [#]	5.0 ^s	
Shokrzadeh et al., 2019 (101)	Iranian	95	Men and women with gastric cancer	46.4	41.0	12.6
		90	Control healthy men and women	57.8	37.8	4.4
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	94.0	6.0 ^s	
González-Martínez et al., 2018 (102)	Colombian	101	Men and women	53.4	43.6	3.0
Białkowska et al., 2018 (88)	Polish	197	Men with prostate cancer	90.9	9.1 ^s	
		197	Control men without prostate cancer	89.3	10.7 ^s	
Yang et al., 2017 (105)	Thai	677	Men and women	79.5	20.5	0.0
Liu et al., 2017 (100)	Chinese	459	Women with breast cancer (various types)	82.3	15.3	2.4
		549	Control healthy women	92.5	7.5	0.0
García et al., 2017 (97)	Spanish	130	Men and women with AMD	88.5	11.5	0.0
		96	Control healthy men and women	97.9	2.1	0.0
Raudenska et al., 2017 (103)	Czech	70	Men and women with type 2 diabetes mellitus	88.6	8.6	0.0
		80	Control healthy men and women	86.3	13.7	0.0
Hattori et al., 2016 (95)	Japanese	2774	Men and women	81.8	17.4	0.8
Adams et al., 2015 (104)	US	170	Premenopausal women	88.0	12.0	0.0
		151	Men and women	84.0	15.0	1.0
Starska et al., 2015 (80)	Polish	130	Men and women with SIP	75.4	23.8	0.8
		418	Control men and women without head or neck tumour	95.5	4.1	0.0
Starska et al., 2015 (84)	Polish	117	Men and women with SIP	76.1	23.1	0.8
		132	Control men and women with normal sinonasal mucosa	87.9	12.1	0.0
Starska et al., 2014 (85)	Polish	323	Men and women with SCC	89.2	9.9	0.9
		116	Control men and women with normal laryngeal mucosa	84.5	14.6	0.9
Starska et al., 2014 (81)	Polish	323	Men and women with laryngeal cancer	89.2	9.9	0.9
		418	Control healthy men and women	84.0	16.0	0.0
Krzeslak et al., 2014 (82)	Polish	534	Women with ductal breast cancer	87.1	12.3	0.6
		556	Control healthy women	92.8	7.2	0.0
Krzeslak et al., 2013 (86)	Polish	412	Men with prostate cancer	76.0	21.1	2.9
		67	Control men without prostate cancer	88.0	12.0	0.0
Wang et al., 2012 (106)	US	239	Men and women	89.1	10.1	0.8
Forma et al., 2012 (87)	Polish	358	Men with prostate cancer	76.8	20.9	2.3
		406	Control men without prostate cancer	88.9	10.6	0.5
Tekin et al., 2012 (89)	Turkish	95	Mother-newborn pairs	87.4	12.6	0.0
Tekin et al., 2012 (90)	Turkish	91	Mother-newborn pairs	86.8	13.2	0.0
Kayaalti et al., 2011 (91)	Turkish	616	Men and women	86.6	12.8	0.6
Kayaalti et al., 2011 (92)	Turkish	354	Men and women	90.4	9.0	0.6
McElroy et al., 2010 (98)	US	142	Black women	97.9	2.1	-
		149	White women	87.3	12.8	-
Kayaalti et al., 2010 (93)	Turkish	122	Men and women (kidney samples)	88.5	10.7	0.8
		186	Men and women (blood samples)	86.0	13.4	0.6
Kayaalti et al., 2010 (94)	Turkish	114	Men and women (kidney samples)	87.7	11.4	0.9
Hayashi et al., 2006 (96)	Japanese	37	Patients with SALS	75.7	24.3	0.0
		206	Control healthy men and women	82.5	17.0	0.5

n – sample size; AA – typical homozygote; AG – heterozygote; GG – atypical homozygote; AMD – age-related macular degeneration; SIP – sinonasal inverted papilloma (Schneiderian papilloma); SCC – squamous cell laryngeal carcinoma; SALS – sporadic amyotrophic lateral sclerosis; [#]A allele frequency; ^sG allele frequency; ^sG allele carriers (AG plus GG genotype)

Table 2 Association between the rs28366003 (*MT2A* -5A/G) single nucleotide polymorphism and human diseases

