Genetic Polymorphisms and Zinc Status: Implications for Supplementation in Metabolic Diseases

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Abstract: ***Background:*** Zinc is an essential component for all living organisms, representing the second most abundant trace element, after iron. This element is widely distributed in the tissues of human body where it is involved in the normal growth, reproduction and several biological functions including immunity, energy metabolism and antioxidant processes. Because of its essential role, zinc levels in human body must remain constant, independently of dietary intake fluctuations. The homeostasis of zinc is a well-regulated cellular process and has been reported to be chiefly mediated by the expression and activity of zinc-binding proteins such as metallothioneins and zinc transporters. Genes encoding for these proteins are subjected to genetic variants.

Methods: We performed a multi-database electronic search to provide an overview on the relationship between specific polymorphisms (SNP) of genes encoding for metallothioneins and zinc transporters and their relationship with zinc status, immune function and some non-communicable diseases.

Results: A number of SNP are implicated in a range of metabolic disease. Some SNP may affect the impact of zinc supplementation on immune function, diabetes, obesity.

Conclusion: New studies are needed to clarify the interaction between individual genetic profile and zinc status. Moreover, there is a need to a better interaction between the scientific bodies and health professionals to allow better dietary and behavioural recommendations to promote human health, with particular concern to elderly people.

Keywords: Zinc, Genetic Polimorphisms, Metallothionein, Zinc transporters, Immune function, Non-communicable disease

1. INTRODUCTION

Zinc (Zn) is recognised as an essential micronutrient involved in the structural and functional components that underpin a wide range of biological processes involved in cellular metabolism, growth, development, cellular physiology and immune function [1]. To date more than 300 enzymes and 100 transcription factors have been identified to have a requirement of Zn for their activity. Zn plays a key role as a structural component for the stabilization of the tertiary structure of many proteins, such as transcription factors containing "zinc finger" (ZNF) domains, which interact with a variety of proteins, lipids and nucleic acid. Moreover, as cofactor of numerous enzymes present in animal tissues, it ensures the catalytic activity of six main classes of enzymes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [2, 3].

Because of its essential pleiotropic role, human body needs to maintain constant levels of zinc, independently of dietary intake fluctuations. In humans, the daily turnover corresponds to about 1% of the total zinc content. Zinc is lost through urine and by the non-avoidable skin and intestinal cell desquamation and hair. In specific harsh conditions, significant amounts can be lost through perspirations and together with occasional loss of fluids during menstruations and ejaculation. The homeostasis is therefore obtained thanks to its daily replacement by the diet and is closely controlled by the intestinal absorption system and excretion through pancreatic and intestinal secretions. The homeostasis of zinc is a well-regulated cellular

process. In fact, the expression of the membrane conveyors and zinc sequestering proteins varies greatly and rapidly, depending on the intracellular concentration of zinc [4].

The homeostasis of zinc has been reported to be chiefly mediated through the expression and action of zinc binding proteins such as metallothioneins (MTs) and zinc transporters [5, 6]. According to membrane topology data, transporters of zinc have been grouped into two major families:

(a) 10 zinc "exporters" (ZnT1-10) of the SLC305 family, which are associated with lowering of cytoplasmic zinc by transportation of zinc either out of the cell or into intracellular organelles, and

(b) 14 zinc "importers" of the SLC39s family (Zrt (zinc regulated transporter)-like Irt (iron-regulated transporter)-like proteins, ZIP 1-14), the activity of which result in increased cytoplasmic zinc [7].

Zinc associated to enzymes and transcription factors accounts for about 90% of the zinc proteome – indicating the key role of this trace element in the regulation of the processes of catalysis and transcription [6]. MTs are a cysteine-rich family of proteins, mainly localized at the level of Golgi apparatus having the capacity to bind either Zinc and other transition elements (Selenium and Copper) or potentially toxic heavy metals such as mercury and arsenic [8]. By binding and releasing zinc, MTs may regulate zinc levels within the body and are involved in Zn detoxification. Thanks to its protein sequence, rich in cysteines, and its quaternary structure, MTs are able to bind 7 zinc atoms, sequestering them from the surrounding environment, in order to protect the cell from its toxic action [9]. MTs expression is ubiquitous but is particularly high in parenchymal cells of the intestine, pancreas, kidney and liver.

Very few data are available on the molecular mechanisms involved in MTs' and ZnTs' functions. MTs' cellular protection mechanisms were initially shown to involve interaction with antioxidant proteins [10-12]. Then, a role of reactive oxygen species (ROS) [13], anti-inflammatory [14], antiapoptotic proteins [15] and of the mitogen-activated protein kinase [16] was reported. More recent findings show an indirect involvement of uncoupling proteins in the MTs-dependent attenuation of the free radical-induced cardiac toxicity [17]. With respect to ZnTs, some data is available for ZnT7 that has been shown to upregulate insulin gene expression through MTF1 activation [18] and Irs2 and Akt phosphorylation [19]. MTF1 is believed to act as a buffer influencing the cellular sensitivity to zinc through the modulation of MT and ZnT expression [20], however, no information is available on the relationships between ZnTs, zinc and MTF1. Similarly, ZnT9 functions as a transcriptional coactivator moving into the nucleus upon activation by hormone stimulation, binding to nuclear receptors complexes to regulate gene transcription [21] and activating Wnt signaling through β-catenin interaction [22]. However, no information is available on ZnT9 transcriptional activation.

