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ROZPRAWA DOKTORSKA

GENETYCZNE ASPEKTY OTYŁOŚCI I ZESPOŁU METABOLICZNEGO U OSÓB ZAWODOWO NARAŻONYCH NA ARSEN I NIEKTÓRE METALE CIĘŻKIE

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WPROWADZENIE

Związki arsenu, ołowiu i kadmu w znamienny sposób zaburzają prawidłową czynność organizmu człowieka. Znaczenie kliniczne toksyczności arsenu, ołowiu i kadmu dotyczy przede wszystkim narażenia przewlekłego: narażenia zawodowego osób pracujących w niektórych gałęziach przemysłu, oraz narażenia środowiskowego, zwłaszcza populacji osób zamieszkujących obszary przemysłowe.

Następstwa przewlekłego narażenia na arsen dotyczą układu oddechowego (perforacja przegrody nosa, zapalenie górnych dróg oddechowych), układu krążenia (zmiany elektrokardiograficzne i zespół Raynauda), skóry (hiperpigmentacja, hiperkeratoza, „choroba czarnej stopy"), układu pokarmowego (uszkodzenie wątroby), układu nerwowego (neuropatia obwodowa, utrata słuchu). Arsen jest uznanym kancerogenem; powoduje nowotwory płuc, skóry, pęcherza moczowego, nerek i wątroby. Posiada słabe właściwości mutagenne.

Zaburzenia będą konsekwencją przewlekłej ekspozycji na ołów dotyczą układu krwiotwórczego (niedokrwistość i retikulocytoza), układu oddechowego, układu nerwowego (encefalopatia, neuropatia, zaburzenia neuropsychologiczne), nerek (nefropatia i niewydolność nerek), układu pokarmowego (podwyższenie aktywności aminotransferaz wątrobowych) i układu sercowo-naczyniowego (nadciśnienie tętnicze, zaburzenia rytmu serca, miażdżyca). W klasyfikacji substancji o działaniu kancerogennym ołów uznano za przypuszczalnie kancerogenny; narażenie na ołów może skutkować rozwojem nowotworów układu oddechowego, pęcherza moczowego i nerek.

Przewlekła ekspozycja na kadm skutkuje niekorzystnymi zmianami dotyczącymi w pierwszej kolejności nerek (uszkodzenie nerek typu kanalikowego, kamica nerkowa) i układu

kostnego (osteoporoza, osteopenia, osteomalacja, „choroba Itai-itai”); mogą wystąpić ponadto nieprawidłowości układu oddechowego (rozedma płuc), układu krążenia (nadciśnienie tętnicze, niedokrwistość) i układu nerwowego (utrata węchu). Kadm uznany został za czynnik o udowodnionym działaniu rakotwórczym u ludzi; w pierwszej kolejności w stosunku do nowotworów płuc, być może również nowotworów przewodu pokarmowego i jąder. Prenatalne narażenie na kadm jest czynnikiem teratogennym.

Według Światowej Organizacji Zdrowia na świecie żyje około 1,6 miliarda ludzi z nadwagą, osoby otyłe to ponad 522 mln. Otyłość jest nie tylko problemem medycznym, który przyczynia się bezpośrednio do 10-13% przedwczesnych zgonów w regionie europejskim, ale również ekonomicznym – koszty związane z otyłością stanowią od 2 do 7% nakładów na ochronę zdrowia. Otyłość stanowi składową tzw. zespołu metabolicznego, definiowanego jako współwystępowanie czterech uznanych czynników ryzyka sercowo-naczyniowego: otyłości brzusznej, hiperglikemii, nadciśnienia tętniczego i dyslipidemii.

Badania, które mają na celu wyjaśnienie zarówno przyczyn powstawania zespołu, jak i epidemicznego występowania jego wieloobjawowych form, z jednej strony opierają się na analizach genetycznych i genotypowych, a z drugiej strony na obserwacjach wpływów środowiska zewnętrznego. Wykryto wiele zaburzeń genetycznych kształtujących predyspozycję do powstawania zaburzeń ujawnianych przez wpływy środowiskowe. Wśród genów o najsilniejszym związku z zespołem metabolicznym wyróżnia się następujące geny: LEP – gen promotora leptyny, FTO- gen otyłości, MC4R – gen receptora 4 melanokortyny i CNR1 – gen receptora kannabinoidowego typu 1. Samo stężenie arsenu w organizmie jest zależne również od niewielkich zmian genetycznych – polimorfizmu genów, biorących udział w metabolizmie arsenu. Mogą to być polimorfizmy genów PNP (fosforylaza nukleozydów

purynowych), GSTO-1 (glutationowa S-transferaza omega 1) czy As3MT (metylotransferaza arsenowa).

Istnieje coraz więcej doniesień na temat wpływu metali ciężkich na rozwój zespołu metabolicznego. W dotychczasowych badaniach stwierdzano m. in. prawdopodobne istnienie zależności pomiędzy stężeniem arsenu a występowaniem cukrzycy typu 2, istotny związek pomiędzy ekspozycją na ołów a rozwojem zespołu metabolicznego czy istotny wpływ kadmu w patogenezie dyslipidemii.

ZAŁOŻENIA I CELE PRACY

W ujęciu globalnym, narażenie na metale jest narażeniem mieszanym, dotyczy przede wszystkim arsenu, ołowiu i kadmu, a jego źródłem jest szeroko pojęte zanieczyszczenie środowiska. Duży problem stanowią jednak narażenia spowodowane lokalnymi uwarunkowaniami, które, choć o mniejszym zasięgu, mogą dotyczyć tysięcy czy nawet milionów osób. Specyficzną i szczególną grupę stanowią osoby zawodowo narażone na arsen, ołów i kadm. Wszelkie uwarunkowania mogą być u nich widoczne wyraźniej i być łatwiejsze do zidentyfikowania niż w populacji ogólnej. Z drugiej strony, otyłość i zespół metaboliczny dotykają tej grupy w tym samym stopniu, co populacji ogólnej.

Wpływ arsenu, ołowiu i kadmu na rozwój zespołu metabolicznego jest już znany od dłuższego czasu. Kolejne, nowe badania tłumaczą mechanizmy komórkowe tego zjawiska oraz pokazują jego złożoność. Przede wszytkim podkreślają jednak, że ten wpływ jest istotniejszy, niż do niedawna sądzono i wskazują na konieczność ograniczenia narażenia populacji na ksenobiotyki. Narażenie to ocenia się mierząc stężenie ołowiu we krwi pełnej, stężenie wolnych cynkoprotoporfiryn w erytrocytach, będących czułym markerem tkankowych depozytów ołowiu, stężenie kadmu we krwi pełnej oraz stężenie arsenu (lub jego metabolitów – MMA i DMA) w moczu.

Odrębnym aspektem jest indywidualna wrażliwość na toksyczność metali i zdolność ich absorpcji. Warunkowana jest ona między innymi drobnymi zmianami genetycznymi, takimi jak polimorfizm pojedynczego nukleotydu w poszczególnych genach. Zmiany te mogą dotyczyć zarówno genów odpowiedzialnych za rozwój zespołu metabolicznego (jak geny FTO, LEP, MC4R, CNR1), jak i za metabolizm arsenu (PNP, GSTO-1, As3MT), ołowiu (ALAD, HFE, VDR), czy kadmu (cgMT1).

Celem rozprawy było oszacowanie związku pomiędzy stężeniem niektórych metali i metaloidów z określonymi konstelacjami polimorficznymi genów, uczestniczących w rozwoju cukrzycy i innych elementów składowych zespołu metabolicznego (otyłości, insulinooporności) oraz metabolizmie arsenu w populacji osób narażonych zawodowo.

Cele poszczególnych prac składających się na rozprawę doktorską stanowiły:

1. Ocena zależności pomiędzy zmiennością genów odpowiedzialnych za rozwój zespołu metabolicznego (genu receptora kannabinoidowego 1 - CNR1, genu receptora melanokortyny 4 - MC4R, genu promotora leptyny - LEP G-2548A, genu dioksygenazy zależnej od alfa-ketoglutaranu – FTO, oraz genu receptora witaminy D – FokI) a stężeniem arsenu, ołowiu i kadmu we krwi.
2. Ocena relacji pomiędzy stężeniem arsenu (wyrażonym jako stężenie całkowitego arsenu w moczu) a polimorfizmem genów odpowiedzialnych za metabolizm arsenu (genu fosforylazy nukleozydów purynowych - PNP, genu glutationowej S-transferazy omega - GSTO-1, czy genu metylotransferazy arsenowej - As3MT).
3. Uporządkowanie i ugruntowanie posiadanej dotychczasowego stanu wiedzy dotyczącej genetycznych aspektów otyłości i zespołu metabolicznego u osób zawodowo narażonych na arsen i niektóre metale ciężkie wraz ze wskazaniem potencjalnych kierunków dalszych badań.

OMÓWIENIE

W pierwszej pracy włączonej do cyklu stanowiącego rozprawę doktorską zatytułowanej *,,The relationship between selected CNR1, MC4R, LEP G-2548A, FTO and VDR FokI gene polymorphisms and several basic toxicological parameters among persons occupationally exposed to arsenic, cadmium and lead*" przebadano 85 pracowników huty miedzi zawodowo narażonych na ołów, arsen i kadm, w wieku 26-67 lata.

U wszystkich badanych osób wykonano oznaczenia podstawowych markerów laboratoryjnych i toksykologicznych. Określono stężenia ołowiu (Pb-B), kadmu (Cd-B), protoporfiryn cynkowych (ZnPP), cynku i miedzi (Zn-S i Cu-S) we krwi. Oceniano także całkowite stężenie arsenu (As-U) w moczu. Wykonano morfologię krwi obwodowej oraz określono stężenia kreatyniny, mocznika, aminotransferaz, bilirubiny, glukozy, cholesterolu całkowitego, cholesterolu LDL, cholesterolu HDL i triglicerydów we krwi. Oceniane były również markery gospodarki wapniowo-fosforanowej, określano stężenia wapnia, fosforanów, 25-OH witaminy D i parathormonu we krwi. W zasadniczym etapie dokonano natomiast oceny wybranych polimorfizmów pojedynczego nukleotydu dla genu receptora kannabinoidowego 1 (CNR1), genu receptora melanokortyny 4 (MC4R), leptyny (LEP G-2548A), genu dioksygenazy zależnej od alfa-ketoglutaranu (FTO) i receptora witaminy D FokI. Oceniano cztery polimorfizmy CNR1: rs806381, rs806368, rs1049353, rs12720071, jeden polimorfizm genu MC4R: rs17782313, jeden polimorfizm genu leptyny LEP G-2548A: rs7799039, jeden polimorfizm genu MC4R: rs17782313, jeden polimorfizm genu leptyny LEP G-2548A: rs7799039 oraz jeden polimorfizm genu FTO V39609I rs358.

Wykazano, że homozygotyczność AA w locus rs806381 w genie CNR1 wiąże się ze znamiennie niższym stężeniem arsenu w moczu w porównaniu z heterozygotycznością AG

i homozygotycznością GG. Obecność natomiast allelu G w tym locus wiąże się ze znamiennie wyższym stężeniem arsenu w moczu. W locus rs12720071 genu CNR1, homozygoty GG charakteryzowały się znamiennie wyższym stężeniem arsenu w moczu niż heterozygoty AG i homozygoty AA. Allel A w tym locus może być powiązany ze znamiennie niższym stężeniem arsenu w moczu. Analiza wykazała również, że allel A w locus rs1049353 genu CNR1 może odpowiadać za niższe stężenia arsenu w moczu, a allel G w locus rs7799039 genu LEP G-2548A może być odpowiedzialny za istotnie wyższe stężenie arsenu w moczu badanych. Wykazano także, że allel T w locus rs10735810 genu VDR FokI może być czynnikiem odpowiedzialnym za znamiennie niższe stężenie kadmu we krwi badanych. W badanej grupie homozygoty GG w locus rs1049353 genu CNR1 miały znamiennie wyższe stężenie ołowiu we krwi w porównaniu z heterozygotami AG i homozygotami AA. Stwierdzono, że heterozygotyczność AG w locus rs1049353 genu CNR1 może skutkować znamiennie niższym stężeniem ZnPP we krwi w porównaniu z homozygotycznością AA i GG, a allel A może odpowiadać za niższe stężenie ZnPP we krwi. Poza tym wykazano, że allel A w locus rs9939609 genu FTO jest związany ze znamiennie wyższym stężeniem ZnPP we krwi.

W drugiej pracy oryginalnej włączonej do cyklu zatytułowanej *,,The relationship between PNP, GSTO-1, AS3MT and ADRB3 gene polymorphisms and urinary arsenic concentration among copper smelter and refinery employers"* przebadano 113 pracowników huty miedzi zawodowo narażonych na ołów, arsen i kadm, w wieku 26-63 lata.

W grupie badanej określano stężenia we krwi ołowiu (Pb-B), kadmu (Cd-B), protoporfiryn cynkowych (ZnPP), cynku i miedzi (Zn-S i Cu-S). Oceniano także całkowite stężenie arsenu (As-U) w moczu. Wykonano także morfologię krwi obwodowej i podstawowe

badania biochemiczne, w tym stężenie kreatyniny, mocznika, aminotransferaz, bilirubiny, glukozy, cholesterolu całkowitego, cholesterolu LDL, cholesterolu HDL i triglicerydów. W zasadniczym etapie badań wykonano oznaczenia polimorfizmów genów PNP (rs1130650), GSTO-1 (rs4925), As3MT (rs11191439) i ADRB3 (rs4994).