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	Findings
Shokrzadeh et al., 2019 (101)	Iranian	95	Men and women with gastric cancer	Leukocytes	SNP <i>MT2A</i> -5A/G increase the risk of gastric adenocarcinoma
		90	Control healthy men and women		
Bialkowska et al., 2018 (88)	Polish	197	Men with prostate cancer	Whole blood	No association was found between SNP <i>MT2A</i> -5A/G and prostate cancer
		197	Control men without prostate cancer		
Liu et al., 2017 (100)	Chinese	459	Women with breast cancer	Whole blood	SNP <i>MT2A</i> -5A/G was associated with different types of breast cancer
		549	Control healthy women		
García et al., 2017 (97)	Spanish	130	Men and women with AMD	Whole blood	AG genotype subjects had 5.5-fold higher risk for AMD; G allele was associated with dry form of AMD
		96	Control healthy men and women		
Raudenska et al., 2017 (103)	Czech	70	Men and women with type 2 diabetes mellitus	Whole blood	No association was found between SNP <i>MT2A</i> -5A/G and type 2 diabetes mellitus
		80	Control healthy men and women		
Hattori et al., 2016 (95)	Japanese	165	Men and women with DM	Serum	GG genotype associated with CKD and AG genotype with DM; no association of <i>MT2A</i> -5A/G and HT
		417	Men and women with CKD		
		2192	Healthy men and women		
Starska et al., 2015 (80)		130	Men and women with SIP		SNP <i>MT2A</i> -5A/G was related to SIP (Schneiderian papilloma); G allele increased 7.7-fold occurrence of SIP (Schneiderian papilloma); SNP <i>MT2A</i> -5A/G was associated with SIP phenotype
		418	Control men and women without head or neck tumour		
Starska et al., 2015 (84)	Polish	117	Men and women with SIP	Tissue of nasal cavities or paranasal sinuses	Heterozygotes vs. homozygotes had increased risk of SIP
		132	Control men and women with normal sinonasal mucosa		
Starska et al., 2014 (81)		323	Men and women with laryngeal cancer		AG genotype subjects had 1.6-fold higher risk for laryngeal cancer development; Association between SNP <i>MT2A</i> -5A/G and tumour aggressiveness
		418	Control healthy men and women		
Krzyszlak et al., 2014 (82)	Polish	534	Women with ductal breast cancer	Whole blood	SNP <i>MT2A</i> -5A/G was associated with ductal breast cancer
		556	Control healthy women		
Krzyszlak et al., 2013 (86)	Polish	412	Men with prostate cancer	Prostate tissue	AG genotype had higher risk for occurrence of prostate cancer
		67	Control men without prostate cancer		
Forma et al., 2012 (87)	Polish	358	Men with prostate cancer	Prostate tissue	SNP <i>MT2A</i> -5A/G was associated with prostate cancer and Gleason score
		406	Control men without prostate cancer		
Kayaalty et al., 2011 (92)	Turkish	354	Healthy men and women	Whole blood	SNP <i>MT2A</i> -5A/G was associated with longevity
Hayashi et al., 2006 (96)	Japanese	37	Patients with SALS	Whole blood	No association between SNP <i>MT2A</i> -5A/G and SALS and progression rate
		206	Control healthy men and women		

n – sample size; AG – heterozygote; GG – homozygote-atypical; *MT2A* – metallothionein 2A; AMD – age-related macular degeneration; CKD – chronic kidney disease; DM – diabetes mellitus; HT – hypertension; SIP – sinonasal inverted papilloma (Schneiderian papilloma); SALS – sporadic amyotrophic lateral sclerosis

Table 3 Association between the rs28366003 (*MT2A* -5A/G) single nucleotide polymorphism and element concentrations in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	<i>MT2A</i> genotype			Findings
					AA	AG	GG	
As concentrations (µg/g creatine)								
Stajniko et al., 2019 (99)	Croatian	136	Pregnant women	Urine	3.07*	4.58* [§]		No differences between genotypes
González-Martínez, et al., 2018 (102)	Colombian	101	Men and women	Urine	(Not available)			No association between As and SNP <i>MT2A</i> -5A/G
Cd concentrations (µg/L* or µg/kg)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	0.87±0.99*	0.73±0.60* [§]		No difference in either sample between genotypes
				Cord blood	0.06±0.03*	0.05±0.03* [§]		
				Placenta	10.1±5.1	8.80±3.70 [§]		
Hattori et al., 2016 (95)	Japanese	2774	Men and women	Serum	(Graphical illustration: Cd ≈ 0.001*)		No differences between genotypes	
Adams et al., 2015 (104)	US	321	Men and women	Urine	116±79	376±126	393	↓Cd in urine of G allele carriers
				Tissue of nasal cavities or paranasal sinuses (dry)	62.2±41.2	96.2±57.1	-	AG vs. AA genotype ↑Cd in SIP samples; no association between Cd and SNP <i>MT2A</i> -5A/G in control samples
Starska et al., 2015 (84)	Polish	132	Control men and women with normal sinonasal mucosa					
Starska et al., 2014 (85)	Polish	116	Control men and women with normal laryngeal mucosa	Tissue of laryngeal mucosa (dry)	87.2±32.2	113±26	117	AG vs. AA genotype ↑Cd in both sample types; GG vs. AA and AG vs. GG genotype ↑Cd in SCC samples
				Tissue of laryngeal mucosa (dry)	198±87	369±128	509±57	
Krześlak et al., 2013 (86)	Polish	412	Men with prostate cancer	Prostate tissue (dry)	720±330	970±460	1090±220	AG vs. AA genotype ↑Cd in both sample types; GG vs. AA genotype ↑Cd in prostate cancer samples
Tekin et al., 2012 (89)	Turkish	95	Mother-newborn pairs	Maternal blood	1.60±0.94*	2.54±2.72*	-	AG vs. AA genotype ↑Cd in maternal blood and ↓Cd in placenta
				Cord blood	0.95±0.32*	0.98±0.28*	-	
				Placenta	20.8±19.7	8.65±6.70	-	
Kayaalti et al., 2011 (91)	Turkish	616	Men and women	Whole blood	1.60±1.44*	2.09±1.85*	5.98±4.38*	G allele carriers ↑Cd
Kayaalti et al., 2010 (94)	Turkish	114	Men and women	Kidney samples (dry)	87.7±62.9†	151±60†	153†	↑Cd in G allele carriers
Pb concentrations (µg/L* or µg/kg)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	13.7±6.6*	12.0±3.6* [§]		No difference in either sample between genotypes
				Cord blood	8.3±5.5*	7.1±4.0* [§]		
				Placenta	6.9±4.9	5.5±2.8 [§]		