Zinc deficiency has been associated with insufficient dietary intakes or impaired intestinal function in groups of population either aged or suffering of a wide spectrum of different chronic conditions and also related to specific dietary profiles such as vegetarian/vegan [23,24]. Given that there are no specialised storage reserves of zinc within the body, it is necessary to maintain an adequate, regular supply [25]. Specifically, people in developing countries are at particular risk and it has been estimated that globally close to 2 billion people may be zinc deficient [3]. A broad range of clinical manifestations have been associated either to nutritional zinc deficiency or to inherited phenotypes concerning zinc absorption and metabolism. When zinc homeostasis is concerned, several important pathologies have been reported including growth retardation, testicular hypofunction, compromised immune function, oxidative stress, and an increase in the production of inflammatory cytokines [2, 5, 7, 26-28]. Accordingly with the evidence of the association between zinc deficiency or malnutrition and diarrheal disease, zinc supplementation has been used for the treatment and prevention of diarrhoea in infants and children [3,29]. The occurrence of a more marginal zinc deficiency has been observed in vegetarians and vegans and is thought to be the result of the consumption of high levels of zinc-chelating agents present in cereals, legumes or plant parts. In fact, lignins and phytate, found in these types of foods, have been reported to be able to bind Zinc and counter its absorption therefore reducing its bioavailability [6, 30,31]. It has also been reported that a rise in Cu to Zn ratio (CZr) is a common feature associated with a number of age-related chronic conditions; something which has been postulated to be more dependent on physiological alterations arising with age as opposed to regular nutritional intake [32,33].

Elderly people are a group of population at an increased risk of nutritional disorders owing to a combination of the impact of the ageing on physiological/physical/biochemical capacities, as well as behavioural and dietary factors [24,34]. People aged more than 60-65 years have been

reported to have intakes of zinc significantly below the recommended intakes, and about 30% of them has been assessed as "zinc deficient" [6].This deficiency, is likely to result in impaired immune function and in a significant increase of the risk of a spectrum of degenerative disease [24].

Zinc has low toxicity and is generally considered to be safe even though very high, excessive supplementations can have detrimental consequences possibly due to inadequate absorption of copper [35]. Therefore, numerous zinc supplementation trials have shown a wide range of health benefits, including decrease diarrhoea mortality in children, incidence of infections and immune functions improvement [36-39]. Moreover, the efficacy of zinc supplements in boosting health and well-being has been further confirmed by the meta-analysis addressing growth and body weight gain in children [40] and also dealing with the incidence of blindness and the risk of developing age related macular degeneration [41].

Single nucleotide polymorphisms (SNPs) are a common type of genetic variation with high prevalence (>1%) among people. Each SNP is characterized by a specific substitution of a single DNA base occurring with high frequency. Overall, population studies conducted by multicentric consortia have recent contributed to the identification of about 60 million SNPs in the human genome [42]. These allelic variants may fall within coding and in non-coding sequences of genes as well or in the intergenic regions (regions between genes). SNPs within a coding sequence do not necessarily change the amino acid sequence of the encoded protein due to degeneracy of the genetic code: in fact, synonymous SNPs do not affect the protein sequence. SNPs not occurring in protein-coding regions may still affect gene splicing, transcription factor binding, messenger RNA degradation, or the sequence of noncoding RNA. SNPs may determine an individual's response to certain drugs, and the susceptibility to environmental factors such as toxins, and risk of developing particular diseases. So far several SNPs have been associated with complex diseases such as heart disease, diabetes, and cancer.

Several SNPs have been identified to modulate zinc intake/ status [43]. Costarelli e colleagues [44] have reported that gene expression of MTs, zinc-transporters and inflammatory cytokines are regulated by zinc intake. In addition to zinc-regulated transcription, genetic polymorphisms of MTs and zinc transporters have been associated with age-related diseases, such as chronic inflammation [45], type 2 diabetes ([46] and cardiovascular diseases (CVD) [47].

In this review, we will focus on the relationship between specific polymorphisms of genes encoding for MTs and zinc transporters and their relationship with zinc status, immune function and some non-communicable diseases.

2. GENETIC POLYMORPHISMS OF METALLOTHIONEINS AND ZINC TRANSPORTERS

As mentioned above, the physiological requirement of trace metal elements is regulated by proteins specialized in the transport and deposit of metals in non-toxic forms. MTs have been very well characterized for their high content in cysteine, an amino acid that plays a key role in the formation of complexes with transition metals resulting in a high capacity to bind heavy metals [48]. At the same time, zinc transporters (ZnTs) family of membrane transport proteins of the solute carrier family control the membrane transport of zinc and regulate its intracellular and cytoplasmic concentrations [49,50]. ZnTs belong to two major groups: i) zinc transporters (ZnT) involved in the controls the efflux of zinc from the cytoplasm out of the cell and from the cytoplasm into vesicles; and ii) zinc importers, Zrt- and Irt-like protein (ZIP), controlling the influx of zinc into the cytoplasm from outside the cell and from vesicles [48,50].

SNPs in genes encoding for MTs and ZnTs can modify different aspects of gene product including its transcription, and also the specific molecular characteristics of the protein resulting e.g. in changes of their Zn binding affinity [51,52].

Such finding, already 20 years ago, encouraged for the search of genetic variants affecting the functioning of MTs and ZnTs, both necessary for cellular zinc homeostasis.

2.1 Metallothioneins

MTs can be considered "cellular buffer" of metals regulating their cellular amounts and being in turn regulated by metals, protecting organisms from harmful effects of highly toxic heavy metals, such as cadmium and mercury, but also regulating the cellular amounts of trace metal micronutrients including zinc, copper, iron, manganese. MT are low molecular weight (6-8 kDa) proteins with high content of cysteine, a low or null content of aromatic amino acids, and spectroscopic characteristics typical of metal-thiolate clusters. Cysteine content and its particular sequence arrangement is crucial for the MTs' ability to form

complexes with heavy metal ions, those three-dimensional structure is influenced in turn by presence of the metal [53]. MTs are particularly abundant in liver and kidney tissues and they are also present in the central nervous system and in mammary, olfactory and thyroid glands, in gastric and intestinal tissues, in hair follicles and in circulating monocytes.

As mentioned above, the main role of MTs is linked to their ability to bind and regulate the homeostasis of essential trace elements, in order to prevent their harmful effects [54]. MTs are polymorphic genes, clustering together on a single chromosomal locus (16q12-22 for homo sapiens [55]), and coded proteins have very similar amino acid sequences. Accordingly, experiments on transgenic mice [56] suggested already 20 years ago that single MT isoforms are not essential for life but rather that MT functions are redundant and compensated by homeostatic mechanisms.

Several studies have demonstrated that MT isoforms are differentially transcribed, translated and maintained in the cell, with a different response to metals. MT1 and MT2 isoforms, which are present in all mammalian tissues, are inducible, with MT2 appearing to be expressed more than MT1 [57], constituting the 50% of the total expressions of all metallothionein isoforms [58] and being frequently overexpressed in invasive human breast cancers [59].