Porównanie podgrup wyodrębnionych w oparciu o kryterium polimorfizmu typu rs1130650 genu PNP wykazało, że w podgrupach homozygot CC i heterozygot CT w porównaniu do podgrupy homozygot TT znamiennie częściej wartości As-U były równe lub wyższe od mediany uzyskanej dla całej badanej grupy. Przy pomocy analizy porównawczej podgrup wyodrębnionych w oparciu o kryterium polimorfizmu rs4925 genu dla GSTO-1 wykazano, że homozygoty AA charakteryzowały się znamiennie wyższymi średnimi wartościami As-U niż heterozygoty AC i homozygoty CC. Ponadto w podgrupie homozygot AA istotnie statystycznie częściej w stosunku do podgrup heterozygot AC i homozygot CC wartości As-U mieściły się w zakresach: równe lub wyższe od mediany uzyskanej dla całej badanej grupy; równe lub wyższe od 3. kwartyla uzyskanego dla całej badanej grupy oraz wyższe od wartości MAC. Stosując analizę porównawczą podgrup nie wykazano natomiast znamiennych różnic pomiędzy podgrupami wyodrębnionymi w oparciu o kryterium polimorfizmu typu rs11191439 genu As3MT oraz polimorfizmu typu rs4994 genu ADRB3.

W 3. pracy cyklu, którą stanowi praca poglądowa pt. *,,Genetyczne aspekty otyłości i zespołu metabolicznego u osób zawodowo narażonych na arsen i niektóre metale ciężkie*" zwrócono uwagę, że otyłość jest powszechnym problemem zdrowotnym i dotyczy ponad 650 milionów osób na świecie. Jest integralnym elementem zespołu metabolicznego, prowadzi do

rozwoju nadciśnienia tętniczego i cukrzycy typu 2, ale też wielu różnych innych schorzeń, w tym licznych nowotworów. Podkreślono, że jednocześnie coraz większą wagę przywiązuje się do wpływu narażenia na arsen, ołów i kadm na rozwój zespołu metabolicznego. Zaznaczono, że wpływ ten środowiskowo dotyczy całej populacji, jednak jest szczególnie widoczny w populacji narażonej zawodowo. W pracy podkreślono, że analizując zarówno zespół metaboliczny, jak i narażenie na metale, nie sposób ominąć dużej zmienności genetycznej w populacji. Dotyczy ona polimorfizmu pojedynczych nukleotydów genów współodpowiedzialnych za rozwój zespołu metabolicznego, genów odpowiedzialnych za metabolizm arsenu, ale też ekspresji mikro RNA. Dlatego też nie sposób mówić o wpływie metali na rozwój zespołu metabolicznego nie uwzględniając tej zmienności genetycznej. Przedstawiono wybrane, najlepiej poznane elementy zmienności genetycznej i jej znaczenie w rozwoju zespołu metabolicznego z uwzględnieniem narażenia na arsen, ołów i kadm. Polimorfizm pojedynczych nukleotydów jest dość dobrze poznany. Poszczególne warianty genów leptyny, CNR1, FTO związane są z większym ryzykiem rozwoju zespołu metabolicznego, a pewnie warianty genów odpowiedzialnych za metabolizm arsenu (As3MT) i ołowiu (ALAD) związane są z ich większą toksycznością. Natomiast wiedza o związku narażenia na arsen, ołów i kadm a polimorfizmami genów odpowiedzialnych za rozwój zespołu metabolicznego jest niewielka. Luki pozbawione są nowsze badania, oparte o ocenę ekspresji mikro RNA. W pracy zauważono, że ekspresja pewnych miRNA pozwala wykryć zarówno narażenie na arsen, jak i zwiększone ryzyko epizodów sercowo-naczyniowych w przyszłości, a także istnienie uszkodzeń narządowych już w chwili obecnej. Zasugerowano, że ugruntowanie i wykorzystanie wiedzy o miRNA wydaje się słusznym kierunkiem, jednak nie powinno zapominać się o posiadanej już wiedzy o polimorfizmie pojedynczych nukletotydów.

WNIOSKI

1. Polimorfizm pojedynczych nukleotydów w genach odpowiedzialnych za rozwój zespołu metabolicznego może mieć wartość prognostyczną dla osób zawodowo narażonych na ołów, kadm i arsen.
2. U osób zawodowo narażonych na arsen, ołów i kadm allel G w locus rs806381 genu CNR1 i w locus rs7799039 genu LEP mogą być odpowiedzialne za wyższe stężenia arsenu, natomiast allel A w locus rs1049353 i rs12720071 genu CNR1 może odpowiadać za niższe stężenia arsenu.
3. Stężenie kadmu u osób narażonych może być uwarunkowane polimorfizmem genu VDR FoK1. Allel T w locus rs10735810 genu VDR FokI może być odpowiedzialny za niższe stężenia kadmu.
4. U osób narażonych na ołów, kadm i arsen istnieją zależności pomiędzy markerami narażenia na ołów (stężenie ołowiu i cynkoprotoporfiryn we krwi) a polimorfizmami genu CNR1 i FTO. Allel A w locus rs1049353 genu CNR1 może odpowiadać za niższe stężenia ołowiu i cynkoprotoporfiryn a allele A w locus rs9939609 genu FTO może odpowiadać za wyższe stężenia cynkoprotoporfiryn.
5. Polimorfizm rs17782813 genu MC4R nie jest powiązany ze stężeniami arsenu, ołowiu i kadmu w badanej grupie osób.
6. Wykazano istnienie zależności pomiędzy wybranymi polimorfizmami genów PNP i GSTO-1 a stężeniem arsenu w moczu u osób zawodowo narażonych na związki arsenu, co może potwierdzać hipotezę o genetycznie uwarunkowanej skłonności do absorpcji metali toksycznych przez osoby eksponowane na nie zawodowo.

7. Osoby zawodowo narażone na związki arsenu posiadające allel T w układzie homozygotycznym w locus rs 1130650 genu PNP zdają się być predysponowane do niższych stężeń As-U.
8. Homozygotyczność AA w locus rs 4925 genu dla GSTO-1 może skutkować wyższymi stężeniami As-U.
9. Wśród osób zawodowo narażonych na arsen genotypy locus rs 11191439 genu As3MT oraz locus rs4994 genu ADRB3 nie wpływają istotnie na stężenie arsenu w moczu, jednak obecność niektórych alleli w tych locus determinuje skłonność do większej ustrojowej absorpcji arsenu.
10. U osób szczególnie predysponowanych do wchłaniania arsenu, posiadających określone konfiguracje polimorficzne w zakresie genów PNP, As3MT, GSTO-1 i ADRB-3, należy prowadzić systematyczną ocenę odległych skutków zdrowotnych ekspozycji, w szczególności skrining onkologiczny, kontrolę ciśnienia tętniczego oraz analizę parametrów gospodarki lipidowej i węglowodanowej. W grupie tej należy także wprowadzać wcześnie metody leczenia niefarmakologicznego nadciśnienia tętniczego i innych elementów zespołu metabolicznego (cukrzycy, hiperlipidemii i otyłości).
11. Ugruntowanie i wykorzystanie wiedzy o mikroRNA wydaje się słusznym kierunkiem badań nad zależnościami pomiędzy narażeniem na arsen, ołów i kadm a rozwojem zespołu metabolicznego, jednak nie powinno zapominać się o znaczeniu polimorfizmów pojedynczych nukletotydów, próbując jednocześnie identyfikować kolejne genotypy predysponujące do potencjalizacji ryzyka sercowo-naczyniowego w aspekcie narażenia środowiskowego i zawodowego.

STRESZCZENIE

Otyłość nazywana jest cichą plagą XXI wieku. Dotyczy milionów osób na świecie (a nadwaga miliardów), jest podstawową składową zespołu metabolicznego i pociąga za sobą szereg kolejnych schorzeń. Jest bardzo istotnym czynnikiem ryzyka rozwoju całego spektrum chorób układu sercowo-naczyniowego i zwiększa częstość występowania nowotworów, przyczyniając się tym samym do zwiększania liczby zgonów wśród osób dorosłych w krajach rozwiniętych. Wpływ na rozwój otyłości mają zarówno czynniki genetyczne, jak i środowiskowe. Znanych jest szereg genów, których różne warianty, na przykład określone polimorfizmy pojedynczych nukleotydów, związane są z rozwojem otyłości. Geny te mogą być odpowiedzialne na przykład za regulację uczucia głodu i sytości, a osoby z pewnymi wariantami polimorficznymi tych genów mają wyższy wskaźnik BMI czy spożywają więcej kilokalorii na dobę niż osoby z innymi, korzystniejszymi wariantami genetycznymi. Innym czynnikiem modyfikującym powikłania otyłości i zespołu metabolicznego, są czynniki środowiskowe, a w szczególności narażenie na arsen, ołów i kadm. Arsen jest znanym czynnikiem wpływającym na powstawanie insulinooporności i cukrzycy typu 2, kadm powoduje uszkodzenie naczyń i działa aterogennie, a stężenie ołowiu jest na przykład związane ze stężeniem trójglicerydów. Liczne badania pokazują z jednej strony wpływ arsenu, ołowiu i kadmu na rozwój składowych zespołu metabolicznego, z drugiej natomiast - wpływ polimorfizmu genów na rozwój otyłości. Brakuje jednak badań łączących te elementy. Celem badań było oszacowanie związku pomiędzy stężeniem niektórych metali ciężkich i arsenu z określonymi konstelacjami polimorficznymi, uczestniczącymi w rozwoju cukrzycy i innych elementów składowych zespołu metabolicznego (otyłości, insulinooporności) oraz metabolizmie arsenu. W pierwszej pracy poszukiwaliśmy związku pomiędzy polimorfizmami

genów, odpowiedzialnych za rozwój otyłości, a stężeniami arsenu, ołowiu i kadmu. Wykazaliśmy liczne zależności pomiędzy wszystkimi badanymi metalami a genami wpływającymi na rozwój otyłości. W drugiej pracy wykazaliśmy natomiast związek pomiędzy stężeniem arsenu a polimorfizmami genów, odpowiedzialnych za produkcję enzymów uczestniczących w jego metabolizmie. Uzyskane zależności wskazują, że polimorfizmy badanych genów mogą mieć znaczenie prognostyczne nie tylko w kontekście rozwoju otyłości, ale także przy niekorzystnym działaniu metali. Trzecia praca podsumowuje dotychczasową wiedzę na temat narażenia na arsen, ołów i kadm oraz wskazuje potencjalne dalsze kierunki badań.

Uzyskane wyniki pozwalają zidentyfikować osoby szczególnie narażone na rozwój otyłości i zespołu metabolicznego oraz w większym stopniu absorbujące arsen, ołów i kadm ze środowiska. Szczególnie niedogodną sytuacją jest istnienie szeregu niekorzystnych wariantów polimorficznych u jednej osoby. U takich osób z jednej strony zwiększa się ryzyko rozwoju zespołu metabolicznego i jego powikłań związane z istnieniem niesprzyjających form genów, a z drugiej strony wyższe stężenia biologiczne metali mogą te powikłania nasilać i przyśpieszać. W chwili obecnej polimorfizmy genetyczne i ekspozycja na ksenobiotyki nie są uwzględniane w żadnej stratyfikacji oceny ryzyka sercowo-naczyniowego. Zidentyfikowanie osób predysponowanych genetycznie do powstania zespołu metabolicznego pozwoli na objęcie ich szczególną opieką i zintensyfikowanie działań mających na celu obniżenie ryzyka sercowo-naczyniowego, zarówno poprzez edukację, postępowanie niefarmakologiczne, jak i wcześniejszą interwencję farmakologiczną. Umożliwi także, w przypadku osób zawodowo narażonych na arsen, ołów i kadm, racjonalne planowanie i organizację stanowiska pracy i skuteczniejszą profilaktykę za pomocą dedykowanych środków ochrony osobistej. Niniejsza

praca pokazuje również, iż analiza wyników badań toksykologicznych i genetycznych w oparciu o wzajemne zależności daje możliwość lepszej i wielokierunkowej identyfikacji czynników ryzyka, a to z kolei niesie szansę na zmniejszenie śmiertelności wśród osób szczególnie narażonych na rozwój chorób układu sercowo-naczyniowego.

SUMMARY

Obesity is called a silent plague of 21st century. It affects millions of people (and billions are overweight) and it is a basic compound of metabolic syndrome, leading to further illnesses. Obesity is a major risk factor of cardiovascular diseases and increases risk of many tumors, therefore contributing to an increase in deaths in developed societies. Both genetic and environmental factors affect development of obesity. Numerous variants of genes are known to contribute to obesity, including single nucleotide polymorphisms of certain loci. These genes are responsible for instance for hunger – satiety regulation or individuals with certain polymorphic variants have higher BMI or consume more kilocalories than others, with more profitable variants. Other factors modifying complications from obesity and metabolic syndrome are environmental factors, especially exposure to arsenic, lead and cadmium. Arsenic is known to induce insulin resistance and type 2 diabetes; cadmium causes damage to arteries and leads to faster atherosclerosis and lead is for example correlated with higher triglycerides. There are numerous studies showing how arsenic, cadmium and lead contribute to metabolic syndrome and other studies that show relations between single nucleotide polymorphism and obesity. There are though very few studies that combine these two factors. The purpose of this study was to assess relationships between arsenic, cadmium and lead concentration and polymorphic variants of genes that take part in development of obesity, metabolic syndrome and arsenic metabolism. In our first study we were looking for relationships between polymorphisms of genes affecting development of obesity and arsenic, cadmium and lead concentration. As in previous work, we were able to detect many correlations between studied metals and genetic variations. In our second study we were able to show relationship between arsenic concentration and polymorphisms of genes responsible

for its metabolism. It demonstrates that harmful exposure effect may depend on individual's genetic variations. It shows that polymorphisms of these genes may have prognostic value not only concerning obesity, but also toxic effect of metals. Third study summarizes our knowledge about arsenic, cadmium and lead exposure and shows eventual directions for further studies.