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	MT2A genotype			Findings
					AA	AG	GG	
Yang et al., 2017 (105)	Thai	677	Men and women	Whole blood	122±122*	105±113*	-	No differences between genotype
Tekin et al., 2012 (90)	Turkish	91	Mother-newborn pairs	Maternal blood	3.53±1.43*	5.13±2.79*	-	AG vs. AA genotype ↑Pb in maternal blood
				Cord blood	2.42±1.00*	2.94±1.49*	-	
				Placenta	7.79±2.55	9.75±4.14	-	
Krzyszlak et al., 2013 (86)	Polish	412	Men with prostate cancer	Prostate tissue (dry)	3.11±1.27†	4.66±1.82†	5.11±2.52†	GG vs. AA genotype ↑Pb in prostate cancer
					67	Control men without prostate cancer	1.67±0.61†	
Kayaalti et al., 2011 (91)	Turkish	616	Men and women	Plasma	30.1±13.9*	32.9±14.9*	50.4±11.5*	G allele carriers ↑Pb
Hg concentrations (µg/L*or µg/kg)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	13.7±6.6*	12.0±3.6**§	-	No difference in either sample between genotypes
				Cord blood	8.3±5.5*	7.1±4.0**§	-	
				Placenta	6.9±4.9	5.5±2.8§	-	
Wang et al., 2012 (106)	US	239	Men and women	Urine	1.03*	0.76*	0.34*	No difference between genotypes
				247	Men and women	440	390	
Fe concentrations (mg/L*or mg kg⁻¹)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	422±61*	418±56**§	-	G allele carriers (AG+GG) vs. AA genotype ↓Fe in placenta
				Cord blood	552±61*	539±57**§	-	
				Placenta	83±22	74±18§	-	
Tekin et al., 2012 (89)	Turkish	95	Mother-newborn pairs	Maternal blood	343±89*	373±103*	-	AG vs. AA genotype ↑Fe in cord blood
				Cord blood	271±130*	456±214*	-	
				Placenta	527±194	624±162	-	
Zn concentrations (mg/L*or mg kg⁻¹)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	5.58±0.92*	5.51±0.82**§	-	No difference in either sample between genotypes
				Cord blood	2.78±0.46*	2.58±0.53**§	-	
				Placenta	13.7±3.0	13.4±1.8§	-	
Raudenska et al., 2017 (103)	Czech	70	Men and women with diabetics	Whole blood	(Graphical illustration: Zn ≈ 3* in AG vs. ≈ 7.5* in AA)			AG vs. AA genotype ↓Zn in blood in diabetics no association between Zn and SNP MT2A -5A/G in the control samples
					80	Control healthy men and women	(Graphical illustration: Zn ≈ 5* in AG vs. ≈ 6* in AA)	
Hattori et al., 2016 (95)	Japanese	2774	Men and women	Serum	(Graphical illustration: Zn ≈ 0.850*)			No differences between genotypes
Adams et al., 2015 (104)	US	321	Men and women	Urine	(Graphical illustration)			↓Zn in urine of G allele carriers