2.1.1 MT polymorphisms

2.1.1.1 MT1A polymorphisms

According to the database of The National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/SNP/, May 2018), 98 validated SNPs are present in the human MT1A gene region. Ten are located in the 5'UTR, 14 in the coding sequence, 64 in introns and 10 in the 3'UTR (May 2018). Of these, seven SNPs are mentioned in the literature (rs11076161, rs8052394, rs7196890, rs11640851, rs7190725, rs11076160, rs8049883 and rs11647171) and three have an impact on metabolic disease [60, 61] (table 1). rs11076161 and rs8052394 were considered in a search for an association between MT and type 2 diabetes mellitus (T2DM) in a population of 851 Chinese people of Han descent (397 diabetes and 454 controls) [60]. An association of the SNPs was found with the disease and its clinical symptoms, i.e. neuropathy (rs11076161) and serum superoxide dismutase activity (rs8052394) [60].

Table 1. MT polymorphisms, SNP variant and association with metabolic disease

MT polymorphisms	SNP variant	Associated with	Ref.
MT1A			
rs11076161	intron	Diabetic neuropathy	[60]
rs8052394	missense	T2DM, Serum superoxide dismutase	[60]
rs11640851	missense	Longevity, Cardiovascular disease, T2DM, MT levels, IL-6 plasma concentration	[61]
MT1B			
rs964372	intron	Hyperlipidemia, Diabetic neuropathy	[60]
rs7198427	5'UTR	Advanced glycation end-products	[62]
rs7197489	5'UTR	Advanced glycation end-products	[62]
MT2A			
rs28366003	5'UTR	CKD and DM B-MT and -Zn levels in T2DM	[66] [46]
rs1610216	5'UTR	B-Zn levels, T1DM and cardiovascular complications Diabetes	[45] [68]
rs10636	3'UTR	Atherosclerosis, B-Zn, B-Cu and inflammatory cytokines levels Diabetic neuropathy and hyperlipidemia in T2DM	[69] [66]
MT4			
rs396230	intron	Blood pressure, serum uric acid	[73]

T2DM: Type 2 diabetes mellitus; CKD: chronic kidney disease; DM: diabetes mellitus; MT: Metallothionein; IL-6: Interleukin 6; T1DM: Type 1 diabetes mellitus.

2.1.1.2 MT1B polymorphisms:

103 SNPs are present in the human MT1B gene region. Seven are located in the 5'UTR, 25 in the coding sequence, 54 in introns and 17 in the 3'UTR. Of these, nine SNPs are mentioned in the literature (rs964372, rs8052334, rs7191779, rs2070839, rs1875232, rs7197489, rs7198427,

rs12051311 and rs61744104) with the first four having a significant association with diseases. As for variants of MT1A, significant association of rs964372 exists with diabetic neuropathy (linked to hyperlipidemia) [60] (as for MT2A, see below). More recently, rs7198427 and rs7197489, initially attributed to the MT1A gene portion, were identified by genome-wide association study (GWAS) as associated to advanced glycation end-products (AGEs) in diabetic subjects [62].

2.1.1.3 MT1E polymorphisms

The MT1E gene regions contains 188 validated SNPs, ten are located in the 5'UTR, 63 in the coding sequence, 87 in introns and 28 in the 3'UTR. Of these, only four SNPs are mentioned in the literature (rs7403881, rs34166523, rs2070836 and rs708274) and an association of rs7403881 was reported with sporadic amyotrophic lateral sclerosis (SALS) [63]. None of these have been considered to be associated to metabolic disorders.

2.1.1.4 MT1F polymorphisms

112 SNPs have been identified so far in the human MT1F gene region. Eight are located in the 5'UTR, 27 in the coding sequence, 51 in introns and 26 in the 3'UTR. Of these, only one SNP is mentioned in the literature (rs2291956) and still lacks any association with metabolic diseases.

2.1.1.5 MT1G and MT1H polymorphisms

A total of 258 validated SNPs have been found in human MT1G and MT1H gene regions, including 55 variants spread along all regions, but not in the 3'UTRs of the two genes, that bear 17 and 11 SNPs respectively. Twenty 5'UTR SNPs are entirely shared between the two genes. SNPs in intron regions are discretely 94 and 78, plus 25 shared. Finally, coding regions contain respectively 27 and 26 SNPs, plus 10 shared variants. Of these, only five SNPs are mentioned in the literature for both genes, including two in shared gene regions (rs12448654 and rs4784708), and rs2298847, rs2298846 and rs12315 for MT1G and rs9934181, rs2062546 and rs2062545 for MT1H. None of these has been reported so far to have any association with metabolic diseases.

2.1.1.6 MT1M polymorphisms

MT1M variants, whose genomic region partially overlaps with that of the MT1JP pseudogene, include a total of 116 SNPs, 11 in the 5'UTR, 34 in the coding sequence, 59 in introns, and 12 in the 3'UTR region. Five are mentioned in the literature, three with no associations with diseases (rs2270837, rs1827210 and rs1827208), and two linked with mercury levels in the urine (rs2270836) and in hair (rs9936741) [64]. Activation of MT1M in Hep-G2 cells, upon heavy metals or glucocorticoids exposition, was shown to affect NF-kB activity, therefore potentially involving also metabolic dysfunctions [65].

2.1.1.7 MT1X polymorphisms

139 SNPs have been already reported in the human MT1X gene region. Eleven are located in the 5'UTR, 21 in the coding sequence, 93 in introns and 14 in the 3'UTR. Of these, two SNPs are mentioned in the literature (rs2301234 and rs8051405) but none of them has been associated to metabolic diseases.

2.1.1.8 MT2A polymorphisms

According to the NCBI dbSNP database, the human MT2A gene region contains 68 validated SNPs. Eight are located in the 5'UTR, 14 in the coding sequence, 34 in introns and 8 in the 3'UTR. Among those, three SNPs are cited in the literature having association with diseases: rs28366003, rs1610216 and rs10636.