Obtained results allow us to identify individuals particularly at risk of development of obesity with metabolic syndrome and individuals that absorb more arsenic, lead and cadmium from environment. Especially unfavorable situation takes place when one individual has numerous detrimental genetic variations. Those persons have increased cardiovascular risk due to genetic variants responsible for development of obesity and, on the other hand, greater exposure to metals will increase this risk even more. Currently, genetic polymorphisms and exposure to xenobiotics are not considered in current typical cardiovascular risk assessment. Identifying individuals genetically predisposed to metabolic syndrome will let us provide them with extensive care to lower their cardiovascular risk. It could be done by education, non-pharmacological treatment, and earlier pharmacological intervention. It enables, in persons occupationally exposed to arsenic, cadmium and lead, to rationally plan and organize their workplace and provide them with personalized protective equipment. This work shows that analysis of both toxicological and genetic parameters based on mutual dependencies gives us a possibility to identify cardiovascular risk better and in a more multidirectional way, therefore giving us a chance to lower mortality amongst individuals particularly at risk of development of cardiovascular diseases.

PIŚMIENNICTWO

1. Baker BA, Cassano VA, Murray C; ACOEM Task Force on Arsenic Exposure. Arsenic exposure, assessment, toxicity, diagnosis, and management: guidance for occupational and environmental physicians. J Occup Environ Med. 2018;60(12):e634-e639.
2. Charkiewicz AE, Backstrand JR. Lead toxicity and pollution in Poland. Int J Environ Res Public Health. 2020;17(12):4385.
3. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. Int J Environ Res Public Health. 2020;17(11):3782.
4. WHO. Obesity and overweight – key facts. https://www.who.int/newsroom/fact-sheets/detail/obesity-and-overweight (dostęp: 2020.01.14).
5. Kuo CC, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr Diab Rep. 2013;13(6):831-849.
6. Agusa T, Fujihara J, Takeshita H, et al. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. Int J Mol Sci. 2011; 12: 2351–2382.
7. Rhee SY, Hwang YC, Woo JT, Sinn DH et al. Blood lead is significantly associated with metabolic syndrome in Korean adults: an analysis based on the Korea National Health and Nutrition Examination Survey (KNHANES), 2008. Cardiovasc. Diabetol. 2013, 12, 9.
8. Rafati Rahimzadeh M, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 2017;8(3):135-145.

9. Antonelli R, Shao K, Thomas DJ et al. AS3MT, GSTO, and PNP polymorphisms: impact on arsenic methylation and implications for disease susceptibility. Environ Res 2014; 132: 156–167.
10. Thomas DJ: Arsenolysisandthiol-dependent arsenate reduction. Toxicol.Sci. 2010, 117,249–252.
11. Yan J, Wang X, Tao H, Yang W, Luo M, Lin F. Lack of association between leptin G-2548A polymorphisms and obesity risk: Evidence based on a meta-analysis. Obes Res Clin Pract. 2015; 9(4): 389–97.
12. Manriquez V, Aviles J, Salazar L, Saavedra N, Seron P, Lanas F, et al. Polymorphisms in Genes Involved in the Leptin-Melanocortin Pathway are Associated with Obesity-Related Cardiometabolic Alterations in a Southern Chilean Population. Mol Diagn Ther. 2018; 22(1): 101–113.
13. Nowzari Z, Masoumi M, Nazari-Robati M, Akbari H, Shahrokhi N, Asadikaram G. Association of polymorphisms of leptin, leptin receptor and apelin receptor genes with susceptibility to coronary artery disease and hypertension. Life Sci. 2018; 207: 166–171.
14. Pawlak M, Milewicz A. Rola układu endokannabinoidowego i polimorfizmów genu CNR1 w powstawaniu otyłości. Endokrynologia, Otyłość i Zaburzenia Przemiany Mater. 2011: 192–196.
15. de Luis DA, Izaola O, Aller R, Lopez JJ et al. Association of G1359A polymorphism of the cannabinoid receptor gene (CNR1) with macronutrient intakes in obese females. J Hum Nutr Diet. 2016; 29(1): 118–123.

16. Doaei S, Mosavi Jarrahi SA, Sanjari Moghadam A et al. The effect of rs9930506 FTO gene polymorphism on obesity risk: a meta-analysis. Biomol Concepts. 2019; 10(1): 237–242.
17. Lappalainen TJ, Tolppanen A-M, Kolehmainen M et al. The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the Finnish Diabetes Prevention Study. Obesity (Silver Spring). 2009; 17(4): 832–836.
18. Tschritter, O, Haupt A, Preiss H. An obesity risk SNP (rs17782313) near the MC4R gene is associated with cerebrocortical insulin resistance in humans. J. Obes. 2011, 2011, 283153.
19. Wysoczańska-Klaczyńska, A, Ślęzak A, Hetman M, Barg E. The impact of VDR gene polymorphisms on obesity, metabolic changes, bone mass disorders and neoplastic processes. Pediatr. Endocrinol. Diabetes Metabol. 2018, 24, 96–105.

WYKAZ PUBLIKACJI WŁĄCZONYCH DO ROZPRAWY DOKTORSKIEJ

Tomasz Matys

Cykl publikacji stanowiących podstawę doktoratu

Lp.	Tytuł, autorzy, źródło	IF	PK
1.	**The relationship between selected CNR1, MC4R, LEP, FTO and VDR gene polymorphisms and several basic toxicological parameters among persons occupationally exposed to arsenic, cadmium and lead.** [AUT.] TOMASZ MATYS, ANNA SZYMAŃSKA-CHABOWSKA, KATARZYNA BOGUNIA-KUBIK, BEATA SMYK, MAŁGORZATA KAMIŃSKA, GRZEGORZ MAZUR, RAFAŁ PORĘBA, [AUT. KORESP.] PAWEŁ GAĆ. *J.Clin.Med.* 2020 Vol.9 no.4 art.1040 [15 s.], tab. bibliogr. 27 poz. summ. DOI: 10.3390/jcm9041040	3,303	140,00
2.	**The relationship between PNP, GSTO-1, AS3MT and ADRB3 gene polymorphisms and urinary arsenic concentration among copper smelter and refinery employers.** [AUT.] A[NNA] SZYMAŃSKA-CHABOWSKA, T[OMASZ] MATYS, Ł[UKASZ] ŁACZMAŃSKI, K. CZERWIŃSKA, A[GNIESZKA] JANUS, B[EATA] SMYK, G[RZEGORZ] MAZUR, R[AFAŁ] PORĘBA, [AUT. KORESP.] P[AWEŁ] GAĆ. *Hum.Exp.Toxicol.* 2020 Vol.39 no.11 s.1443-1453, tab. bibliogr. 37 poz. summ. DOI: 10.1177/0960327120925891	2,067	70,00
3.	**Genetic aspects of obesity and metabolic syndrome in people occupationally exposed to arsenic and certain heavy metals.** [AUT.] TOMASZ MATYS, ANNA SZYMAŃSKA-CHABOWSKA, RAFAŁ PORĘBA, GRZEGORZ MAZUR, PAWEŁ GAĆ. *Med.Śr.* 2019 T.22 nr 1-2 s.29-32, bibliogr. 30 poz. streszcz. summ, Publikacja w czasopiśmie spoza listy MNiSW. DOI: 10.26444/ms/122202	0,000	5,00
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The Relationship Between Selected CNR1, MC4R, LEP, FTO and VDR Gene Polymorphisms and Several Basic Toxicological Parameters Among Persons Occupationally Exposed to Arsenic, Cadmium and Lead

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Abstract: The purpose of this work was to assess the influence of selected CNR1, MC4R, LEP, FTO and VDR FOKI gene polymorphisms on blood and urine concentration markers of lead, cadmium and arsenic in a population directly exposed to these metals. Eighty-five people exposed to lead, arsenic and cadmium were qualified to take part in the study. Standard urine samples and 25mL of venous blood from each worker were collected to assay basic laboratory and toxicological markers as well as selected single nucleotide polymorphisms (SNPs) within CNR1—cannabinoid receptor 1 gene (*rs806368*, *rs806381*, *rs1049353*, *rs12720071*), MC4R—melanocortin 4 receptor gene (*rs17782313*), LEP—leptin promoter gene (*rs7799039*), FTO—alpha-ketoglutarate-dependent dioxygenase gene (*rs9939609*) and VDR—vitamin D receptor (*rs10735810*) genes. It appeared that, except for the MC4R SNP, all the other polymorphisms were found to be associated with various laboratory parameters. Arsenic concentration in urine was associated with all four CNR1 and LEP SNPs, while cadmium concentration in blood was affected by the VDR polymorphism. Moreover, some significant relationships were also observed between CNR1 *rs1049353* and FTO *rs9939609* gene variants and markers of lead exposure. These results imply SNPs within genes coding for proteins involved in development of metabolic syndrome may be of prognostic value for persons directly exposed to lead, cadmium and arsenic.

Keywords: arsenic; cadmium; lead; single nucleotide polymorphism; zinc protoporphyrin

1. Introduction

Metabolic syndrome is an important global clinical problem and a challenge for modern medicine [1]. Metabolic syndrome was firstly described in specified detail in 1988 by Reaven. He suggested that it has four

components: visceral obesity, hyperglycemia, arterial hypertension and dyslipidemia, described as hyper triglycerides, low HDL cholesterol fraction and high non-HDL fraction [2,3].

Research that aims to explain the causes of metabolic syndrome and its epidemic occurrence is based, on the one hand, on genetic and genotypic analyses and, on the other hand, observation of influence of the external environment [4,5].

Metabolic syndrome is multi-genetic. Among the genes with the strongest relationship with metabolic syndrome, CNR1—cannabinoid receptor 1 gene, LEP—leptin promoter gene, FTO—alpha-ketoglutarate-dependent dioxygenase gene, MC4R—melanocortin 4 receptor gene and VDR—vitamin D receptor stand out.

CNR1 (cannabinoid receptor 1) is a G protein-coupled receptor activated by endogenous and exogenous cannabinoids. They are found mostly in the central nervous system, in the cerebellum, nucleus accumbens and several other brain regions responsible for hunger, satiety and the reward system [6]. Several specific single nucleotide polymorphisms of the CNR1 gene (located in 6q15) were found (*rs806381*, *rs806368*, *rs1049353*, *rs12720071*), as well as several possible combinations. The most popular, A to G transition in *rs1049353,* leads to higher body mass index and wider waist circumference [7]. Another studied polymorphism was leptin (LEP), a hormone made mostly by adipocyte cells. It inhibits hunger and stimulates the sympathetic nervous system. Despite its seemingly straightforward effect on the human organism, current research suggests that leptin polymorphism is not a relevant obesity marker [8]. Next, MC4R polymorphism was studied. Melanocortin 4 receptor is a known genetic obesity marker [9]. Yet, the mechanism remains unclear, and homozygous CC (*rs17782313*) tends to be associated with higher body mass index and insulin resistance [10]. Another typical obesity polymorphism is the FTO gene responsible for fat mass and an obesity-associated protein also known as alpha-ketoglutarate-dependent dioxygenase. Certain variants of this enzyme, active mostly in the central nervous system, are also correlated with higher BMI and obesity [11]. Finally, VDR (vitamin D receptor) FokI polymorphism was studied. This particular receptor is responsible for most of the comprehensive activity of vitamin D. Some variants are known to be responsible for different bone density, some are correlated with renin activity and some could even directly induce obesity [12].

According to current knowledge, exposure to metals may affect the development of metabolic syndrome. There are more and more reports confirming the existence of such a relationship.

Using data from the 2011–2014 National Health and Nutrition Examination Survey, Bulka et al. evaluated associations between essential and toxic metals exposure and metabolic syndrome [13]. The positive correlations observed for arsenic exposure were due to an elevated prevalence of high blood pressure, low HDL cholesterol and high triglycerides among people with greater exposures. On the other hand, greater lead and cadmium co-exposures were related to a lower prevalence of dyslipidemia and abdominal obesity.

On the contrary, an analysis based on the Korea National Health and Nutrition Examination Survey (KNHANES) found that a higher prevalence of metabolic syndrome was associated with higher blood lead levels in the Korean population [14].

In a study by Wang et al., blood and urinary markers of 18 heavy metals among 9537 adults in NHANES 2003–2014 were evaluated. This study suggests that cumulative exposure to heavy metals as mixtures is associated with obesity and its related to chronic conditions such as hypertension and diabetes type II [15].

Luzhetskyi and co-authors proved that children with higher serum levels of cadmium and arsenic (1.4–2.0 times vs. the reference group) demonstrated 2.2 times more frequent endocrine diseases, up to 2.7 times more frequent obesity-related diseases, when compared to the reference group. Metabolic disorders in that group were associated with some lipid metabolism changes [16].