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	MT2A genotype			Findings
					AA	AG	GG	
Starska et al., 2015 (84)	Polish	117	Men and women with SIP	Tissue of nasal cavities or paranasal sinuses (dry)	52.2±41.2	127±76	136	AG vs. AA genotype ↑Zn in SIP tissue samples;
		132	Control men and women with normal sinonasal mucosa		199±44	204±52	-	No association between Zn and SNP MT2A -5A/G in control samples
Starska et al., 2014 (85)	Polish	323	Men and women with SCC		86.4±38.1	184±57	194±74	AG vs. AA genotype ↑Zn in both sample types; GG vs. AA genotype
		116	Control men and women with normal laryngeal mucosa	Tissue of laryngeal mucosa	97.6±30.0	133±27	129	↑Zn in SCC samples
Krześlak et al., 2013 (86)	Polish	412	Men with prostate cancer		135±48	239±80	243.7±64.4	AG vs. AA genotype ↑Zn in both sample types; GG vs. AA genotype
		67	Control men without prostate cancer	Prostate tissue (dry)	485±119	927±317	-	↑Zn in cancer samples
Tekin et al., 2012 (89)	Turkish	95	Mother- newborn pairs	Maternal blood	4.33±1.13*	4.82±1.44*	-	No difference between genotypes
				Cord blood	1.32±0.55*	1.48±0.53*	-	
				Placenta	50.5±10.1	46.1±7	-	
Kayaalti et al., 2011 (91)	Turkish	616	Men and women	Plasma	1.01±0.48*	0.84±0.50*	0.39±0.33*	G allele carriers ↓Zn
Kayaalti et al., 2010 (94)	Turkish	114	Men and women	Kidney tissue (dry)	180.2±84.6	192±115	142	No difference between genotypes
Cu concentrations (mg/L* or mg kg⁻¹)								
Sekovanić et al., 2018 (4)	Croatian	268	Mother-newborn pairs	Maternal blood	1.52±0.30*	1.53±0.09*§	-	No difference in either sample between genotypes
				Cord blood	0.59±0.09*	0.58±0.12*§	-	
				Placenta	0.78±0.18	0.74±0.08§	-	
Adams et al., 2015 (104)	US	321	Men and women	Urine	(Graphical illustration)			↓Cu in urine of G allele carriers
Starska et al., 2015 (84)	Polish	117	Men and women with SIP	Tissue of nasal cavities or paranasal sinuses (dry)	24.2±13.6	27.1±11.6	26.2	No differences between genotypes in SIP tissue samples; AG vs. AA genotype
		132	Control men and women with normal sinonasal mucosa		11.0±2.98	17.2±5.2	-	↑Cu in control samples
Starska et al., 2014 (85)	Polish	323	Men and women with SCC		14.4±7.83	26.6±12.5	29.7±0.72	AG vs. AA genotype ↑Cu in both sample types;
Starska et al., 2014 (85)	Polish	116	Control men and women with normal laryngeal mucosa	Tissue of laryngeal mucosa	9.85±4.10	12.7±3.56	11.5	GG vs. AA genotype ↑Cu in SCC samples

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	MT2A genotype			Findings
					AA	AG	GG	
Krzyszlak et al., 2013 (86)	Polish	412	Men with prostate cancer	Prostate tissue (dry)	10.3±4.2	21.1±9.6	25.6±5.8	AG vs. AA genotype ↑Cu in both sample types; GG vs. AA genotype ↑Cu in cancer samples
Tekin et al., 2012 (89)	Turkish	95	Control men without prostate cancer	Maternal blood Cord blood Placenta	1.67±0.34*	1.84±0.50*	-	No difference between genotypes
Kayaalti et al., 2011 (91)	Turkish	616	Mother-newborn pairs	Plasma	0.69±0.25*	0.69±0.28*	-	No difference between genotypes
Kayaalti et al., 2010 (94)	Turkish	114	Men and women	Kidney tissue (dry)	5.90±2.59	6.63±1.73	31.9	No difference between genotypes

n – sample size; MT2A – metallothionein 2A; AA – typical homozygote; AG – heterozygote; GG – atypical homozygote; SIP – sinonasal inverted papilloma (Schneiderian papilloma); SCC – squamous cell laryngeal carcinoma; ⁶⁵G allele carriers (AG plus GG genotype); †μg g⁻¹; ‡ – increased concentration; ↓ – decreased concentration

MT2A rs1610216 polymorphism

The rs1610216 (*MT2A* –209A/G) SNP also occurs in the promoter region. Unlike rs28366003, however it has received considerably less attention and most of the studies were done in Polish population. Table 4 summarises its genotype frequencies. The frequencies of the AA, AG, and GG genotype in healthy Polish population ranged from 72.0 % to 73.9 %, 25.3 % to 27.8, and 0.2 % to 0.8 %, respectively (80–82, 87). Similar AA genotype frequencies were reported for healthy Italian population, while their GG genotype frequencies were somewhat higher, from 3.0 % to 3.7 % (112, 113). The highest AA genotype frequency of 90.5 % was reported in healthy Bulgarian population (114). The same study also reported higher percentage of the AG genotype in patients with type 2 diabetes mellitus and coronary artery disease than in healthy persons.

Table 5 shows the association between *MT2A* rs1610216 and human diseases. Studies conducted in Polish population found no association between rs1610216 and either Schneiderian papilloma or laryngeal cancer (80, 81). No associations were also reported for this SNP and breast or prostate cancers (82, 87), carotid artery stenosis and hypertension (112, 113), or coronary artery disease (114). Positive association was reported with type 2 diabetes mellitus (114) and subjects with the AA genotype ran a higher risk of ischaemic cardiomyopathy and hyperglycaemia (112). There are no data on association between the rs1610216 SNP and element concentrations in human organism, except the one study (112) dealing with an association between this SNP and Zn in plasma (Table 6).