Recently, the rs28366003 SNP has been associated with increased risk of chronic kidney disease (CKD), and diabetes mellitus (DM) in a large Japanese population [66],

According to the previously reported association of MTs to diabetes and metabolic diseases [67], an association with the disease in elderly patients was reported with two more SNPs within MT2A. The first one, rs1610216, was linked to higher risk for type 1 diabetes mellitus (T1DM) and cardiovascular complications (chronic inflammation, higher plasma levels of IL-6 and glycosylated hemoglobin), together with lower plasma zinc levels, in Italian atherosclerotic patients [45]. rs1610216 has been confirmed to be associated to diabetes also in Bulgarian diabetic patients [68]. The second one, rs10636, was linked to higher risk of atherosclerosis and carotid plaques, increased inflammatory cytokines and decreased zinc and copper plasma levels [69]. rs10636 role in diabetes has been confirmed by Yang and coworkers that observed variants of the SNP in T2DM patients with diabetic neuropathy and hyperlipidemia [60].

2.1.1.9 MT3 polymorphisms

The expression of MT3 (also known as growth inhibitory factor), is primary in the central nervous system [70]. According to the NCBI dbSNP database, the human MT3 gene contains 131 validated SNPs. 26 are located in the 5'UTR, 22 in the coding sequence, 72 in introns and 11 in the 3'UTR. Among those, three SNPs are cited in the literature and two (rs45570941 and rs11644094)

have been reported to be associated with distinct pathologies, but not to metabolic dysfunction.

2.1.1.10 MT4 polymorphisms

MT4 was isolated from digestive and neonatal skin epithelia [71] and showed better Cu binding properties than MT1 [72]. Two MT4 SNPs are present in the coding sequence and 55 in introns. Only one association has been reported, i.e. that of rs396230, with blood pressure and serum uric acid values in car battery workers [73].

2.2 ZnT zinc transporters

ZnT are membrane proteins that regulate its intracellular and cytoplasmic concentrations of zinc through its transport. They differ from the zinc importers, Zrt- and Irt-like proteins (ZIP), because they control the efflux of zinc from the cytoplasm out of the cell (Znt1) and into vesicles (all other except ZnT9, considered a misnomer) [74], while the latter, not considered in this review, control the influx of zinc into the cytoplasm from outside the cell and from vesicles. This review will not specifically address on ZIP and will be mainly focus on ZnT.

The SLC30 family of ZnT zinc transporters family comprises 10 mammalian members that belong to the large superfamily of CDF transporters that also includes zinc transporters in bacteria, fungi, nematodes, insects and plants [75]. Based on structural information obtained in the bacterial homolog YiiP [76], CDF family members are predicted to have six trans-membrane domains (TM) and a histidine/serine-rich loop between TM4 and TM5, except for ZnT5 which contains additional TM domains at the N-terminal. At the C-terminal, ZnTsbear long tails, from 82 amino acids for ZnT7 to 203 for ZnT6. Differently from MTs, high length heterogeneity exists for the amino acid sequences upstream of the first TM domain where different subcellular targeting signals are present. Based on sequence similarities, ZnT family members are grouped into four subfamilies with SLC30A5 (ZnT5) and SLC30A7 (ZnT7) in subfamily I, SLC30A2/3/4 and SLC30A8 (ZnT2, ZnT3, ZnT4 and ZnT8) in subfamily II, SLC30A1 (ZnT1) and SLC30A10 (ZnT10) in subfamily III, and SLC30A6 (ZnT6) and SLC30A9 (ZnT9) in subfamily IV.

Expression of ZnT is in general ubiquitous (ZnT1) but ZnT5, ZnT6, ZnT7, and ZnT9 are highly expressed in certain districts and tissue-specific with respect to other tissues and characterized by specific temporal patterns. For example, the members of the subfamily II are restricted to secretory tissues such as lactating mammary glands, glutamatergic neurons, prostate, and pancreatic β-cells. A high expression of ZnT5, ZnT6 and ZnT7 has been reported in the heart [77], in the brain [78] in the intestine [79], respectively while ZnT10 expression is limited to brain and liver [80].

ZnT1 expression in the placenta and other tissues is induced by dietary zinc intake [81] and is switched on during post-implantation period in trophoblasts and in the maternal deciduum [82]. The activation depends on MTF1 binding to MRE of ZnT1 promoter [83]. On the other hand, dietary zinc intake reduces ZnT5 expression both *in vitro* in Caco2 cells and *in vivo* in the ileal mucosa [84].

Differently from MT KO, ZnT silencing has serious consequences and ZnT KO is hardly compatible with life, indicating a crucial role of these transporters. For instance, ZnT1 KO mice are embryonically unviable because of impaired zinc transfer from the mother [85]. ZnT5 [86] and ZnT7 [79] are localized on the membrane of the Golgi apparatus and in cytoplasmic vesicles. ZnT7 KO mice display poor growth, with decreased adiposity and insulin resistance [87]. Znt8 KO mice have an impaired insulin secretion and zinc-insulin crystals [88,89].

2.2.2 ZnT polymorphisms

2.2.2.1 ZnT1, ZnT4, ZnT5, ZnT6, ZnT7 and ZnT9 polymorphisms

According to the NCBI database (May 2018), 152 and 1482 validated SNPs are present in the human ZnT1 and ZnT4 gene regions, respectively. With respect to ZnT1, four are located in the 5'UTR, 93 in the coding sequence, 49 in introns and 4 in the 3'UTR. Regarding ZnT4, 109 are located in the 5'UTR, 90 in the coding sequence, 1256 in introns and 27 in the 3'UTR.

1527 SNPs are present in the ZnT5 gene region, 14 in the 5'UTR, 172 in the coding, 1265 in introns and 76 in the 3'UTR. Among those, only two SNPs have publications in PubMed, rs337253 and rs164578, with no association with diseases. However, rs337253 has been proposed to be associated with the expression of antioxidant response element (ARE)-regulated genes, and in particular to NRF2-mediated antioxidant response pathway [90], which is involved in metabolic diseases [91].