Similarly, study of Kawakami et al. demonstrated that exposure to cadmium caused a reduction of adipocyte size and the modulation of adipokine expression. The reduction in adipocyte size by Cd may arise from an imbalance between lipid synthesis and lipolysis. In addition, the expression levels of leptin, adiponectin and resistin decreased in adipocytes. So, exposure to Cd may induce unusually small adipocytes and modulate the expression of adipokines differently from

the case of physiologically small adipocytes, and it may accelerate the risk of developing insulin resistance and type 2 diabetes [17].

Numerous data concerning potential arsenic, cadmium and lead effects on development of obesity and metabolic syndrome have inspired us to pose a hypothesis that concentrations of these metals in people occupationally exposed are related to selected gene polymorphisms and determine metabolic disorders.

The purpose of this work was to assess the influence of selected single nucleotide polymorphisms (SNPs) coding for proteins involved in development of metabolic syndrome with the previously mentioned selected gene polymorphisms on blood and urine concentration markers of lead, cadmium and arsenic in a population directly exposed to these metals.

2. Materials and Methods

Eighty-five people, employees of a copper smelter and refinery, were qualified to be in the study. Inclusion criteria for the study were employment in workplaces exposed to arsenic, cadmium and lead (metal concentrations >0.1 maximum admissible concentration (MAC)) and work-related exposure to metals for at least 0.25 years. Size and quality of environmental exposure for all subjects included in the study were similar because all subjects resided in the same region. Only people who lived in the region for a long time (for at least 3 years) were included in the study group. The data and biological sample were collected in June 2016. There were 67 men and 18 women, and age ranged from 26 to 67 years. Thirty-three participants were obese, 34 of them were previously diagnosed with hypertension and 37 of them were smokers. Full clinical characteristics of the group are shown in Table 1, and basic laboratory characteristics of the population are shown in Table 2.

Table 1. Clinical characteristics of the study population.

	X	Me	SD	Min	Max
Age (years)	49.04	50.00	11.08	26.00	67.00
Height (cm)	174.24	175.50	7.64	156.00	190.00
Weight (kg)	87.39	86.00	15.93	53.00	127.00
BMI (kg/m^2)	28.68	27.91	4.29	19.00	40.90
Waist circumference (cm)	100.44	100.00	12.55	72.00	125.00
Pack-years	465.41	340.00	386.40	60.00	1500.00
	n			%	
Number	85			100.0	
Gender					
Male	67			78.8	
Female	18			21.2	
Weight					
Normal	18			21.2	
Overweight	33			38.8	
Obese	33			38.8	
Smokers	37			43.5	
Hypertension	34			40.0	
Diabetes	10			11.8	

Max-maximal value; Me-median value; Min-minimal value; SD-standard deviation; X-arithmetic mean.

Table 2. Conventional lab tests and calcium-phosphate balance in the study population.

	X	Me	SD	Min	Max
WBC (K/μL)	7.25	6.85	1.83	3.83	13.37
RBC (M/μL)	5.04	5.05	0.36	4.28	5.85
Hemoglobin (g/dL)	15.21	15.30	1.01	12.10	17.00
Hematocrit (%)	44.58	44.70	2.70	37.90	50.00
Platelets (K/μL)	249.93	246.00	52.44	129.00	406.00
Glucose (mg/dL)	96.70	93.00	20.80	68.00	181.00
HbA1C (%)	5.71	5.40	1.21	4.60	13.60
Total cholesterol (mg/dL)	234.24	231.00	49.34	102.00	405.00
HDL cholesterol (mg/dL)	51.02	49.00	11.08	27.00	86.00
LDL cholesterol (mg/dL)	138.00	134.00	41.43	27.00	311.00
Triglycerides (mg/dL)	236.58	196.00	155.31	46.00	824.00
Calcium (mg/dL)	9.69	9.70	0.33	9.10	10.60
Phosphorus (mg/dL)	3.43	3.30	0.66	2.20	5.60
25-OH-D_3 (μg/L)	20.81	20.49	7.69	5.21	45.00
Parathormone (ng/L)	46.68	43.30	20.02	15.50	108.60

All the participants were asked to fill in a questionnaire about their medical history and lifestyle. Next, basic anthropometric measurements were taken. We also took standard urine samples and 25mL of venous blood from each worker just after finishing their work shift to assay basic laboratory and toxicology markers as well as selected single nucleotide polymorphisms.

Blood count, fasting glucose, HbA1C, lipids (total cholesterol, HDL and LDL cholesterol, triglycerides) and calcium-phosphate balance markers (calcium, phosphorus, 25-OH-D_3 and parathormone) were determined by standard methods in accordance with the manufacturer's instructions.

We also determined concentrations of lead (Pb-B), cadmium (Cd-B), zinc protoporphyrins in blood (ZnPP) and arsenic in urine (As-U). Blood lead and cadmium concentrations were measured by graphite furnace atomic absorption spectrometry Solaar M6 (Thermo Elemental, UK). The calibration curves of lead and cadmium were prepared with blood standards of certified reference material (BCR IRMM). Both methods are routinely monitored by determination of reference material (Recipe) and participation in an intercomparison program for toxicological analyses in biological materials, G-EQUAS. The measurement was calculated as micrograms per liter (μg/L), and the biological exposure limits were 500 μg/L for lead and 5 μg/L for cadmium according to recommendations of the National Hygiene Institute and Institute of Occupational Health. ZnPP was measured using a rapid fluorometric screening method by means of Hematofluorymeter ProtoFluor (Helena Laboratories, Beaumont, Texas, USA). Total urine arsenic concentration was measured by Hydride Generation Atomic Absorption Spectrometry (HGAAS) using the VP100 Continuous Flow Vapour System. To determine the calibration curve, the reference material ClinCal® Urine Calibrator (Recipe) was used. We monitored the accuracy of the analytical method by analyzing samples of a reference material, Seronorm Trace Elements Urine (SERO AS, Oslo Norway), and participation in an intercomparison program for toxicological analyses in biological materials, G-EQUAS. The biological exposure limit proposed by the National Hygiene Institute and Institute of Occupational Health for arsenic in urine is 35 μg/L (35 μg/g creatinine).

In the study we analyzed selected single nucleotide polymorphisms (SNPs) for cannabinoid receptor 1 gene (CNR1), melanocortin 4 receptor gene (MC4R), leptin (LEP), alpha-ketoglutarate-dependent dioxygenase gene (FTO) and vitamin D receptor FokI. In the present study, the following eight SNPs were selected: *rs17782313* (T>C), located upstream of the MC4R gene on chromosome 18q22; *rs7799039* (G>A), a common promoter polymorphic site within the LEP gene on chromosome 7q31.3; *rs9939609* (G>A), a SNP within intron 1 of the FTO gene on chromosome 16q12.2; *rs10735810* (FokI) (C>T), located within exon 2 of the VDR gene on chromosome 12q11; and four polymorphisms of the CNR1 gene located on chromosome 6q15 (one intronic SNP *rs806381* A>G, *rs806368* T>C within 3′UTR, *rs1049353* a synonymous G>A polymorphism (within the splicing site), and *rs12720071* A>G, a substitution in 3′UTR). Genotyping was performed by real-time polymerase chain reaction (PCR)

amplifications and a melting curve analysis using a LightSNiP typing assay (TIB-MolBiol, Berlin, Germany). Real-time PCR was carried out on a LightCycler 480 Real-Time PCR system (Roche Diagnostics, Rotkreuz, Switzerland) in accordance with the conditions recommended by the manufacturers.

The research was compliant with Good Clinical Practice guidelines, The Declaration of Helsinki and was based on consent from a local Bioethical Committee (No KB-398/2018, date: 25.06.2018).

Statistical analyses were calculated using the statistical program STATISTICA 13 (Dell Inc., Tulsa, Oklahoma, USA). For the quantitative variables, the arithmetic mean (X), the median value (Me), the standard deviation (SD) as well as the minimal (Min) and maximal (Max) values of assayed parameters were calculated in the studied groups. Distribution of variables was tested using the Lilliefors and W-Shapiro–Wilk tests. In the case of independent, quantitative variables having normal distributions, a t test for independent variables and the analysis of variances ANOVA (unifactorial parametric) were used in the further statistical analysis. The U test of Mann–Whitney or a non-parametric equivalent of Kruskal–Wallis ANOVA analysis of variance test were used in the case of variables with non-normal distributions. The significant differences between the arithmetic means were estimated using a post-hoc Newman–Keuls test. Results for the nominal variables were presented in percentages. In order to assess the relations between studied variables, a correlation analysis was performed. In the case of variables having a normal distribution, Pearson's r was calculated, and for the variables with a distribution other than normal, the Spearman's r correlation coefficient was used. Results at the level of $p < 0.05$ were regarded as statistically significant.

3. Results

The mean blood calcium, phosphorus, 25-hydroxyvitamin D and parathormone concentrations in the occupationally exposed group were accordingly 9.69 ± 0.33 mg/dL, 3.43 ± 0.66 mg/dL, 20.81 ± 7.69 μg/L and 46.68 ± 20.02 ng/L. These are presented in Table 2.

Arsenic concentration was 11.74 ± 9.37 μg/L, cadmium was 0.84 ± 0.80 μg/L, lead was 199.23 ± 117.02 μg/L and ZnPP was 47.94 ± 30.64 μg/dL. A total of 3.5% of employees had a urine arsenic concentration higher than the norm of the allowable concentration in biological material (determined as a maximum of 35 μg/L). Totals of 1.2% and 16.5% of workers, respectively, had a blood lead concentration and blood zinc protoporphyrin concentration higher than the norms of the allowable concentration in biological material (determined as maximums of 500 and 70 μg/L, respectively). Nobody in the study group was characterized by exceeding the norm of the permissible blood cadmium concentration. These are presented in Table 3.

Table 3. Basic toxicological parameters in the study population.

	X	Me	SD	Min	Max
Exposure period (years)	17.64	13.00	13.33	0.25	46.00
As-U (μg/L)	11.74	9.93	9.37	0.27	46.15
Cd-B (μg/L)	0.84	0.55	0.80	0.22	4.61
Pb-B (μg/L)	199.23	193.80	117.02	22.20	520.90
ZnPP (μg/dL)	47.94	35.00	30.64	21.00	160.00
	n			**%**	
Studied population	85			100.0	
As-U >acceptable biological concentration (>35 μg/L)	3			3.5	
Cd-B >acceptable biological concentration (>5 μg/L)	0			0.0	
Pb-B >acceptable biological concentration (>500 μg/L)	1			1.2	
ZnPP>acceptable biological concentration (>70 μg/dL)	14			16.5	

Distributions of alleles and genotypes of selected SNPs in the studied population are shown in Table 4. Most genotypes had frequencies exceeding 10%. The CRN1 *rs12720071* GG homozygosity was the rarest and was detected in one individual only (1.2%). The other rare genotypes were MC4R *rs17782313* GG and CNR1 *rs1049353* AA homozygosity observed both in five (5.9%) cases,

and the CNR1 *rs806386* CC genotype was found in seven (8.2%) cases. These distributions closely resemble those described for other European populations.

Table 4. Selected polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI in the study population.

SNP	genotype	*n*	%	allele	*n*	%
rs806381 gene CNR1	homozygote AA	27	31.8	allele A	70	82.4
	heterozygote AG	43	50.6	allele G	58	68.2
	homozygote GG	15	17.6			
rs806368 gene CNR1	homozygote CC	7	8.2	allele C	30	35.3
	heterozygote CT	23	27.1	allele T	78	91.8
	homozygote TT	55	64.7			
rs1049353 gene CNR1	homozygote AA	5	5.9	allele A	39	45.8
	heterozygote AG	34	40.0	allele G	80	94.1
	homozygote GG	46	54.1			
rs12720071 gene CNR1	homozygote AA	69	81.2	allele A	84	98.8
	heterozygote AG	15	17.6	allele G	16	18.8
	homozygote GG	1	1.2			
rs17782313 gene MC4R	homozygote CC	5	5.9	allele C	32	37.6
	heterozygote CT	27	31.8	allele T	80	94.1
	homozygote TT	53	62.4			
rs7799039 gene LEP	homozygote AA	14	16.5	allele A	54	63.5
	heterozygote AG	40	47.1	allele G	70	82.3
	homozygote GG	30	35.3			
rs9939609 gene FTO	homozygote AA	23	27.1	allele A	66	77.6
	heterozygote AT	43	50.6	allele T	62	72.4
	homozygote TT	19	22.4			
rs10735810 gene VDR FokI	homozygote CC	25	29.4	allele C	66	77.6
	heterozygote CT	41	48.2	allele T	60	70.6
	homozygote TT	19	22.4			

The results of comparative analyses of arsenic urine concentrations of subgroups based on genotype criteria and alleles of single nucleotide polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI are presented in Table 5. We have proved that homozygosity AA in locus *rs806381* in the CNR1 gene is related to a statistically significant lower arsenic concentration, compared to heterozygosity AG and homozygosity GG, and the presence of allele G in this locus is associated with a significantly higher arsenic urine concentration. In locus *rs12720071* of the CNR1 gene, homozygotes GG have statistically significant higher arsenic concentrations than heterozygotes AG and homozygotes AA, and allele A in this locus can be associated with a statistically significant lower arsenic concentration. The analysis also has shown that allele A in locus *rs1049353* of the CNR1 gene can be responsible for lower arsenic concentrations, and allele G in locus *rs7799039* of gene LEP is responsible for significantly higher arsenic concentrations in the studied population.

Table 5. Total arsenic concentration in subgroups divided according to selected polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI.