MT2A rs10636 polymorphism

The rs10636 (*MT2A* +838G/C) SNP occurs in the 3'UTR. Genotype frequencies of this SNP are presented in Table 7. The frequencies of the GG genotype range from 42.9 % in Chinese (115) to 67.7 % in healthy Spanish population (97). Healthy Chinese population had the highest percentage of the CC genotype (9.7 %) (115), whereas the US and Polish populations had the lowest frequency (about 4.0 %) (82, 104). The only study that reported genotype frequencies of the rs10636 SNP in children was in boys and girls with the mean age of 10 years in Portugal, a country known for increased Hg intake through seafood/fish consumption and the risk of related neurotoxic effects in both sexes at young age (116).

Table 8 summarises the findings on associations between rs10636 and diseases. This polymorphism may be associated with higher incidence of neuropathy and hyperlipidaemia in patients with type 2 diabetes mellitus (115), coronary heart disease (117), and breast cancer (100). Krześlak et al. (82) found no association with ductal breast cancer. No association was also reported between rs10636 and macular degeneration related to age (97), Schneiderian papilloma, or laryngeal cancer (80, 81). Giacconi et al. (113)

Table 4 Genotype frequencies of the rs1610216 (*MT2A* –209A/G) single nucleotide polymorphism in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study population	Genotype frequencies (%)		
				AA	AG	GG
Starska et al., 2015 (80)	Polish	130	Men and women with SIP	73.8	25.4	0.8
		418	Control men and women without head and neck tumours	73.9	25.3	0.8
Starska et al., 2014 (81)	Polish	323	Men and women with laryngeal cancer	73.4	26.0	0.6
		418	Control healthy men and women	73.9	25.3	0.8
Krześlak et al., 2014 (82)	Polish	534	Women with breast cancer	76.4	23.4	0.2
		556	Control healthy women	72.3	27.5	0.2
Forma et al., 2012 (87)	Polish	358	Men with prostate cancer	71.8	27.6	0.6
		406	Control men without prostate cancer	72.0	27.8	0.2
Kozarova et al., 2012 (114)	Bulgarian	142	Patients with CAD	89.2	9.4	1.4
		101	Patients with DM	69.7	28.3	2.0
		61	Control healthy volunteers	90.5	0.0	9.5
		100	CS patients	75.0	24.0	1.0
Giacconi et al., 2007 (113)	Italian	188	CS patients without cerebrovascular episodes	73.0	25.0	2.0
		218	Control elderly volunteers	71.0	26.0	3.0
Giacconi et al., 2005 (112)	Italian	91	Men and women with carotid stenosis	86.0	14.0	0.0
		188	Control elderly men and women	70.2	26.1	3.7

n – sample size; AA – typical homozygote; AG – heterozygote; GG – atypical homozygote; SIP – sinonasal inverted papilloma (Schneiderian papilloma); CAD – coronary artery disease; DM – diabetes mellitus; CS – carotid artery stenosis

reported that in the C allele carriers carotid artery disease was more likely to progress to carotid artery stenosis.

The associations between this polymorphism and element concentrations in human organism are presented in Table 9. A weak association was reported for blood Cd in healthy women exposed to Cd (118). Although Hg was not associated with the CC genotype, a multivariate analysis indicated lower Hg in urine in subjects with the CC genotype than those with the GG genotype (106). C allele carriers were found to have lower concentrations of Cd, Cu and Zn in urine (104), Pb in blood (119, 120), and Fe in plasma (113) and higher Zn and Cu in red blood cells (113).

CONCLUDING REMARKS

There is strong evidence that MTs participate in physiological and pathological processes in the human body which involve the homeostasis of intracellular essential element, primarily Zn and Cu. They may chelate divalent toxic metals, such as Cd, Pb, Hg, or Pt with the –SH groups in cysteine and thus detoxify cells, scavenge free radicals, and protect cells against oxidative stress. They also have a role in cell survival and proliferation, angiogenesis, and inhibition of apoptosis. Emerging evidence confirms that MT insufficiency may lead to pathogenic processes and carcinogenesis. Single gene polymorphisms of MTs may be responsible for individual differences in reactions to harmful effects of external chemical and physical stressors and reactive oxygen species in the body.

Identification of individual MT isoforms in human cells and tissues can be applied in prospective tissue, plasma, and urine analyses or retrospectively, using fixed paraffin-embedded tissue samples. In the future, MTs may serve as biomarkers of environmental exposure to toxic metals, such as Cd, as already reported in biomonitoring studies on occupational exposure in humans (121–123) or environmental exposure in animals (124–126). MTs are also intensively studied as potential clinical biomarkers to be used in the diagnosis, prognosis, and selection of efficient therapy/ies for a number of malignant tumours, such as breast, thyroid, head, neck, lung, gallbladder, pancreas, colon, kidney, ovary, prostate, bone, and skin cancers, childhood solid tumours, and various types of leukaemia (29–32, 82, 86, 87, 94, 127–132). Exogenous MTs are already being investigated for the treatment of pathological processes in the central nervous system (59).