Near three thousand SNPs have been identified in the ZnT6 gene region, 99 in the 5'UTR, 173 in the coding, 2309 in introns and 217 in the 3'UTR. Finally, almost four thousand SNPs are present in both Znt7 and Znt9 gene regions. Znt7 has 45 in the 5'UTR, 133 in the coding, 3292 in introns and

267 in the 3'UTR. Similarly, the majority of ZNT9 SNPs are in the intron region (3496), 11 are located in the 5'UTR, 129 in the coding sequence and 43 in the 3'UTR. Several links are present for these three SNPs in PubMed citations, but no associations with metabolic diseases have been reported, so far.

2.2.2.2 ZnT2 polymorphisms

The human ZnT2 gene region presents 422 SNPs. Eighteen are located in the 5'UTR, 107 in the coding sequence, 256 in introns and 41 in the 3'UTR (May 2018). Several are mentioned in the literature and the number is actually underestimated because of the presence of rs-unclassified variants (table 2). So far, no association with metabolic disease has been reported.

Table 2. ZnT polymorphisms, SNP variant and association with metabolic disease

ZnT polymorphisms	SNP variant	Associated with	Ref.
ZnT2			
rs35623192	missense $R^{340}C$	T2DM	(100)
rs35235055	missense $L^{23}P$	T2DM	(100)
Znt8			
rs13266634	missense $R^{325}W$	T2DM	[94- 96, 103-110]
		T2DM drug therapeutic efficacy	[105]
		Proinsulin conversion	[120-121]
		Insulin secretion	[122-123]
		T1DM	[125-127]
		Dyslipidemia	[129]
		Chronic coronary artery disease	[128]
		Muscle strength and size	[130]
rs16889462	missense $R^{325}Q$	T2DM and drug therapeutic efficacy	[105]
rs11558471	3'UTR	T2DM	[131-135]
rs3802177	3'UTR	T2DM	[98,132,136]
rs2466293	3'UTR	T2DM	[137]
		T1DM	[138]
		Gestational diabetes mellitus	[139]

T2DM: Type 2 diabetes mellitus; T1DM: Type 1 diabetes mellitus

2.2.2.3 ZnT3 polymorphisms

According to the NCBI database, 1103 validated SNPs are present in the human ZnT3 gene region. 127 are located in the 5'UTR, 178 in the coding, 759 in introns and 39 in the 3'UTR. Several ZnT3 SNPs have PubMed links in neurological disorders and with Zn status, which is consistent with the phenotype of the mouse KO [92], but none was associated to metabolic disease or diabetes.

2.2.2.4 ZnT8 polymorphisms

According to the NCBI database more than eight thousand validated SNPs are present in the human ZnT8 gene regions, the majority are in the intron region (8021), 56 are located in the 5'UTR, 141 in the coding sequence and 134 in the 3'UTR. 32 SNPs have links with PubMed citations, and six have an association with diseases.

rs13266634 has a number of citations (more than two hundred) related to the $R^{325}W$ variant, a well-known susceptibility locus of T2DM, since its finding ten years ago in several genome-wide association studies conducted in European [93-96], Asian [97-107], Russian [108], Tunisian [109], Mexican Mestizo [110] (but not Mexican [111,112], African [113], Hispanic American [114], Qatari [115] nor south Iranian [116]) subjects (reviewed in [117,118]. These findings are consistent with ZnT8's major role as transporter of zinc, necessary for insulin maturation, into secretory pancreatic β-cells [119]. In fact, impaired proinsulin conversion [120,121] and insulin secretion [122,123] have been found in carriers of the $R^{325}W$ variant. Recent reports demonstrate that T2DM risk depends on different zinc transport kinetics of the variants [124]. Because of its role in β-cell functions, rs13266634 SNP was tested and found implicated also in T1DM [125-127], and in cardiovascular diseases incidence [128]. Other studies of associations of rs13266634 variants include implication with dyslipidemia in

HIV/hepatitis C virus co-infected patients [129] and with skeletal muscle strength and size [130].

Other ZnT8 SNPs implicated in T2DM were reported (frequently in high linkage disequilibrium with rs13266634), *i.e* rs16889462 [105], rs11558471 [131-135] and rs3802177 [98,132,136] and rs2466293 [137]. rs16889462, actually concerning the same codon 325 ($R^{325}Q$) involved in rs13266634, found in a single African-Americans family, but not in Caucasians, was associated like rs13266634 with repaglinide therapeutic efficacy in Chinese patients [105]. rs2466293 has been recently associated with T1DM in Latin Americans [138] and was identified by means of *in silico* as a miR-binding SNP in Chinese pregnant women affected by gestational diabetes mellitus (GDM) [139]. On the other hand, rs13266634 lowered GDM risk in in European women [140] and was excluded as a risk factor for Euro-Brazilian ones [141].

2.2.2.5 **ZnT10 polymorphisms**

According to the NCBI database, the human ZnT10 gene region contains 1799 validated SNPs. 50 are located in the 5'UTR, 125 in the coding sequence, 1571 in introns and 53 in the 3'UTR. Among those, six intron SNPs are cited in the literature to be associated with metal physiology but no reports about any association with metabolic syndrome has been reported.

3. GENETIC POLYMORPHISMS, ZINC STATUS AND ALTERATIONS IN IMMUNE AND INFLAMMATORY FUNCTION

Zinc has been demonstrated to have both antioxidant and anti-inflammatory roles. It has been described as a "second messenger" for immune cells, with a range of transcription factors associated with the gene expression of inflammatory cytokines and with adhesion molecules having been reported to be zinc dependent [3]. Zinc has been demonstrated to have a role in both innate and adaptive immune function [23] with a long established relationship recognised between zinc deficiency and immune dysfunction [142]. Via its availability, which is closely regulated by a number of transporters and regulators, zinc functions as a modulator of immune response. Perturbations of these processes may alter zinc availability, impacting on the survival, proliferation and maturation of cells associated with both innate and adaptive immunity [143].