SNP	genotype	As-U (μg/L)	allele	As-U (μg/L)
rs806381 gene CNR1	homozygote AA	8.58 ± 5.38	allele A	11.18 ± 8.72
	heterozygote AG	12.81 ± 10.00	allele G	13.24 ± 10.47
	homozygote GG	14.56 ± 12.11		
	AA vs. AG: p=0.044 AA vs. GG: p=0.041		G (GG or AG) vs. non-G (AA): p=0.032	
rs806368 gene CNR1	homozygote CC	11.43 ± 9.61	allele C	12.19 ± 11.27
	heterozygote CT	12.39 ± 11.85	allele T	11.77 ± 9.42
	homozygote TT	11.51 ± 8.31		
	ns		ns	
rs1049353 gene CNR1	homozygote AA	8.99 ± 4.68	allele A	9.48 ± 6.66
	heterozygote AG	9.56 ± 6.96	allele G	11.92 ± 9.58
	homozygote GG	13.70 ± 10.91		
	ns		A (AA or AG) vs. non-A (GG): p=0.039	
rs12720071 gene CNR1	homozygote AA	11.04 ± 8.44	allele A	11.53 ± 9.22
	heterozygote AG	13.72 ± 12.26	allele G	14.72 ± 12.49
	homozygote GG	29.58 ± 0.00		
	AA vs. GG: p=0.034 AG vs. GG: p=0.039		A (AA or AG) vs. non-A (GG): p=0.045	
rs17782313 gene MC4R	homozygote CC	14.06 ± 5.78	allele C	10.84 ± 8.06
	heterozygote CT	10.25 ± 8.36	allele T	11.60 ± 9.56
	homozygote TT	12.30 ± 10.13		
	ns		ns	
rs7799039 gene LEP	homozygote AA	7.76 ± 5.27	allele A	10.98 ± 9.15
	heterozygote AG	12.11 ± 9.98	allele G	12.31 ± 9.72
	homozygote GG	12.60 ± 9.52		
	ns		G (GG or AG) vs. non-G (AA): p=0.043	
rs9939609 gene FTO	homozygote AA	10.13 ± 7.14	allele A	12.09 ± 9.81
	heterozygote AT	13.10 ± 10.87	allele T	12.31 ± 10.04
	homozygote TT	10.54 ± 7.79		
	ns		ns	
rs10735810 gene VDR FokI	homozygote CC	13.58 ± 11.47	allele C	11.34 ± 9.36
	heterozygote CT	9.97 ± 7.64	allele T	10.96 ± 8.32
	homozygote TT	13.21 ± 9.54		
	ns		ns	

ns-non-significant statistically.

The results of comparative analyses of cadmium blood concentrations of subgroups based on genotype criteria and alleles of single nucleotide polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI are presented in Table 6. We have proved that allele T in locus *rs10735810* of VDR FokI gene can be a factor responsible for the significantly lower cadmium concentration in the studied population.

Table 6. Cadmium concentration in subgroups divided according to selected polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI.

SNP	genotype	Cd-B (μg/L)	allele	Cd-B (μg/L)
rs806381 gene CNR1	homozygote AA	0.89 ± 0.98	allele A	0.86 ± 0.84
	heterozygote AG	0.85 ± 0.75	allele G	0.82 ± 0.71
	homozygote GG	0.75 ± 0.60		
	ns			ns
rs806368 gene CNR1	homozygote CC	0.66 ± 0.35	allele C	0.84 ± 0.84
	heterozygote CT	0.89 ± 0.94	allele T	0.86 ± 0.83
	homozygote TT	0.85 ± 0.79		
	ns			ns
rs1049353 gene CNR1	homozygote AA	1.08 ± 1.05	allele A	0.78 ± 0.67
	heterozygote AG	0.74 ± 0.61	allele G	0.83 ± 0.79
	homozygote GG	0.90 ± 0.90		
	ns			ns
rs12720071 gene CNR1	homozygote AA	0.83 ± 0.74	allele A	0.85 ± 0.80
	heterozygote AG	0.95 ± 1.07	allele G	0.92 ± 1.04
	homozygote GG	0.35 ± 0.00		
	ns			ns
rs17782313 gene MC4R	homozygote CC	1.08 ± 1.05	allele C	0.79 ± 0.69
	heterozygote CT	0.73 ± 0.61	allele T	0.83 ± 0.79
	homozygote TT	0.88 ± 0.87		
	ns			ns
rs7799039 gene LEP	homozygote AA	0.95 ± 0.75	allele A	0.89 ± 0.85
	heterozygote AG	0.86 ± 0.89	allele G	0.82 ± 0.82
	homozygote GG	0.77 ± 0.73		
	ns			ns
rs9939609 gene FTO	homozygote AA	0.74 ± 0.64	allele A	0.79 ± 0.73
	heterozygote AT	0.81 ± 0.78	allele T	0.88 ± 0.85
	homozygote TT	1.04 ± 1.01		
	ns			ns
rs10735810 gene VDR FokI	homozygote CC	1.12 ± 1.17	allele C	0.85 ± 0.82
	heterozygote CT	0.68 ± 0.46	allele T	0.73 ± 0.56
	homozygote TT	0.82 ± 0.74		
	ns		T (TT or CT) vs. non-T (CC): $p = 0.041$	

Similarly, the results of comparative analyses of lead blood concentrations of subgroups based on genotype criteria and alleles of single nucleotide polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI are presented in Table 7. In the studied population, homozygotes GG in locus *rs1049353* of the CNR1 gene have a significantly higher blood lead concentration compared to heterozygotes AG and homozygotes AA. The presence of allele A in the locus is correlated with a statistically relevant lower lead blood concentration.

Table 7. Lead concentration in subgroups divided according to selected polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI.

SNP	genotype	Pb-B (μg/L)	allele	Pb-B (μg/L)
rs806381 gene CNR1	homozygote AA	180.50 ± 97.72	allele A	194.23 ± 123.09
	heterozygote AG	202.85 ± 137.05	allele G	207.95 ± 124.82
	homozygote GG	222.58 ± 82.32		
	ns		ns	
rs806368 gene CNR1	homozygote CC	207.09 ± 50.81	allele C	180.20 ± 101.05
	heterozygote CT	172.01 ± 111.61	allele T	198.53 ± 121.37
	homozygote TT	209.61 ± 124.52		
	ns		ns	
rs1049353 gene CNR1	homozygote AA	171.58 ± 117.21	allele A	161.48 ± 107.35
	heterozygote AG	160.00 ± 107.64	allele G	200.96 ± 117.53
	homozygote GG	231.23 ± 116.39		
	AG vs. GG: *p*=0.006 AA vs. GG: *p*=0.045		A (AA or AG) vs. non-A (GG): p=0.005	
rs12720071 gene CNR1	homozygote AA	193.85 ± 115.37	allele A	199.31 ± 117.72
	heterozygote AG	224.46 ± 129.17	allele G	222.44 ± 125.05
	homozygote GG	192.20 ± 0.00		
	ns		ns	
rs17782313 gene MC4R	homozygote CC	244.16 ± 99.42	allele C	185.52 ± 114.97
	heterozygote CT	174.66 ± 116.00	allele T	196.42 ± 118.00
	homozygote TT	207.51 ± 118.55		
	ns		ns	
rs7799039 gene LEP	homozygote AA	208.89 ± 137.93	allele A	197.13 ± 123.09
	heterozygote AG	193.02 ± 119.09	allele G	196.51 ± 114.08
	homozygote GG	201.17 ± 108.88		
	ns		ns	
rs9939609 gene FTO	homozygote AA	205.35 ± 107.83	allele A	206.01 ± 119.89
	heterozygote AT	206.36 ± 127.10	allele T	196.96 ± 121.01
	homozygote TT	175.69 ± 106.02		
	ns		ns	
rs10735810 gene VDR FokI	homozygote CC	224.20 ± 139.97	allele C	194.11 ± 121.91
	heterozygote CT	175.76 ± 107.16	allele T	188.83 ± 105.60
	homozygote TT	217.04 ± 99.02		
	ns		ns	

The results of comparative analyses of zinc protoporphyrin blood concentrations of subgroups based on genotype criteria and alleles of single nucleotide polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI are presented in Table 8. It was proven that heterozygosity AG in locus *rs1049353* of the CNR1 gene may result in a statistically lower ZnPP concentration compared to homozygosity AA and GG, and allele A is responsible for lower ZnPP concentrations. Apart from that, we documented that allele A in locus *rs9939609* of the FTO gene is responsible for higher ZnPP concentration.

In the correlation analysis we found statistically significant, linear correlations between cadmium concentration and white blood cell count (r = 0.22, p = 0.040), ZnPP concentration and platelets (r = 0.25, p = 0.020), ZnPP concentration and phosphorus in blood (r = 0.23, p = 0.035) as well as ZnPP and vitamin D (r= −0.22, p = 0.032).

Table 8. Zinc protoporphyrins (ZnPP) concentration in subgroups divided according to selected polymorphisms of genes CNR1, MC4R, LEP, FTO and VDR FokI.

SNP	genotype	ZnPP (μg/dL)	allele	ZnPP (μg/dL)
rs806381 gene CNR1	homozygote AA	42.48 ± 25.15	allele A	45.99 ± 27.84
	heterozygote AG	48.19 ± 29.47	allele G	50.48 ± 32.78
	homozygote GG	57.07 ± 41.30		
	ns		ns	
rs806368 gene CNR1	homozygote CC	64.86 ± 48.64	allele C	47.10 ± 32.03
	heterozygote CT	41.70 ± 24.01	allele T	46.42 ± 28.49
	homozygote TT	48.40 ± 30.15		
	ns		ns	
rs1049353 gene CNR1	homozygote AA	56.60 ± 42.48	allele A	39.64 ± 23.11
	heterozygote AG	37.15 ± 18.61	allele G	47.40 ± 30.03
	homozygote GG	54.98 ± 34.51		
	AG vs. GG: p=0.009 AA vs. AG: p=0.037		A (AA or AG) vs. non-A (GG): p=0.020	
rs12720071 gene CNR1	homozygote AA	47.38 ± 30.92	allele A	47.39 ± 30.40
	heterozygote AG	47.47 ± 28.91	allele G	50.38 ± 30.26
	homozygote GG	94.00 ± 0.00		
	ns		ns	
rs17782313 gene MC4R	homozygote CC	51.20 ± 43.25	allele C	45.88 ± 32.92
	heterozygote CT	44.89 ± 31.59	allele T	47.74 ± 30.05
	homozygote TT	49.19 ± 29.43		
	ns		ns	
rs7799039 gene LEP	homozygote AA	53.57 ± 32.82	allele A	46.53 ± 30.42
	heterozygote AG	42.75 ± 25.72	allele G	45.56 ± 27.82
	homozygote GG	51.57 ± 35.59		
	ns		ns	
rs9939609 gene FTO	homozygote AA	52.26 ± 37.44	allele A	51.08 ± 32.33
	heterozygote AT	50.44 ± 29.70	allele T	46.34 ± 27.89
	homozygote TT	37.05 ± 21.13		
	ns		A (AA or AT) vs. non-A (TT): p=0.038	
rs10735810 gene VDR FokI	homozygote CC	52.96 ± 29.89	allele C	46.26 ± 29.02
	heterozygote CT	42.17 ± 28.05	allele T	45.85 ± 30.95
	homozygote TT	53.79 ± 35.97		
	ns		ns	

In the last part of the study, multivariate regression analysis was performed, and the following significant models were observed:

As-U = 1.330 allele G in *rs806381* gene CNR1 − 4.274 allele A in *rs1049353* gene CNR1 − 18.415 allele A in *rs12720071* gene CNR1 + 2.291 allele G in *rs7799039* gene LEP + 0.424 BMI + 0.160 age + 5.324 diabetes ± 4.877.

Cd-B = − 0.439 allele T in *rs10735810* gene VDR FokI + 0.814 smoking − 0.023 HDL cholesterol + 0.014 BMI ± 0.709.

Pb-B = − 77.411 allele A in *rs1049353* gene CNR1 + 62.804 hypertension + 82.478 diabetes − 44.553 phosphorus ± 0.709.

Based on the obtained regression models it was shown that allele G in *rs806381* gene CNR1, allele G in *rs7799039* gene LEP, higher BMI, older age and diabetes were independently associated with higher As-U concentration; while allele A in *rs1049353* gene CNR1 and allele A in *rs12720071* gene CNR1 were independently associated with lower As-U concentration. It was shown that allele T in *rs10735810* gene VDR FokI and higher HDL cholesterol concentration were independently associated with lower Cd-B concentration, while smoking and higher BMI were independently associated with higher Cd-B concentration. Finally, allele A in *rs1049353* gene CNR1 and higher phosphorus concentration were

independently associated with a lower Pb-B concentration, while hypertension and diabetes were independently associated with a higher Pb-B concentration (Table 9).

Table 9. Results of estimation for the final model obtained from multivariate regression analysis.

