Review DOI: 10.2478/aiht-2020-71-3349

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

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[Received in October 2019; Similarity Check in October 2019; Accepted in March 2020]

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).

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## BIOLOGICAL SIGNIFICANCE OF METALLOTHIONEINS

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

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 homedomain 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 supress 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*, *MT1M/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 ($H_2O_2$) 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 |
| Stajnko et al., 2019 (99) | Croatian | 136 | Pregnant women | 93.0[#] | 7.0[$] | |
| | Slovenian | 176 | Non-pregnant women | 95.0[#] | 5.0[$] | |
| 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[§] | |
| 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[§] | |
| | | 197 | Control men without prostate cancer | 89.3 | 10.7[§] | |
| 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 |
| Krześlak 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 |
| Krześlak 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; [$]G allele frequency; [§]G 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 *MT2A* −5A/G increase the risk of gastric adenocarcinoma |
| | | 90 | Control healthy men and women | | |
| Białkowska et al., 2018 (88) | Polish | 197 | Men with prostate cancer | Whole blood | No association was found between SNP *MT2A* −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 *MT2A* −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 *MT2A* −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 *MT2A* −5A/G and HT |
| | | 417 | Men and women with CKD | | |
| | | 2192 | Healthy men and women | | |
| Starska et al., 2015 (80) | Polish | 130 | Men and women with SIP | Tissue of nasal cavities or paranasal sinuses | SNP *MT2A* −5A/G was related to SIP (Schneiderian papilloma); G allele increased 7.7-fold occurrence of SIP (Schneiderian papilloma); SNP *MT2A* −5A/G was associated with SIP phenotype |
| | | 418 | Control men and women without head or neck tumour | | |
| Starska et al., 2015 (84) | | 117 | Men and women with SIP | | 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 *MT2A* −5A/G and tumour aggressiveness |
| | | 418 | Control healthy men and women | | |
| Krześlak et al., 2014 (82) | Polish | 534 | Women with ductal breast cancer | Whole blood | SNP *MT2A* −5A/G was associated with ductal breast cancer |
| | | 556 | Control healthy women | | |
| Krześlak 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 *MT2A* −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 *MT2A* −5A/G was associated with longevity |
| Hayashi et al., 2006 (96) | Japanese | 37 | Patients with SALS | Whole blood | No association between SNP *MT2A* −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 | *MT2A* genotype | | | Findings |
|---|---|---|---|---|---|---|---|---|
| | | | | | AA | AG | GG | |
| **As concentrations (μg/g creatine)** | | | | | | | | |
| Stajnko 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 *MT2A* −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 | (Graphical illustration) | | | ↓Cd 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) | 116±79 | 376±126 | 393 | AG *vs*. AA genotype ↑Cd in SIP samples; no association between Cd and SNP *MT2A* −5A/G in control samples |
| | | 132 | Control men and women with normal sinonasal mucosa | | 62.2±41.2 | 96.2±57.1 | - | |
| Starska et al., 2014 (85) | Polish | 323 | Men and women with SCC | Tissue of laryngeal mucosa (dry) | 198±87 | 369±128 | 509±57 | AG *vs*. AA genotype ↑Cd in both sample types; GG *vs*. AA and AG *vs*. GG genotype ↑Cd in SCC samples |
| | | 116 | Control men and women with normal laryngeal mucosa | | 87.2±32.2 | 113±26 | 117 | |
| 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 | - | |
| Krześlak 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† | 1.72±0.67† | - | |
| 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 | Hair | 440 | 390 | 430 | |
| **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; No association between Zn and SNP *MT2A* −5A/G in control samples |
| | | 132 | Control men and women with normal sinonasal mucosa | | 199±44 | 204±52 | - | |
| Starska et al., 2014 (85) | Polish | 323 | Men and women with SCC | Tissue of laryngeal mucosa | 86.4±38.1 | 184±57 | 194±74 | AG *vs*. AA genotype ↑Zn in both sample types; GG *vs*. AA genotype ↑Zn in SCC samples |
| | | 116 | Control men and women with normal laryngeal mucosa | | 97.6±30.0 | 133±27 | 129 | |
| Krześlak et al., 2013 (86) | Polish | 412 | Men with prostate cancer | Prostate tissue (dry) | 135±48 | 239±80 | 243.7±64.4 | AG *vs*. AA genotype ↑Zn in both sample types; GG *vs*. AA genotype ↑Zn in cancer samples |
| | | 67 | Control men without prostate cancer | | 485±119 | 927±317 | - | |
| 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 ↑Cu in control samples |
| | | 132 | Control men and women with normal sinonasal mucosa | | 11.0±2.98 | 17.2±5.2 | - | |
| Starska et al., 2014 (85) | Polish | 323 | Men and women with SCC | Tissue of laryngeal mucosa | 14.4±7.83 | 26.6±12.5 | 29.7±0.72 | AG *vs*. AA genotype ↑Cu in both sample types; GG *vs*. AA genotype ↑Cu in SCC samples |
| | | 116 | Control men and women with normal laryngeal mucosa | | 9.85±4.10 | 12.7±3.56 | 11.5 | |