Patients who are clinically zinc deficient may present range of immune-related symptoms including lymphopenia, decreased ratio of T-helper (Th) cells to cytotoxic T-cells; a decrease in the cellular natural killer cell activity and an increase in cytoxicity. Such deficiency is associated with chronic diarrhoea, administration of parenteral nutrition lacking in zinc or as a result of excessive consumption of alcohol, as well as being found in patients with the malabsorption disorder, Acrodermatitis Enteropathica (AE). Owing to the prevalence of marginal zinc deficiency in older people there is likely to be a correlation between zinc status and impaired immune function in elderly people. Increased risk of inflammatory conditions, increased susceptibility to infection, autoimmune diseases, cardiovascular disease and cancer are all associated with immunosenescence – the age-related alterations in immune function [6, 24, 36].

Changes in immune function associated with zinc deficiency and ageing show many similar features in both innate and adaptive immunity and neutrophil function which led [24] to propose the existence of a tight relationship between zinc deficiency and immunosenescence. Zinc supplementation may, therefore, be particularly relevant in older people in the prevention, reduction or delay of disease. However, the data from intervention studies to date are conflicting. This may be owing to the usage of different doses, durations and forms of zinc supplements utilised. In addition in the presence of high oxidative stress high doses of zinc may trigger apoptosis of immune cells [144] The accumulation of zinc may reach toxic levels resulting in the aberrant activation of zinc-dependent enzymes such as PARP-1 with a role in genomic stability or promote the uptake of excessive levels of calcium into the cells, resulting in cell death [24]

It has been stated that the key factors underpinning intracellular zinc levels during ageing are increased expression of MTs and defective zinc transporters, leading to increased sequestration of zinc and low intracellular free zinc content [145]. In its role as a "second messenger" within the immune system, zinc may alter the signalling cascades that promote antioxidant and immune defence [146]. In the elderly low free zinc ion availability and increased MTs levels may result in compromised antioxidant and immune response, increasing inflammation accompanied by repeated infections and risk of degenerative disease [145]. Although data from intervention studies are contradictory, there is evidence that zinc supplementation may have a positive impact on DNA repair and increase the expression of some

zinc transporters genes, improving zinc homeostasis and reducing infections [145,147,148]. Following a systematic review of the literature examining the relationship between zinc status and autoimmunity Sanna and coworkers [149] have identified a relationship between zinc homeostasis imbalance and the state of autoimmunity, suggesting a need to commence a screening campaign for the evaluation of zinc levels in children. They have also proposed a clinical trial in populations at higher risk of autoimmunity to investigate the possibility of personalised zinc supplementation for the prevention or treatment of autoimmune diseases.

Giacconi *et al.* [47] reviewed the pro-inflammatory genetic factors and zinc status in older atherosclerotic subjects and stated that the identification of polymorphisms of pro-and anti-inflammatory cytokines and their interaction with nutrients, such as zinc that impact on Th1/Th2 balance, represents an opportunity for predicting atherosclerotic plaque formation and targets for future therapies. Nonetheless, conflicting data exist between polymorphisms, the occurrence of atherosclerosis and the influence on zinc homeostasis.

Genetic make-up may influence the response to zinc supplementation, with recent studies indicating that polymorphisms in IL-6-174 G/C and MT1a +647A/C loci may affect the impact of zinc supplementation on antioxidant and immune function in older people [24, 150,151]. In the rare, inborn disorder of zinc metabolism, AE, a mutation in ZIP 4 (SLC 49A4) importer gene results in severe zinc deficiency symptoms [152]. A variety of other polymorphisms in zinc-related proteins have been implicated in a range of disorders and it has been suggested that zinc, via zinc transporters, may affect DNA stability resulting in altered zinc homeostasis and metabolism [43]. Through zinc fingers, extraction part of several transcription factors, zinc is involved in the DNA replication processes which are necessary for a wide range of key cellular processes [145]. Some of these are transcription factors which are involved in the regulation of pro-inflammatory cytokines (IL6 and TNF_{α}) and heat shock proteins (Hsp70) [153]

Giacconi *et al.* [154] investigated the association of Hsp70 1267 A/G and TNF_{α} -308 G/A polymorphisms with pro-inflammatory mediators and zinc status in elderly people. Utilizing a linear regression modelling approach to examine additive, dominant or recessive associations of each SNP with pro-inflammatory mediators, MT and zinc status measures they concluded that HsP70 A/G is linked to the production of pro-inflammatory cytokines in healthy elderly people, which might be involved in determining how vulnerable individuals are to inflammatory disease. They also concluded that TNF_{α} -308 G/A SNP do not impact on production of pro-inflammatory cytokines but that both SNP are associated with levels of creatinine. Giacconi and colleagues [52] also investigated the role of ZIP 2 Gln/Arg/Leu (rs2234632) polymorphism on zinc homeostasis and inflammatory response following zinc supplementation, again in elderly volunteers. Enhanced IL-6, TNF_{α} and RANTES plasma level were demonstrated in ZIP2 Leu^{-} (Arg43Arg) carriers. This was associated with decreased free cytosolic zinc and an up-regulation of the ZIP2, ZIP8 and Znt1 transporters. Upon zinc supplementation, the volunteers who were Leu- showed a decrease in inflammatory mediators.

Wong *et al.*[155] investigated the impact of zinc deficiency on cellular immune activation and the epigenetic mechanisms which might enhance inflammation. They concluded that the zinc deficiency induced inflammatory responses at least partly by promoting irregular immune cell activation and altering promoter methylation.

Borghaei *et al.* [156] demonstrated that the zinc-binding protein -89 (ZIP-89) cooperates with NF-kB to regulate matrix metalloproteins (MMPs) expression as a response to inflammatory cytokine. MMPs have important roles in physiological tissue remodelling and wound healing as well as roles in the pathology of a range of disease conditions and transcriptional mechanisms are key in regulating healthy physiological level. ZBP-89 has been reported to bind the MMP-3 promoter at a site which is polymorphic, namely 5A/6A along with NF-kB. Tissue MMP-3 protein levels are affected by this polymorphism. They reported that ZBP-89 was necessary for maximal induction of both genes by IL-B and TNF_{α} and suggested a role for ZBP-89 in expression of MMP-1 and in inflammatory processes via interaction with NF-kB [156,157]. In 2010 Mocchegiani *et al.* [158] reported that the contradictory data from studies investigating the association between IL-6 polymorphisms, longevity and age-related diseases appear to be as a result of the interaction of these inflammatory processes with dietary intake, providing further evidence for a link among gene interaction and frailty in older age. Mocchegiani *et al.* [145] provide further supporting evidence of micronutrient-gene interactions related to inflammatory/immune response and antioxidant activity in ageing and propose that elucidating these associations could give a formula of personalised zinc supplementation or chelation to promote healthy ageing and long life.