	Independent Variable	Regression Coefficient	Standard error of Regression Coefficient	*p*-Value	*p* Value of the Model	Standard Error of the Model
model for As-U (µg/L)	allele G in *rs806381* gene CNR1	1.330	0.666	0.041	0.043	4.877
	allele A in *rs1049353* gene CNR1	−4.274	2.095	0.037		
	allele A in *rs12720071* gene CNR1	−18.415	9.136	0.037		
	allele G in *rs7799039* gene LEP	2.291	1.072	0.045		
	BMI (kg/m^2)	0.424	0.173	0.025		
	age (years)	0.160	0.070	0.015		
	diabetes	5.324	2.269	0.026		
model for Cd-B (µg/L)	allele T in *rs10735810* gene VDR FokI	−0.439	0.187	0.022	0.006	0.709
	smoking	0.814	0.184	0.001		
	HDL cholesterol (mg/dL)	−0.023	0.011	0.045		
	BMI (kg/m2)	0.014	0.002	0.046		
model for Pb-B (µg/L)	allele A in *rs1049353* gene CNR1	−77.411	26.320	0.004	0.014	112.131
	hypertension	62.804	39.182	0.014		
	diabetes	82.478	58.742	0.016		
	phosphorus (mg/dL)	−44.553	21.391	0.041		

4. Discussion

The relationship between some metals and body mass, obesity development and central hunger regulation is widely discussed and confirmed in numerous studies. Many studies focus on genetic and epigenetic mechanisms of obesity evolution. In the study of Tyrrell et al., Pb-exposed animals showed elevated hepatic triglyceride levels and increased expression of the gluconeogenic genes PEPCK and glucose-6-phosphatase [18]. In cultured rat hepatoma cells, treatment with Pb stimulated PEPCK and glucose-6-phosphatase gene expression, suggesting a possible direct effect of Pb on hepatic gluconeogenic gene expression. Vidal et al. proved that elevated maternal blood Cd levels were associated with lower birth weight, and higher maternal blood Cd levels were also associated with lower methylation at the PEG3 or at the MEG3 in methylated regions of newborn DNA [19].

Our study is either the first or one of the very few studies trying to determine the relationship between single nucleotide polymorphisms of genes involved in development of metabolic syndrome and toxicological parameters. Although the literature is poor, we decided to proceed with selected genes as they are the best studied in the aspect of metabolic syndrome. We found several statistically important correlations. However, we were unable to compare our results with other studies, as there have been none. On the other hand, there are numerous studies concerning studied polymorphisms and various other parameters. A comprehensive discussion of the subject will extend the framework

of this research. Therefore, we decided to discuss a few examples to show the scientific and clinical importance of our study, as well as the need to continue the research.

Until now, we were unable to find other studies showing correlations between CNR1 polymorphism locus *rs1049353* and arsenic concentration. Moreover, data concerning this polymorphism and its correlation with BMI parameters are inconclusive and often contradictory [7]. In our research we were able to determine a significant correlation: allele G is correlated with higher arsenic concentration. This poses the following question: what is the reason for such correlation? Do people with allele G consume more food, as some studies show [20], therefore absorbing more arsenic and resulting in a higher arsenic concentration? Or, on the other hand, does a greater amount of fat tissue enable a higher arsenic concentration? Whatever the mechanism could be, we have found a genetic predisposition to higher arsenic concentration. This requires further study, especially in light of the growing obesity epidemic.

In our study we discovered that allele G in locus *rs7799039* of gene LEP is correlated with a markedly higher arsenic concentration. The authors were not able to find any other research to support or undermine our result. Nevertheless, the leptin polymorphism has been heavily studied recently. Carriers of A allele in the studied locus tend to have lower LDL and total cholesterol [21]. Another recent study shows association between leptin polymorphism and coronary artery disease and hypertension [22]. This finally leads us to the question about the role of arsenic in development of metabolic syndrome and cardiovascular diseases. This role is yet to be determined.

We were also able to find that allele T in locus *rs10735810* of VDR FokI gene is associated with lower cadmium concentration. Again, it seems that it is the first attempt to assess this correlation. There are single studies on metals and VDR FokI polymorphism. In the study by Szymanska-Chabowska et al., they were able to determine an association between another locus of VDR FokI gene (*rs2228570*) and concentration of lead and ZnPP [23]. Our study provides more input on the matter, especially that some research suggests there is no simple connection between cadmium concentration and vitamin D concentration [24].

In studied population, we were able to determine a significant correlation between the CNR1 polymorphism and lead and ZnPP concentrations. Again, this is the first research proving this relation. However, polymorphism of CNR1 is being thoroughly studied. It was found that *rs1049353* polymorphism was associated with specific changes in brain morphology as well as with evolution of positive symptoms in schizophrenia [25]. This poses a question about the role of lead in the development of psychiatric disorders and brain reconstruction.

As for the linear correlation between cadmium concentration and white blood cell count, our research is compliant with many previous studies, including the most recent ones [26,27]. Correlations of ZnPP concentration and platelets, ZnPP concentration and phosphorus in blood as well as ZnPP and vitamin D were not found in previous studies [23].

Finally, it should be mentioned that, when analyzing multiple polymorphisms affecting a single variable (e.g., blood concentration of a particular metal), it is necessary to consider the possible compensatory effects of polymorphisms. In the current study, this compensation in individuals may be due to the effect of polymorphisms on urinary arsenic levels. If a person is a carrier of allele G in loci *rs806381* CNR1 gene and *rs7799039* LEP gene, and at the same time a carrier of allele A in loci *rs1049353* and *rs12720071* CNR1 gene, the increase in As-U concentration due to the impact of the first two alleles may be reduced as a result of the impact of the second two alleles.

The authors of this work see two main limitations of this study. First, there is little or no research to compare and confront our data. Our study was conducted with the highest standards; we found statistically significant correlations, but still, further research needs to be conducted to confirm our results. Secondly, our study is based on a relatively small population (85 persons) for genetic study. This results in poor representation of certain alleles (allele G in locus *rs12720071* CNR1—18.8%; allele C in locus *rs17782313* MC4R—37.6%). As for the first allele, we were able to obtain statistically significant correlation. If statistically important correlations are found in a relatively small group, the correlation is strong and it will be even more visible in larger groups. Finally, showing specific

correlations between polymorphisms and metal concentrations is just the first step in understanding their complex role in the human organism; yet, it is an important step forward.

Relationships that we found between some genetic polymorphisms and arsenic, lead and cadmium exposure levels may indicate their role in the promotion of obesity and metabolic disorders. These metals may be one of many environmental factors that, in an unfavorable genetic constellation, contribute to higher cardiovascular risk resulting from obesity, diabetes and atherogenic lipid profile. Immunological processes modulated by vitamin D also have their impact on this risk. The relationship between lower cadmium levels and polymorphism of one of the vitamin D receptors proves that this polymorphism is beneficial in reducing cardiovascular risk in persons occupationally exposed to cadmium.

5. Conclusions

(1) Single nucleotide polymorphisms within genes coding for proteins involved in development of metabolic syndrome may be of prognostic value for persons directly exposed to lead, cadmium and arsenic.

(2) In the group occupationally exposed to arsenic, cadmium and lead, certain associations between polymorphisms *rs806381*, *rs1049353* and *rs12720071* of gene CNR1 and polymorphism *rs7799039* of gen LEP and arsenic concentration in urine were acquired:

- Allele G in locus *rs806381* CNR1 and locus *rs7799039* LEP can be responsible for higher arsenic concentrations;
- Allele A in locus *rs1049353* and *rs12720071* CNR1 can be responsible for lower arsenic concentrations.

(3) Cadmium concentration in blood in people occupationally exposed can be determined by polymorphism of *rs10735810* VDR FokI gene:

- Allele T in locus *rs10735810* VDR FokI gene can be responsible for lower cadmium concentration.

(4) In people occupationally exposed to arsenic, cadmium and lead, there are certain interactions between polymorphisms *rs1049353* gene CNR1 and *rs9939609* gene FTO and markers of lead exposure (lead and zinc protoporphyrin in blood):

- Allele A in locus *rs1049353* CNR1 gene can be responsible for lower lead and ZnPP concentrations;
- Allele A in locus *rs9939609* FTO gene can be responsible for higher ZnPP concentration.

(5) Polymorphism *rs17782813* MC4R gene, as the only one in our study, did not affect concentrations of selected markers amongst workers occupationally exposed to lead, cadmium and arsenic.

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References

1. Saklayen, M.G. The Global Epidemic of the Metabolic Syndrome. *Curr. Hypertens. Rep.* **2018**, *20*, 12.
2. Taton, J.; Bernas, M. Metabolic syndrome—Controversies between academic debate and real world medical practice. *Endokrynol. Otyłość Zaburzenia Przemiany Mater.* **2008**, *5*, 13–26.
3. Engin, A. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv. Exp. Med. Biol.* **2017**, *960*, 1–17.
4. Jeong, H.S. The Relationship between Workplace Environment and Metabolic Syndrome. *Int. J. Occup. Environ. Med.* **2018**, *9*, 176–183.

5. Ziki, M.D.A.; Mani, A. Metabolic syndrome: genetic insights into disease pathogenesis. *Curr. Opin. Lipidol.* **2016**, *27*, 162–171.
6. Hryhorowicz, S.; Walczak, M.; Zakerska-Banaszak, O.; Słomski, R.; Skrzypczak-Zielińska, M. Pharmacogenetics of cannabinoids. *Eur. J. Drug Metabol. Pharmacokinet.* **2017**, *43*, 1-12.
7. Doris, J.M.; Millar, S.A.; Idris, I.; O'Sullivan, S.E. Genetic polymorphisms of the endocannabinoid system in obesity and diabetes. *Diabetes Obes. Metabol.* **2019**, *21*, 382–387.
8. Yan, J.; Wang, X.; Tao, H.; Yang, W.; Luo, M.; Lin, F. Lack of association between leptin G-2548A polymorphisms and obesity risk: Evidence based on a meta-analysis. *Obes. Res. Clin. Pract.* **2015**, *9*, 389–397.
9. Krashes, M.J.; Lowell, B.B.; Garfield, A.S. Melanocortin-4 receptor-regulated energy homeostasis. *Nat. Neurosci.* **2016**, *19*, 206–219.
10. Tschritter, O.; Haupt, A.; Preiss, H. An obesity risk SNP (rs17782313) near the MC4R gene is associated with cerebrocortical insulin resistance in humans. *J. Obes.* **2011**, *2011*, 283153.
11. Speakman, J.R. The fat mass and obesity related (FTO) gene: mechanisms of impact on obesity and energy balance. *Curr. Obes. Rep.* **2015**, *4*, 73–91.
12. Wysoczańska-Klaczyńska, A.; Ślęzak, A.; Hetman, M.; Barg, E. The impact of VDR gene polymorphisms on obesity, metabolic changes, bone mass disorders and neoplastic processes. *Pediatr. Endocrinol. Diabetes Metabol.* **2018**, *24*, 96–105.
13. Bulka, C.M.; Persky, V.W.; Daviglus, M.L.; Durazo-Arvizu, R.A.; Argos, M. Multiple metal exposures and metabolic syndrome: A cross-sectional analysis of the National Health and Nutrition Examination Survey 2011–2014. *Environ. Res.* **2019**, *168*, 397–405.
14. Rhee, S.Y.; Hwang, Y.C.; Woo, J.T.; Sinn, D.H.; Chin, S.O.; Chon, S.; Kim, Y.S. Blood lead is significantly associated with metabolic syndrome in Korean adults: an analysis based on the Korea National Health and Nutrition Examination Survey (KNHANES), 2008. *Cardiovasc. Diabetol.* **2013**, *12*, 9.
15. Wang, X.; Mukherjee, B.; Park, S.K. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003–2014. *Environ. Int.* **2018**, *121 Pt 1*, 683–694.
16. Luzhetskyi, K.P.; Ustinova, O.Y.; Shtina, I.E.; Vekovshinina, S.A.; Ivashova, Y.A.; Tsinker, M.Y. Lipid metabolism changes in population residing in area influenced by storage of ore-processing waste containing lead, cadmium and arsenic. *Med. Tr. Prom. Ekol.* **2016**, *8*, 32–37.
17. Kawakami, T.; Nishiyama, K.; Kadota, Y.; Sato, M.; Inoue, M.; Suzuki, S. Cadmium modulates adipocyte functions in metallothionein-null mice. *Toxicol. Appl. Pharmacol.* **2013**, *272*, 625–636.
18. Tyrrell, J.B.; Hafida, S.; Stemmer, P.; Adhami, A.; Leff, T. Lead (Pb) exposure promotes diabetes in obese rodents. *J. Trace Elem. Med. Biol.* **2017**, *39*, 221–226.
19. Vidal, A.C.; Semenova, V.; Darrah, T.; Vengosh, A.; Huang, Z.; King, K.; Nye, M.D.; Fry, R.; Skaar, D.; Maguire, R.; et al. Maternal cadmium, iron and zinc levels, DNA methylation and birth weight. *BMC Pharmacol. Toxicol.* **2015**, *16*, 20.
20. De Luis, D.A.; Izaola, O.; Aller, R. Association of G1359A polymorphism of the cannabinoid receptor gene (CNR1) with macronutrient intakes in obese females. *J. Hum. Nutr. Diet.* **2016**, *29*, 118–123.
21. Manriquez, V.; Aviles, J.; Salazar, L.; Saavedra, N.; Seron, P.; Lanas, F.; Fajardo, C.M.; Hirata, M.H.; Hirata, R.D.C.; Cerda, A. Polymorphisms in genes involved in the leptin-melanocortin pathway are associated with obesity-related cardiometabolic alterations in a southern Chilean population. *Mol. Diagn. Ther.* **2018**, *22*, 101–113.
22. Nowzari, Z.; Masoumi, M.; Nazari-Robati, M.; Saavedra, N.; Seron, P.; Lanas, F.; Fajardo, C.M.; Hirata, M.H.; Hirata, R.D.C.; Cerda, A. Association of polymorphisms of leptin, leptin receptor and apelin receptor genes with susceptibility to coronary artery disease and hypertension. *Life Sci.* **2018**, *207*, 166–171.
23. Szymańska-Chabowska, A.; Łaczmański, Ł.; Jędrychowska, I.; Chabowski, M.; Gać, P.; Janus, A.; Gosławska, K.; Smyk, B.; Solska, U.; Mazur, G.; et al. The relationship between selected VDR, HFE and ALAD gene polymorphisms and several basic toxicological parameters among persons occupationally exposed to lead. *Toxicology* **2015**, *334*, 12–21.
24. Chen, X.; Dai, Y.; Wang, Z.; Zhu, G.; Ding, X.; Jin, T. The association between serum vitamin D levels and renal tubular dysfunction in a general population exposed to cadmium in China. *PLoS ONE* **2018**, *13*, e0195682.
25. Suárez-Pinilla, P.; Roiz-Santiañez, R.; Ortiz-García de la Foz, V.; Guest, P.C.; Ayesa-Arriola, R.; Córdova-Palomera, A.; Tordesillas-Gutierrez, D.; Crespo-Facorro, B. Brain structural and clinical changes after first episode psychosis: Focus on cannabinoid receptor 1 polymorphisms. *Psychiatry Res.* **2015**, *233*, 112–119.