To date, the rs28366003, rs10636, and rs1610216 SNPs in the *MT2A* gene have been associated with various physiological and pathological conditions. These involve ageing and chronic diseases, such as metabolic syndrome (including type 2 diabetes mellitus and obesity), cardiovascular diseases, osteoporosis, and psychiatric disorders. They also seem to interfere with the effects of toxic drugs and pollutants. However, their use as risk predictors remains controversial. Identifying a single specific allelic variant associated with an individual trait, health or disease by gene-specific, candidate-driven studies (133) may fail to provide full information and risk assessment of certain diseases, which, as a rule, have

Table 5 Association between the rs1610216 SNP (MT2A -209A/G) single nucleotide polymorphism and human diseases

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	Findings
Starska et al., 2015 (80)	Polish	130	Men and women with SIP	Tissue of nasal cavities or paranasal sinuses	No association between SNP <i>MT2A</i> -209A/G and SIP
		418	Control men and women without head and neck tumours		
Starska et al., 2014 (81)	Polish	323	Men and women with laryngeal cancer	Tissue of squamous cell laryngeal cancer	No association between SNP <i>MT2A</i> -209A/G and development of laryngeal cancer
		418	Control volunteers (men and women)		
Krzyszlak et al., 2014 (82)	Polish	534	Women with breast cancer	Whole blood	No associations between SNP <i>MT2A</i> -209A/G and breast cancer
		556	Control healthy women		
Forma et al., 2012 (87)	Polish	358	Men with prostate cancer	Whole blood	No association between SNP <i>MT2A</i> -209 A/G and prostate cancer
		406	Control men without prostate cancer		
Kozarova et al., 2012 (114)	Bulgarian	142	Patients with CAD	Leukocytes	Positive association between G allele carriers and DM; No association between SNP <i>MT2A</i> -209 A/G and CAD
		101	Patients with DM		
		61	Control healthy volunteers		
Giacconi et al., 2007 (113)	Italian	100	CS patients	Blood	No association between SNP <i>MT2A</i> -209 A/G and CS or cerebrovascular episodes
		188	CS patients without cerebrovascular episodes		
		218	Control elderly volunteers		
Giacconi et al., 2005 (112)	Italian	91	Men and women with carotid stenosis	Whole blood	No association between SNP <i>MT2A</i> -209A/G and hypertension, higher risk of ischaemic cardiomyopathy and hyperglycaemia in AA genotype subjects
		188	Control elderly men and women		

n – sample size; AA – typical homozygote; AG – heterozygote; GG – atypical homozygote; MT2A – metallothionein 2A; DM – diabetes mellitus; CAD – coronary artery disease; SIP – sinonasal inverted papilloma (Schneiderian papilloma); CS – carotid artery stenosis

Table 6 Association between the rs1610216 (*MT2A* -209A/G) single nucleotide polymorphism and element concentrations in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	<i>MT2A</i> genotype			Findings
					AA	AG	GG	
Giacconi et al., 2005 (112)	Italian	91	Patients: elderly men and women with type 2 diabetes and carotid stenosis	Plasma	0.77±0.15	0.88±0.18 [§]	↓Zn in AA vs. AG+GG in patients; in AA genotype subjects ↓Zn in patients vs. control	
			Control: healthy elderly men and women		0.87±0.25	0.80±0.15 [§]		

n – sample size; *MT2A* – metallothionein 2A; AA – typical homozygote; AG – heterozygote; GG – atypical homozygote; §G allele carriers (AG plus GG genotype); ↓ – decreased concentration

polygenic origins (134, 135) and therefore need genome-wide association studies (136, 137). More comprehensive studies are needed to determine the role and potential for the clinical use of specific *MT2A* gene polymorphisms. These should recruit a large number of participants (several hundreds and more) with well-defined pathological process and take into account other factors and risks, such as specific environmental exposure and personal habits, genetic characteristics, and epigenetic makeup.

Conflict of interests

None to declare.