4. GENETIC POLYMORPHISMS INVOLVED IN ZINC STATUS AND NON COMMUNICABLE DISEASE

Noncommunicable diseases (NCDs), also known as chronic diseases are the result of a combination of genetic, physiological, environmental and behaviours factors. The main types of NCDs are cardiovascular diseases, metabolic disorders, cancers and chronic respiratory diseases [159]. The metabolic disorder frequently known as the "metabolic syndrome" (MetS), is defined by a clustering of abdominal obesity, high level in serum concentration of triglycerides, a lower quantity level of high-density lipoprotein (HDL) cholesterol, high blood pressure and an increased fasting blood glucose level [160,161] .

In Europe the MetS prevalence is approximately 25% of adults, with a progressive increase in the elderly population [162]. Moreover, the heritability for MetS ranges from 10% to 30% [163,164]. Meta-analyses revealed a combined heritability estimate for Adult Treatment Panel-III MetS of 0.24 (95% CI, 0.11-0.36) and for the MetS severity score of 0.50 (95% CI, -0.05 to 0.99) [165], indicating that this syndrome is in part inheritable. Knowledge of the exact genetic factors underlying MetS development may help to explain why the features of MetS frequently co-occur within one individual [166].

Several studies have demonstrated a relationship between obesity and Zn homeostasis. In particular, a significant decrease of blood Zn levels and an increase of urinary concentrations have been found in obese patients [167-169]. Moreover, erythrocyte Zn levels have been found to be associated with BMI and waist circumference [170]. In obesity, low nutritional Zn status is also associated with the aggravation of obesity-related metabolic disturbances such as insulin resistance, inflammation, and altered lipid profile [44, 171] and numerous studies dealing with zinc supplementation reported an improvement of blood pressure, glucose, and LDL cholesterol serum level [172]. Zinc plays a role in insulin signal transduction through the modulation of insulin receptor phosphorylation and of the activities of phosphoinositide-3-kinase, phosphoinositide-dependent kinase 1, Akt/protein kinase B and glycogen synthase kinase 3, which are part of the insulin signaling pathway [173]. The interaction between zinc status, obesity, altered glucose metabolism and other metabolic disorders may be at least partially mediated by the pathological-induced modulation of zinc transporters that regulate cellular and intracellular Zn fluxes [174].

In recent years, polymorphisms in the solute carrier family 30 member 8 (SLC30A8) gene (ZnT8) with increased type 1 [175-180] and type 2 diabetes [177, 181-184] susceptibility were found (see also paragraph 2). In fact, a connection between the functionality of SLC30A8 has been observed and zinc concentration in plasma was shown able to influence glucose tolerance [185].

Type 1 diabetes is characterized by a destruction of pancreatic β-cells, resulting in absolute insulin deficiency causing hyperglycemia through the involvement of autoimmune-mechanisms and genetic and environmental factors, that can accelerate or slow down the clinical course of the disease [179]. The mechanisms involved in the autoimmune reaction against specific antigens of the pancreatic islands may involve ZnT8 which has been identified as a new target of cell mediated and humoral autoimmunity in T1DM [186]. The C allele of the rs13266634 SNP was found associated with younger age onset of T1DM patients [176] but not in a Swedish population [187]. Furthermore, Swedish subjects had a lower frequency of the R325 (C/C) genotype than the non-Swedes, which could reduce genetic predisposition to T1DM [186]. While rs11203203 was identified as a genetic marker in children, AA genotype conferred a higher risk of persistent islet autoimmunity and type 1 diabetes [188]. Both SLC30A8 polymorphisms were found to be associated with the HLA-DQ gene. However, further research will serve to better understand the role of SLC30A in the pathogenesis of type 1 diabetes across different population and its association with other genes and HLA which may permit major advances in future diagnostic and therapeutic approaches.

Type 2 diabetes is a progressive and chronic metabolic disease and it is characterized by peripheral insulin resistance and pancreatic beta β cell dysfunction with a growing interest in the role of zinc signaling in this disease [173,189]. GWAS of the past decade have identified variants in the human SLC30A8 gene as affecting the risk of Type 2 Diabetes [189]. Genome-wide association studies demonstrate an association of the above mentioned rs13266634 SNP of ZnT8 with decreased insulin release and T2DM susceptibility [43]. In particular, the common allele C of rs13266634 was associated with increased odds of T2DM in Europeans and Asians populations [186]. However, when Zn intake was taken into consideration, the C allele of rs13266634 was associated with lower odds of T2DM after zinc supplementation [182]. On the

other hand, Maruthur et al [190] reported that carriers of the T allele have increased insulin response after supplementation with Zn and thus may benefit more from Zn supplementation. A recent study identified 44 novel SLC30A8 variants; the minor alleles of rs2464591, rs2466296, rs2466297, and rs2466299 were associated with improvement in β-cell function, while carriers of the minor allele of rs2466293 had worsened functions. However, no association was observed between Zn intake and SNPs on diabetes incidence suggesting a limited role for dietary manipulation in affecting risk in relation to the SNPs identified [191].

As systematically reported in paragraph 2, there is another common SNP rs11558471 A/G in 3'-UTR of the SLC30A8 gene. A cross-sectional meta-analysis on 14 cohorts assessed the interaction of 20 genetic variants known to be related to glucose metabolism traits among individuals of European ancestry [192]. The strongest interaction effect was detected for rs11558471, where carriers of the A allele had increased fasting glucose. Moreover, a strong linkage disequilibrium between rs11558471 and rs13266634 was observed in this study. The results show that an increase in Zn intake decreases diabetes risk in A allele carriers, suggesting that Zn intake has an inverse association with fasting glucose plasma concentration.