26. Arikan, T.A.; Kelles, M. Plasma selenium and cadmium levels in patients with chronic otitis media in a Turkish population and their relation to inflammation markers. *Biol. Trace Elem. Res.* **2019**, *189*, 55–63.
27. Rabha, R.; Ghosh, S.; Padhy, P.K. Indoor air pollution in rural north-east India: Elemental compositions, changes in hematological indices, oxidative stress and health risks. *Ecotoxicol. Environ. Saf.* **2018**, *165*, 393–403.

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The relationship between PNP, GSTO-1, AS3MT and ADRB3 gene polymorphisms and urinary arsenic concentration among copper smelter and refinery employers

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Abstract

Introduction: The aim of this study was to assess the relationship between polymorphisms of genes encoding enzymes involved in arsenic metabolism and urinary arsenic concentration in people occupationally exposed to arsenic.

Materials and Methods: The data from 113 employers directly exposed to lead, cadmium, and arsenic in copper smelter in Legnica and Glogow were collected. Urinary arsenic concentration was measured. In addition, blood level of cadmium, lead, and zinc protoporphyrins was assayed. Genetic analyses included polymorphism of PNP (rs 1130650), GSTO-1 (rs 4925), AS3MT (rs 11191439), and ADRB3 (rs4994) genes.

Results: Individuals occupationally exposed to arsenic compounds, who have allele T in homozygous constellation in locus rs 1130650 of PNP gene, are predisposed to lower urinary arsenic concentration, while AA homozygosity in locus rs 4925 of GSTO-1 gene may result in statistically significant higher urinary arsenic concentration. Polymorphisms of AS3MT and ADRB3 genes showed no statistically significant correlation with urinary arsenic, however, there was a tendency to higher arsenic concentration in allele A carriers in locus rs4994 of ADRB3 gene and in allele T carriers in rs 11191439 of AS3MT gene.

Conclusion: This study indicates that arsenic absorption and metabolism depend on polymorphisms of genes encoding PNP and GSTO-1. Individuals with disadvantageous constellation of polymorphisms are more susceptible to harmful effects of arsenic exposure.

Keywords

Arsenic, polymorphism, gene, neoplasm

Introduction

Arsenic is one of the most common elements incorporated in the Earth's crust. It is emitted to the environment by volcanic and industrial activity. Inorganic arsenic is classified as a group I human carcinogen.[1] The primary route of arsenic exposure is via the ingestion of contaminated food or water. In general, the daily intake of arsenic from food and beverages ranges between 20 μg/day and 300 μg/day.[1–3] The amount of arsenic inhaled from ambient air constitutes a minor exposure for the general population, however, it is the main route of occupational exposure. It is worth noting that the amounts of arsenic consumed per day vary, for instance, the daily arsenic intake in rural areas ranges between 20 ng and 200 ng, it increases to 400–600 ng in cities without substantial industrial emission of arsenic and is even higher in the

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vicinity of industrial sources. Moreover, it is influenced by tobacco consumption and can vary from 1 μg/day in a nonsmoker to approximately 10 μg/day in a smoker.[1,3,4]. Epidemiological studies indicate that long-lasting exposure to arsenic is associated with skin, lung, liver, kidney, prostate, and bladder cancer as well as vascular diseases and diabetes. Arsenic is also hepato- and neurotoxic and is responsible for impaired fetal and child development.[5]

The absorbed dose of arsenic can be measured in hair, nail, blood, or urine samples. Due to the fact that arsenic accumulates in keratin-rich tissue, total arsenic level in hair, fingernails, or toenails is used as the indicator of long-lasting exposure. In contrast, levels of arsenic in blood and urine and urinary arsenic metabolites (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) are typically used as indicators of recent exposure. In addition, it has been reported that average occupational exposure to airborne arsenic trioxide is significantly correlated with the inorganic arsenic metabolites in urine samples collected immediately after shift.[6]

After absorption, arsenic is metabolized by stepwise methylation, mainly in the liver. In general, two pathways of arsenic methylation have been suggested—reductive proposed by Hayakawa et al.[7] and oxidative proposed by Challenger et al.[8] Both models result in DMA or MMA induction.[9] To date, arsenic (+3 oxidation state)-methyltransferase (AS3MT), purine nucleoside phosphorylase (PNP), glutathione *S*-transferase omega 1 (GSTO1), and glutathione *S*-transferase omega 2 (GSTO2) have been proposed to be involved in arsenic metabolism.

AS3MT is thought to catalyze the conversion of arsenite (iAs3+) methylate to MMA5+ and trivalent monomehtylarsonic acid (MMA3+) methylate to DMA5+ using *S*-adenosyl-methionine (SAM) as the methyl donor. However, PNP, GSTO1, and GSTO2 are suggested to be involved in reducing the pentavalent arsenic species, including arsenate (iAs5+), MMA5+, and DMA5+ to trivalent arsenicals.[10–13]

Arsenic methylation produces both highly reactive metabolites containing trivalent arsenic (As3+) and less reactive metabolites containing pentavalent arsenic.[14] Studies on arsenic toxicity yield inconsistent results. Numerous studies indicate that inorganic arsenic, such as iAs5+ and iAs3+, is more toxic than organic arsenic, such as MMA, DMA, arsenobetaine, arsenocholine, and arsenosugars.[15] However, other studies indicate that certain types of organic arsenic, such as the MMA3+, are more toxic than As3+.[13,16]

Arsenic-induced health effects differ greatly between individuals, partly due to the individual ability to metabolize arsenic which affects retention and distribution of toxic metabolites.[17] Arsenic absorption and toxicity are thought to depend on specific configurations of PNP, glutathione-*S*-transferase omega 1, and AS3MT genotypes.[1,4,6,17–22]

According to the literature, there are six polymorphisms in the exonic region of PNP gene which in general contains six exons and is located on chromosome 14q11. Among these, one is located in the 5'UTR (rs17881206), three (His20His, Gly51Ser, Pro57Pro) in exon 2, one in exon 5 (Ala174Ala), and one in the 3'UTR (rs7785).[23] Hsieh et al. found a significantly higher risk of carotid atherosclerosis in people who carried the PNP A–T haplotype.[24]

For AS3MT, several exonic single nucleotide polymorphisms were found on chromosome 10q24 (Arg173Trp, Met287Thr, Thr306Ile, Ile132Phe, Tyr135Asn, Gly140Ala).[9,25] Meza et al. pointed out that in Mexican subjects, three AS3MT gene polymorphisms—in locus rs12767543, rs3740393, and rs11191453 of chromosome 10—were associated with high uDMA/uMMA ratio.[9,26] In studies carried out by Gongand O'Bryant, GG polymorphism in locus rs10748835 was associated with higher incidence of cardiovascular diseases in people exposed to low doses of arsenic in drinking water.[22]

Free oxygen radicals produced in arsenic biotransformation contribute to arsenic toxicity and carcinogenicity. Glutathione *S*-transferase (GST) appears to have a key function in the defense system against oxidative damage and, in consequence, in maintenance of cell homeostasis. GST is a phase II enzyme that can detoxify xenobiotics by catalyzing their conjugation with reduced glutathione. GST is involved in the reduction of As(5+), MMA(5+), and DMA(5+).[14,15]

Two genes from the GST family, GSTO1 and GSTO2, are located on chromosome 10q and contain six exons each. In GSTO1, six exonic polymorphisms have been identified in various world populations (Cys32Tyr, Ala140Asp, –/AGG, Glu208Lys, Thr217Asn, Ala236Val)[27] and there has been a single case of a Thr217Asn variant in the dbSNP, but not yet reported in any population.[12] Four nonsynonymous exonic SNPs (Val114Ile, Cys130Tyr, Asn142Asp, Leu158Ile) were also reported in GSTO2. In a study by Paiva et al., Asn142As polymorphism appears to be correlated with an increase in DMA excretion and seems to modulate arsenic biotransformation and thereby arsenic toxicity.[28]

Objectives

The aim of this study was to analyze the link between single nucleotide polymorphisms in locus rs 1130650 in PNP gene, locus rs 4925 in GSTO-1 gene (Ala140Asp), and locus rs 11191439 in AS3MT gene (Met287Thr) and the total urinary arsenic concentration in people occupationally exposed to arsenic and other heavy metals, particularly lead and cadmium. Moreover, we attempted to find the possible connection between occupational arsenic exposure and polymorphism of Trp64Arg in ADRB3 gene, which is associated with atherogenic risk factors, namely weight gain, insulin resistance, and diabetes.[29–31]

Materials and methods

Study population

The study was carried out in copper smelter and refinery in Legnica and Glogow. Ethical approval from the Bioethics Committee of Wroclaw Medical University (No. KB-156/2010) was obtained. All the study participants provided written informed consent.

The data of 113 workers directly exposed to lead, cadmium, and arsenic were collected. Precisely, the group consisted of refinery workers, smelters, crane operators, converter plant operators, dust removal device operators, electrode molders, electrolyzers, outfall operators, feedstock operator, and electrolyte cleaner operator. A total of 109 males and 4 females were enrolled in the study with the age range between 26 years and 63 years. The mean occupational exposure was 21 years. Fifty-five employees smoked cigarettes. All the subjects participating in the study were asked to complete the special questionnaire to obtain the relevant information on occupational history (work department, type of work, and exposure duration), modifying lifestyle factors (type of diet, physical activity, smoking, and alcohol consumption habits), health conditions, and medical history. Physical examination included general medical checks, blood pressure measurement, and body mass index evaluation. The characteristics of the study population are given in Table 1.

We collected 25 ml of venous blood and standard urine sample from each worker right after the end of a shift. The lab tests included:

- blood lead (Pb-B) and cadmium (Cd-B) concentration,
- urinary total arsenic (As-U) concentration,
- blood zinc protoporphyrins (ZnPP) concentration,
- serum zinc (Zn-S) and copper (Cu-S) concentration,
- conventional biochemical tests detecting serum creatinine, urea, aminotransferases, bilirubin, glucose, total cholesterol with low-density lipoprotein (LDL) / high-density lipoprotein (HDL) fractions and triglycerides, and
- complete blood count.

Table 1. Clinical characteristics of the study population.

	X	Me	SD	Min	Max
Age (years)	46.68	48.00	9.11	26.00	63.00
Height (m)	1.76	1.76	0.07	1.54	1.90
Weight (kg)	88.76	87.00	15.01	59.00	130.00
BMI (kg/m^2)	28.63	27.90	4.18	20.50	42.00
BSA (m^2)	2.05	2.04	0.18	1.61	2.55
Waist circumference (cm)	99.95	99.00	11.84	71.00	140.00
	n		%		
Number	113		100.0		
Gender					
Male	109		96.5		
Female	4		3.5		
Smokers	55		48.7		
Overweight/obesity	93		82.3		
Overweight	56		49.6		
Obesity	37		32.7		
Hypertension	30		26.5		
Ischemic heart disease	1		0.9		
Dyslipidemia	5		4.4		
Peripheral artery disease	2		1.8		
Diabetes	7		6.2		

X: arithmetic mean; Me: median; SD: standard deviations; Min: minimal values; Max: maximal values.

Laboratory and toxicological characteristics of the study population are presented in Tables 2 and 3.

Determination of Pb-B and Cd-B concentrations

Pb-B and Cd-B concentrations were measured by graphite furnace atomic absorption spectrometry (Solaar M6 of Thermo Elemental, UK). Analytical procedures were based on the methods described in the work by Trzcinka-Ochocka et al.[32] Calibration curves of lead and cadmium were prepared with blood standards: certified reference material (BCR® IRMM). Both methods were routinely monitored by determination of reference material (Recipe) and participation in intercomparison programme for toxicological analyses in biological materials G-EQUAS.

Table 2. Conventional lab tests in the study population.