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Table 7 Genotype frequencies of the rs10636 (*MT2A* +838G/C) single nucleotide polymorphism in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Genotype frequencies (%)		
				GG	GC	CC
Yang et al., 2017 (105)	Thai	677	Men and women	52.4	41.6	6.0
Liu et al., 2017 (100)	Chinese	459	Women with breast cancer	52.5	37.5	11.8
		549	Control healthy women	52.8	40.8	6.4
García et al., 2017 (97)	Spanish	130	Men and women with AMD	56.9	36.9	6.2
		96	Control healthy men and women	67.7	27.1	5.2
Fernandes et al., 2016 (119)	Brazilian	221	Workers in car battery factories	62.0	32.0	6.0
Adams et al., 2015 (104)	US	170	Premenopausal women	54.0	42.0	4.0
		151	Men and women	62.0	34.0	4.0
Starska et al., 2015 (80)	Polish	130	Men and women with SIP	44.6	43.1	12.3
		418	Control men and women without head and neck tumours	50.9	41.2	7.9
Starska et al., 2014 (81)	Polish	323	Men and women with laryngeal cancer	45.8	46.1	8.1
		418	Control volunteers (men and women)	50.9	41.2	7.9
Yang et al., 2014 (117)	Chinese	287	Men and women with CHD	46.0	45.3	8.7
		226	Control healthy men and women	57.1	36.7	6.2
Krześlak et al., 2014 (82)	Polish	534	Women with breast cancer	57.1	38.4	4.5
		556	Control healthy women	50.3	45.5	4.2
Woods et al., 2013 (116)	Portuguese	163	Boys average age 10 years	61.3	30.7	8.0
		167	Girls average age 10 years	59.9	33.5	6.6
Chen et al., 2012 (118)	Chinese	465	Men and women	52.3	39.5	8.2
Wang et al., 2012 (106)	US	464	Men and women	54.1	36.8	9.1
Forma et al., 2012 (87)	Polish	358	Men with prostate cancer	48.9	43.3	7.8
		406	Control men without prostate cancer	52.0	40.0	8.0
Gundacker et al., 2009 (120)	Austrian	180	Men and women	58.4	33.3	8.3
Yang et al., 2008 (115)	Chinese	182	Men and women with DM	46.7	42.9	10.4
		196	Control volunteers (men and women)	42.9	47.4	9.70
		100	CS patients	73.0	22.0	5.0
Giacconi et al., 2007 (113)	Italian	188	CS patients without cerebrovascular episodes	66.0	30.0	4.0
		218	Control elderly volunteers	56.0	37.0	7.0

n – sample size; *MT2A* – metallothionein 2A; GG – typical homozygote; GC – heterozygote; CC – atypical homozygote; AMD – age-related macular degeneration; SIP – sinonasal inverted papilloma (Schneiderian papilloma); CHD – coronary heart disease; DM – diabetes mellitus; CS – carotid artery stenosis

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Table 8 Association between the rs10636 (*MT2A* +838G/C) single nucleotide polymorphism and human diseases

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	Findings
Liu et al., 2017 (100)	Chinese	459 549	Women with breast cancer Control healthy women	Whole blood	SNP <i>MT2A</i> +838 G/C was associated with breast cancer
García et al., 2017 (97)	Spanish	130 96	Men and women with AMD Control healthy men and women	Whole blood	No association between SNP <i>MT2A</i> +838 G/C and AMD
Starska et al., 2015 (80)	Polish	130 418	Men and women with SIP Control men and women without head and neck tumours	Tissue of nasal cavities or paranasal sinuses	No association between SNP <i>MT2A</i> +838G/C and SIP
Starska et al., 2014 (81)	Polish	323 418	Men and women with laryngeal cancer Control healthy men and women	Tissue of squamous cell laryngeal cancer	No association between SNP <i>MT2A</i> +838G/C and development of laryngeal cancer
Yang et al., 2014 (117)	Chinese	287 226	Men and women with CHD Control healthy men and women	Blood leukocytes	SNP <i>MT2A</i> +838G/C was associated with CHD
Krzyszlak et al., 2014 (82)	Polish	534 556	Women with breast cancer Control healthy women	Whole blood	No associations between SNP <i>MT2A</i> +838G/C and breast cancer
Yang et al., 2008 (115)	Chinese	397 454	Men and women with DM Control men and women	Whole blood	SNP <i>MT2A</i> +838G/C was associated with higher risk for hyperlipidemia and incidence of DM with neuropathy
Giacconi et al., 2007 (113)	Italian	100 188 218	CS patients CS patients without cerebrovascular episodes Control elderly volunteers	Blood	SNP <i>MT2A</i> +838G/C promote the progression of carotid artery disease to CS

n – sample size; *MT2A* – metallothionein 2A; AMD – age-related macular degeneration; SIP – sinonasal inverted papilloma (Schneiderian papilloma); CHD – coronary heart disease; DM – diabetes mellitus; CS – carotid artery stenosis

Table 9 Association between the rs10636 (*MT2A* +838G/C) single nucleotide polymorphism and element concentrations in humans