All these observations highlight the need to generate personalized recommendations of Zn according to the genotype of SLC30A8 [43]. Similar results were also found in the Asian population [193-195]. In this population, another SNP (rs3802177) has been identified in the SLC30A8 gene which increases the risk of type 2 diabetes [196]. Rs3802177 showed the strongest association with T2D compared to the other SNP in the SLC30A8 gene [194]. Moreover, this SNP is strongly linked (r^2 = 0.83) to another SNP in the SLC30A8 gene, rs13266634 [196]. However only about 10% of the total hereditary risk of T2D can be attributed to SLC30A8 genes and this lack could be due to rare variants and epigenetic factors [197]. Flannick et al. [198] genotyped ~ 150,000 individuals across five ethnicities and identified 12 rare protein-truncating variants in SLC30A8 that overall have reduced risk of T2DM for over 60%. Of the 12 variants, two common protein-truncating variants (p.Arg138X and p.Lys34SerfsX50) were associated with T2DM protection. The association of common alleles of SLC30A8 polymorphisms with T2DM implicates that this is a susceptibility gene in T2DM, while loss of SLC30A8 function may have a protective effect in the diseases. With regard to epigenetics, a significant increase in DNA methylation levels in the SLC30A8 gene has been recently observed in patients with T2DM [197].

Independently of SNPs located within genes encoding for proteins involved in Zn metabolism and trafficking, allelic variants located in the gene region encoding for the zinc finger of transcription factors have been reported to play an important role in determining the genetic risk of metabolic disease. Among genetic polymorphisms involved in lipid metabolism and hypertension and associated with zinc, there is rs964184 within the Zinc Finger Protein 259 (ZNF259). Mirhafez and coworkers [199], in a study performed in an Iranian population, found that the risk of MetS is increased in individuals carrying the G allele with an OR of 2.52 (95% CI= 1.33- 4.77; P= 0.005). Likewise, others authors found a positive association between ZNF259 and serum lipid levels in different populations [200,201]. Wu et al. [202], performed a case-control study on 1,812 MetS patients and 2,036 controls from the Northeastern of China and found significant differences ($p < 0.05$) between the two population groups within the ZNF259 rs964184 and rs2075290 genotypes, that could thus be associated with triglycerides levels, blood pressure, abdominal obesity, fasting hyperglycemia and HDL-C levels. Similar results were found by Ueyama *et al*. [203] on an Japanese population including 1,822 subjects with MetS and 1,096 controls. A GWAS performed on 815 Hispanic children seeking genetic markers associated with obesity-related traits identified among several genes involved in obesity pathogenesis also variants in the APOA5-ZNF259 region, in particular linked with triglycerides levels (p = 2.5-4.8E-08) [204].

5. CONCLUSIONS

A constant daily supply of Zn is necessary to maintain an optimal nutritional status and health. Both zinc deficiency and excess may lead to important health impairment such as in immune function, cardiovascular diseases, type 2 diabetes. Zinc homeostasis is therefore critical to protect against infection and reduce the risk of inflammatory disease and other chronic pathological conditions. There is a growing body of evidence demonstrating links between the genetic factors and requirements for zinc and the consequential impact on immune function and other aspects of human health, especially and critically in ageing.

Zinc homeostasis is mediated by metallothioneins (MTs) and zinc transporters. Zinc

status and specific polymorphisms of genes coding for zinc-transporters have been in fact associated with chronic diseases. Base mutation experiments have demonstrated that specific DNA mutations can modify the structure and the expression of MTs and affect their ability to bind metal, through the impairment of transcription factors' activity to MTs' regulatory elements [51]. Indeed, as here summarized, SNPs affect several MTs functions linked to metal binding, from cancer to metabolic syndrome-related pathologies and their complications. Overall, 18 MT SNPs with an impact on pathological processes are known and 16 are in non-coding regions. They are present in every MT gene class, excluding 3 out 8 MT1 genes (E, F, G and H) though 12 SNPs occur in this class. Main implications are on metal disbalance (8 SNPs) and cancer (6 SNPs). Metal-related functions are also impaired by specific SNPs in ZnT genes and a total of 28 SNPs are involved in pathological processes. Their effects include insulin levels [120-123] and severe complications like dyslipidemia and diabetes [94-110,125-127, 131-139]. However, differently from MT SNPs, the majority of ZnT variants involves non-synonymous changes in the coding region of genes (16 out of 28). Variants belong mainly to ZnT2 and ZnT8 genes, according to genes' roles in insulin secretion [88,89]. Nonetheless, like for MTs, other functions, linked to cardiovascular [128], are impaired.

New studies are needed to allow a more comprehensive understanding of MT and ZnT mechanisms of action and of the effects of their common genetic variants. Moreover, the clear impact of zinc in diseases strengthens the need of more studies in transporters' role in the regulation of physiological processes, for the set-up of accurate biomarkers able to monitor and detect subtle changes in cellular zinc homeostasis. Genetic studies have demonstrated that common DNA polymorphisms in some metallothioneins and zinc transporters confer susceptibility for some chronic diseases but the information is still limited and there is a need to better clarify the interactions between genes and other molecules. Furthermore, when considering the assumption of Zn the results remain controversial. This highlights the importance in the near future to clarify gene-nutrient interactions and provide a clear understanding of any intervention requirements.

There is a need to communicate this scientific information to health professionals in a way, which will allow them to translate this into dietary and behavioural recommendations which will promote human health, particularly for the elderly.

LIST OF ABBREVIATIONS (not included in the text)

Irs2 = insulin receptor substrate

Akt = Protein kinase B (PKB),

kDa = kilodalton

UTR = Untranslated region

CDF = Cation Diffusion Facilitator

NF-κB =nuclear factor kappa-light-chain-enhancer of activated B cells

IL-6 = Interleukin 6

NCBI = National Center for Biotechnology Information

MRE = Metal response element

KO mice =knockout

NRF2 = Nuclear factor E2-related factor 2

PARP-1 = Poly (ADP-ribose) polymerase-1

HSP70 = Heat Shock Protein 70 kilodaltons

TNFα = Tumor necrosis factor alpha

RANTES = Regulated on activation, normal T-cell expressed, and secreted

APOA5 = Apolipoprotein A5

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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