| Authors and year of publication (reference No.) | Ethnicity | n | Study participants | Sample type | *MT2A* genotype | | | Findings |
|---|---|---|---|---|---|---|---|---|
| | | | | | AA | AG | GG | |
| Krześlak 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 |
| | | 67 | Control men without prostate cancer | | 2.9±1.3 | 7.6±2.9 | - | |
| Tekin et al., 2012 (89) | Turkish | 95 | Mother-newborn pairs | Maternal blood | 1.67±0.34* | 1.84±0.50* | - | No difference between genotypes |
| | | | | Cord blood | 0.69±0.25* | 0.69±0.28* | - | |
| | | | | Placenta | 5.90±2.59 | 6.63±1.73 | - | |
| Kayaalti et al., 2011 (91) | Turkish | 616 | Men and women | Plasma | 1.04±0.44* | 1.02±0.52* | 0.91±0.37* | No difference between genotypes |
| Kayaalti et al., 2010 (94) | Turkish | 114 | Men and women | Kidney tissue (dry) | 17.2±16.9 | 15.3±10.6 | 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; [δ]G allele carriers (AG plus GG genotype); ↑μgg$^{-1}$; ↑ – 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 |
| Giacconi et al., 2007 (113) | Italian | 100 | CS patients | 75.0 | 24.0 | 1.0 |
| | | 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 *MT2A* −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 *MT2A* −209A/G and development of laryngeal cancer |
| | | 418 | Control volunteers (men and women) | | |
| Krześlak et al., 2014 (82) | Polish | 534 | Women with breast cancer | Whole blood | No associations between SNP *MT2A* −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 *MT2A* −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 *MT2A* −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 *MT2A* −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 *MT2A* −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 | *MT2A* genotype | | | Findings |
|---|---|---|---|---|---|---|---|---|
| | | | | | AA | AG | GG | |
| **Zn concentrations (mg/L)** | | | | | | | | |
| 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 |
| | | 188 | 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.

*Acknowledgement*

Financial support for this work started during the research project "Exposure to metals and their effects in pregnancy and postnatal period" (Croatian Ministry of Science, Education and Sports' grant 022-0222148-2135) and continued through the research project "Assessment of Daily Exposure to Metals and Maternal Individual Susceptibility as Factors of Developmental Origins of Health and Disease, METALORIGINS" (Croatian Science Foundation grant HRZZ-IP-2016-06-1998).

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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 |
| Giacconi et al., 2007 (113) | Italian | 100 | CS patients | 73.0 | 22.0 | 5.0 |
| | | 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 | Women with breast cancer | Whole blood | SNP *MT2A* +838 G/C was associated with breast cancer |
| | | 549 | Control healthy women | | |
| García et al., 2017 (97) | Spanish | 130 | Men and women with AMD | Whole blood | No association between SNP *MT2A* +838 G/C and AMD |
| | | 96 | Control healthy men and women | | |
| Starska et al., 2015 (80) | Polish | 130 | Men and women with SIP | Tissue of nasal cavities or paranasal sinuses | No association between SNP *MT2A* +838G/C 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 *MT2A* +838G/C and development of laryngeal cancer |
| | | 418 | Control heathy men and women | | |
| Yang et al., 2014 (117) | Chinese | 287 | Men and women with CHD | Blood leukocytes | SNP *MT2A* +838G/C was associated with CHD |
| | | 226 | Control healthy men and women | | |
| Krześlak et al., 2014 (82) | Polish | 534 | Women with breast cancer | Whole blood | No associations between SNP *MT2A* +838G/C and breast cancer |
| | | 556 | Control healthy women | | |
| Yang et al., 2008 (115) | Chinese | 397 | Men and women with DM | Whole blood | SNP *MT2A* +838G/C was associated with higher risk for hyperlipidemia and incidence of DM with neuropathy |
| | | 454 | Control men and women | | |
| Giacconi et al., 2007 (113) | Italian | 100 | CS patients | Blood | SNP *MT2A* +838G/C promote the progression of carotid artery disease to CS |
| | | 188 | CS patients without cerebrovascular episodes | | |
| | | 218 | Control elderly volunteers | | |

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 | *MT2A* 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 | Men and women | Urine | 1.04 | 1.04 | 1.22 | No difference between genotypes |
| | | 473 | Men and women | Hair | 500* | 430* | 570* | |
| **Fe concentrations (mg/L)** | | | | | | | | |
| Giacconi et al., 2007 (113) | Italian | 288 | CS patients | Plasma | 1.11±0.47 | 0.99±0.32§ | | C allele carriers had ↓Fe in plasma |
| | | | | Erythrocytes | 505±270 | 506±102§ | | |
| **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 | 0.71±0.17 | 0.74±0.15§ | | C allele carriers ↑Zn in erythrocytes |
| | | | | Erythrocytes | 7.4±2.6 | 8.5±2.0§ | | |
| **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 | 1.07±0.29 | 1.11±0.24§ | | C allele carriers ↑Cu in erythrocytes |
| | | | | Erythrocytes | 0.52±0.11 | 0.56±0.13§ | | |

n – sample size; *MT2A* – metallothionein 2A; GG – typical homozygote; GC – heterozygote; CC – 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 istraživan 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