Authors and year of publication (reference No.)	Ethnicity	n	Study participants	Sample type	<i>MT2A</i> genotype			Findings		
					GG	GC	CC			
Cd concentrations (µg/L)										
Adams et al., 2015 (104)	US	321	Men and women	Urine	(Graphical illustration)			↓Cd in urine of C allele carriers		
Chen et al., 2012 (118)	Chinese	311	Women exposed to Cd	Blood/ Urine	(Graphical illustration)			Trends of ↓Cd in blood of C allele carriers in highly polluted area; no difference of Cd in urine		
Pb concentrations (µg/L)										
Yang et al., 2017 (105)	Thai	677	Men and women	Whole blood		116±119	121±121	124±141	No difference between genotypes	
Fernandes et al., 2016 (119)	Brazilian	221	Workers in car battery factories	Whole blood	(Graphical illustration)				C allele carriers ↓Pb in blood	
Gundacker et al., 2009 (120)	Austrian	122	Men and women	Whole blood		20.2	21.3	16.9	CC genotype had ↓Pb in blood	
Hg concentrations (µg/L or µg/kg*)										
Woods et al., 2013 (116)	Portuguese	96	Boys of avg. age 10 years	Urine		2.17±2.15	2.16±2.16 [§]		No difference between genotypes	
Wang et al., 2012 (106)	US	464 473	Men and women Men and women	Urine Hair		1.04	1.04	1.22	570*	No difference between genotypes
Fe concentrations (mg/L)										
Giacconi et al., 2007 (113)	Italian	288	CS patients	Plasma Erythrocytes		1.11±0.47	0.99±0.32 [§]	506±102 [§]	C allele carriers had ↓Fe in plasma	
Zn concentrations (mg/L)										
Adams et al., 2015 (104)	US	321	Men and women	Urine	(Graphical illustration)				↓Zn in urine of C allele carriers	
Giacconi et al., 2007 (113)	Italian	288	Patients with CS	Plasma Erythrocytes		0.71±0.17	0.74±0.15 [§]	8.5±2.0 [§]	C allele carriers ↑Zn in erythrocytes	
Cu concentrations (mg/L)										
Adams et al., 2015 (104)	US	321	Men and women	Urine	(Graphical illustration)				↓Cu in urine of C allele carriers	
Giacconi et al., 2007 (113)	Italian	288	Patients with CS	Plasma Erythrocytes		1.07±0.29	1.11±0.24 [§]	0.56±0.13 [§]	C allele carriers ↑Cu in erythrocytes	

n – sample size; *MT2A* – metallothionein 2A; GG – typical homozygote; GC – atypical homozygote; CS – carotid artery stenosis; § C allele carriers (GC plus CC genotype); ↑ – increased concentration; ↓ – decreased concentration

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Polimorfizmi gena *metalotioneina 2A* u ljudi i njihova povezanost s bolestima i razinama elemenata u tragu

Metalotioneini u ljudskom organizmu povezana su skupina niskomolekularnih unutarstaničnih proteina, čiju sintezu mogu pobuditi esencijalni elementi, ponajprije Zn i Cu, toksični elementi i druge kemijske tvari te razni uvjeti koji izazivaju stres u organizmu. Od četiriju poznatih izoformi metalotioneina u ljudskome tijelu, najčešći oblik je MT2. Izražaj metalotioneina kodira skupina povezanih gena i na to mogu utjecati polimorfizmi pojedinačnoga nukleotida u tim genima. Do sada su otkrivena 24 jednonukleotidna polimorfizma u području gena *MT2A*, s incidencijom od oko 1 % u raznim skupinama stanovništva, a za tri je takva polimorfizma utvrđeno da bi mogli utjecati na fiziološke i patofiziološke procese. U preglednom radu prikazane su dosadašnje spoznaje o trima jednonukleotidnim polimorfizmima u genu *MT2A* i njihove povezanosti s koncentracijama elemenata u zdravih i bolesnih osoba. Najviše istraživani jednonukleotidni polimorfizam gena *MT2A* do sada bio je rs28366003 (*MT2A* -5A/G) i za njega su pokazane povezanosti s duljinom života, nekoliko tipova karcinoma (u dojki, prostati, grkljanu i sinusima) i s bubrežnim bolestima. Za drugi najviše istraživani polimorfizam rs10636 (*MT2A* +838G/C) nađene su povezanosti s rakom dojke, bolestima srca i krvnih žila te dijabetesom tipa 2. Za obje te vrste polimorfizama nađene su povezanosti i s koncentracijama metala i polumetala u organizmu. U samo nekoliko istraživanja ispitivana je povezanost polimorfizma rs1610216 (*MT2A* -209A/G) s dijabetesom tipa 2, kardiomiopatijom, hiperglikemijom i koncentracijama Zn. Podatci u literaturi upućuju na moguću praktičnu primjenu nalaza koncentracija metalotioneina i genskih polimorfizama *MT2A* kao bioloških pokazatelja izloženosti metalima i kliničkih pokazatelja brojnih kroničnih bolesti. Za tu svrhu potrebna su daljnja opsežna istraživanja u velikom broju dobro definiranih skupina ispitanika (nekoliko stotina i tisuća), usredotočenih na određeno fiziološko ili patološko stanje te uzimajući u obzir druge čimbenike, kao što su okolišna izloženost, osobne životne navike te genetičke i epigenetičke značajke.

KLJUČNE RIJEČI: jednonukleotidni polimorfizam; metali; polumetali; rs28366003; rs10636; rs1610216