	X	Me	SD	Min	Max
WBC (K/mL)	7.62	6.81	2.42	5.24	14.37
RBC (M/mL)	4.89	4.86	0.26	4.39	5.46
Hemoglobin (g/dL)	14.98	15.15	0.99	12.60	17.10
Hematocrit (%)	44.38	44.55	2.57	39.90	52.10
Platelets (K/mL)	229.87	232.50	52.80	129.00	376.00
Total bilirubin (mg/dL)	0.55	0.40	0.35	0.30	1.20
Aspartate aminotransferase(U/L)	31.00	28.00	7.59	24.00	45.00
Alanine transaminase (U/L)	32.17	28.00	14.30	21.00	59.00
Glucose (mg/dL)	101.17	95.50	32.63	63.00	161.00
Creatinine (mg/dL)	1.01	1.03	0.15	0.80	1.15
EGFR (mL/min)	77.50	74.00	15.14	61.00	105.00
Uric acid (mg/dL)	5.42	5.55	1.72	3.40	7.60
Total cholesterol (mg/dL)	230.50	233.00	33.79	178.00	283.00
LDL (mg/dL)	136.33	138.00	29.13	93.00	174.00
HDL (mg/dL)	59.17	55.00	15.07	42.00	84.00
Triglycerides (mg/dL)	174.33	175.50	106.76	55.00	297.00

X: arithmetic mean; Me: median; SD: standard deviations; Min: minimal values; Max: maximal values; EGFR: estimated glomerular filtration rate.

Table 3. Basic toxicological parameters in the study population.

	X	Me	SD	Min	Max
Exposure period (years)	21.01	22.00	11.73	2.00	43.00
Cu-S (mg/dL)	103.62	103.00	14.96	49.50	144.00
Zn-S (mg/dL)	86.71	85.30	11.53	56.10	120.00
Cd-B (μg/L)	1.78	1.11	2.05	0.15	10.20
Pb-B (μg/L)	292.11	291.00	112.58	48.20	534.00
ZnPP (μg/dL)	44.32	34.00	29.64	22.00	232.00
As-U (μg/g CREA)	31.34	25.30	27.52	2.44	182.00

X: arithmetic mean; Me: median; SD: standard deviations; Min: minimal values; Max: maximal values; Pb-B: blood lead concentration; Cd-B: blood cadmium concentration; ZnPP: blood zinc protoporphyrins concentration; Zn-S: serum zinc concentration; Cu-S: serum copper concentration; As-U: urinary total arsenic concentration.

All the measurements were presented as micrograms per liter (μg/L) and in accordance to the recommendations of Nofer Institute of Occupational Medicine in Łódź, the biological exposure limit to lead is 500 μg/L and 5 μg/L to cadmium.

Determination of urinary arsenic concentration

Total urinary As concentration was measured by hydride generation atomic absorption spectrometry (HGAAS) using the VP100 Continuous Flow Vapour System (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). The method used was described in the work by Jakubowski et al.[32] To determine the calibration curve, the reference material—ClinCal® Urine Calibrator; Recipe—was used. We monitored the accuracy of the method by analyzing samples of a reference material: Seronorm Trace Elements Urine (SERO AS, Oslo, Norway) and by participating in intercomparison programme for toxicological analyses in biological materials G-EQUAS.

Biological exposure limit to arsenic, measured in urine, proposed by Nofer Institute of Occupational Medicine in Łódź, is 35 μg/L (35 μg/g creatinine).

Determination of zinc and copper serum concentration

Cu and Zn serum concentrations were assessed by flame atomic absorption spectrometry (ThermoElemental, Solaar M6, UK) by measuring the absorbance at wavelength of 324.8 (213.9) nm and using air-acetylene flame with the deuterium background correction. Single-element copper (Zinc) standard 1000 μg/mL certified by the CPI International; Peak Performance USA was used as a reference material for calibration.

These methods were routinely monitored by analyzing the samples of reference material: Seronorm™ Trace Elements Serum and by participating in intercomparison program for toxicological analyses in biological materials G-EQUAS.

Determination of blood zinc protoporphyrins concentration

ZnPP erythrocyte concentration was measured by means of ProtoFluor Z hematofluorometer (Helena Laboratories Corporation, Beaumont, Texas, USA) in accordance with the manufacturer's instructions. The measurement was performed in one drop of patient's whole blood previously collected into test tubes with anticoagulant (ethylenediaminetetraacetic acid (EDTA)). To obtain total oxygen saturation of hemoglobin, ProtoFluor reagent with cyanide salt and stabilizers in aqueous solution were added. Hemoglobin converts into oxyhemoglobin. The wavelengths for fluorescence were as follows: excitation 415 nm, emission 595 nm, range 0–270 µg/dL. Equipment calibration was performed with two ProtoFluor calibrators: ProtoFluor Low and ProtoFluor High. Both solutions contain ZnPP, pyridine, stabilizers, and preservatives. The results were adjusted for patient's hematocrit.

DNA isolation

Blood samples were collected by venipuncture into standard 2.7 mL tubes containing EDTA and then frozen at −80°C.

Genomic DNA was manually extracted from leukocytes by a magnetic-bead technology incorporated in a NucleoMag® Blood of MACHEREY-NAGEL workstation.

Determination of the PNP (rs 1130650), GSTO-1 (rs 4925), and AS3MT (rs 11191439) gene SNP. A multiplex assay based on the polymerase chain reaction (PCR) was used for amplification of PNP, GSTO-1, and AS3MT gene fragments with the following primers:

PNP gene: F/forward/5′-CTC AGT ATA CCT GCC AGC CTT T-3′
R/reverse/5′-CTC TGA CTC ATG GTG GGC TT-3′

GSTO-1 gene: F/forward/5′-GAC CAA GCC AGC ATT TTA GG-3′
R/reverse/5′-AGC AAG CCC ATG ACA AAG TC-3′

AS3MT gene: F/forward/5′-TCG TTT TGT TTC TGC AAC AT-3′
R/reverse/5′-AGG CAA TGC AAA GTC AAG AA-3′.

All the amplified products were digested in the multistage process by the PCR clean-up restriction enzyme, labeled with probes, and finally digested with the FastAP enzyme. PCR products were separated by the ABI 3100 analyzer (Applied Biosystems, Foster City, California, USA). Collected data concerning PCR products were analyzed with Gene Mapper ver. 3.5 (Applied Biosystems).

Determination of the ADRB3 (rs4994) gene SNP. Genotyping was performed by means of Fluidigm 192.24 Dynamic Array with BioMark HD Systems and EP1 (Fluidigm Corp., San Francisco, California, USA). We applied SNP-type assay (Fluidigm Corp.) which includes allele-specifically designed fluorescence primers (fluorescein amidites with emission of wavelength 518 λ_{max}/nm) and a common reverse primer. The data were analyzed by the BioMark SNP Genotyping Analysis software to obtain genotype calls.

Statistical analysis

Statistical analysis was performed using the Statistica 12.0 software. Quantitative variables established for the study group included arithmetic means (X), medians (Me), standard deviations (SD), minimal values (Min), and maximal values (Max). Distribution of variables was tested with the W-Shapiro–Wilk test. For independent, quantitative variables manifesting normal distribution, analysis of variance (ANOVA, unifactorial parametric) was performed. In case of variables manifesting distribution distinct from normal, for independent qualitative variables, a nonparametric equivalent of Kruskal–Wallis analysis of variance was used. Significant differences between the arithmetic means were estimated using post hoc tests. Results for the qualitative variables were expressed in percentages. For the independent qualitative variables, in further statistical analysis, the highest credibility χ^2 test was used. The values of $p < 0.05$ were regarded as statistically significant.

Results

Mean urinary arsenic concentration in the occupationally exposed group was within the range of maximum allowable concentration and amounted to 31.34 ± 27.52 µg/g creatinine (CREA). The job seniority of people exposed to arsenic was 21.01 ± 11.73 years. Concentrations of other toxicological parameters were also within the range of maximum allowable concentration and were as follows: mean Pb-B concentration 292.11µg/L, mean ZNPP concentration in erythrocytes 44.32ug/dL, mean Cd-B concentration

Table 4. Selected polymorphisms of PNP, GSTO-1, AS3MT, and ADRB3 gene in the study population.

	n	%
Locus rs 1130650 gene PNP	110	97.3
Homozygote CC	69	61.1
Heterozygote CT	23	20.3
Homozygote TT	18	15.9
Locus rs 4925 gene GSTO-1	110	97.3
Homozygote CC	67	59.3
Heterozygote AC	32	28.3
Homozygote AA	11	9.7
Locus rs 11191439 gene As3MT	112	99.1
Homozygote TT	97	85.8
Heterozygote CT	14	12.4
Homozygote CC	1	0.9
Locus rs4994 gene ADRB3	110	97.3
Homozygote AA	91	80.6
Heterozygote AG	15	13.2
Homozygote GG	4	3.5

AS3MT: arsenic (+3 oxidation state)-methyltransferase; PNP: purine nucleoside phosphorylase; GSTO1: glutathione *S*-transferase omega 1; ADRB3: beta-3 adrenergic receptor.

1.78μg/L. Basic toxicology parameters of the exposed group are presented in Table 3.

The results of genotypes distribution for PNP, GSTO-1, AS3MT, and ADRB3 gene polymorphisms in exposed individuals are presented in Table 4.

Comparative analysis of subgroups divided on the basis of rs 1130650 PNP gene polymorphism showed that arsenic levels equal or higher than median for the whole group were statistically more common in homozygotes CC and heterozygotes CT when compared to the subgroup of homozygotes TT. As-U values for described subgroups are presented in Table 5.

The comparative analysis of subgroups based on the type of 4925 GSTO-1 gene polymorphism revealed that homozygotes AA were characterized by statistically significant higher mean values of As-U than heterozygotes AC and homozygotes CC. Moreover, the subgroup of homozygotes AA, when compared to heterozygotes CT and homozygotes TT, had statistically significant higher frequency of As-U levels equal or higher than median or the third quartile for the whole group and higher MAC value. As-U values for subgroups with described type of the GSTO-1 gene polymorphism are presented in Table 5.

The comparative analysis of subgroups divided on the basis of rs 11191439 AS3MT gene polymorphism and rs4994 ADRB3 gene polymorphism revealed no statistically significant differences. As-U values in subgroups divided on the basis of rs 11191439 AS3MT and rs4994 ADRB3 gene polymorphisms are presented in Table 5.

The comparative analysis showed no effect of the studied polymorphisms of the PNP, GSTO-1, AS3MT, and ADRB3 genes on the concentrations of Cu, Zn, Cd, Pb, and ZnPP. Cu-S, Zn-S, Cd-B, Pb-B, and ZnPP values in subgroups divided on the basis of PNP, GSTO-1, AS3MT, and ADRB3 gene polymorphisms are presented in Table 6.

Discussion

Humans typically excrete absorbed arsenic in urine; 10–30% as inorganic, 10–20% as MMA, and 60–80% as DMA, although excretion patterns vary among populations.[10,17,25,28] Furthermore, there are significant differences in arsenic excretion among populations exposed to similar arsenic concentration. For instance, Indians from South America tend to excrete lower (about 5%) amounts of MMA when compared to other populations.[33] A number of factors have been hypothesized to impact As metabolism, including exposed dose, age, gender, race, exposure route, nutritional status, and some environmental circumstances of exposure.[13,34] Taking into account the role of AS3MT, GSTO, and PNP in arsenic biotransformation and metabolism, polymorphisms of genes encoding these enzymes are likely to produce interindividual variations in arsenic metabolism and thus influence susceptibility toward arsenic toxicity, in both in vivo and in vitro studies.[9,12]

Glutathione is the most widespread particle with thiol group that can reduce arsenic (5+) to arsenic (3+) and create a stable complex. Both GSTO and PNP are enzymes catalyzing reduction of arsenic (5+). AS3MT catalyzes the methylation of iAs3+ using SAM as the methyldonor.

A comprehensive retrospective meta-analysis concerning influence of selected polymorphisms of AS3MT, PNP, and GSTO on arsenic methylation and its possible toxicity were published in 2014 by Antonelli et al.[10] He concluded that polymorphism of GSTO gene has no influence on arsenic concentration in urine. However, this influence was noticeable in animal studies and in vitro studies. The authors suggested that humans have arsenic reduction pathways other than just GSTO which makes it even more difficult to determine the role of GSTO in arsenic metabolism. On the other hand, Fu et al. discovered a negative influence of Ala/Asp polymorphism of

Table 5. As-U concentration in subgroups divided according to selected polymorphisms of genes.

	Locus rs 1130650 gene PNP			
	Homozygote CC	Heterozygote CT	Homozygote TT	$p < 0.05$
As-U (μg/g CREA)[a]	35.17 ± 29.79	24.31 ± 28.37	28.44 ± 17.93	—
As-U ≥ Q1 (As-U ≥ 13.30 μg/g CREA)[b]	54/78.3	19/82.6	12/66.7	—
As-U ≥ Me (As-U ≥ 25.30 μg/g CREA)[b]	43/62.3	10/43.5	4/22.2	CC-TT, CT-TT
As-U ≥ Q3 (As-U ≥ 41.94 μg/g CREA)[b]	20/29.0	6/26.1	3/16.7	—
As-U > MAC (As-U > 35 μg/g CREA)[b]	26/37.7	8/34.8	3/16.7	—
	Locus rs 4925 gene GSTO-1			
	Homozygote CC	Heterozygote AC	Homozygote AA	$p < 0.05$
As-U (μg/g CREA)[a]	26.87 ± 20.91	28.87 ± 23.10	53.42 ± 30.38	CC-AA, AC-AA
As-U ≥ Q1 (As-U ≥ 13.30 μg/g CREA)[b]	48/71.6	24/75.0	11/100.0	—
As-U ≥ Me (As-U ≥ 25.30 μg/g CREA)[b]	28/41.8	16/50.0	11/100.0	CC-AA, AC-AA
As-U ≥ Q3 (As-U ≥ 41.94 μg/g CREA)[b]	13/19.4	8/25.0	7/63.6	CC-AA, AC-AA
As-U > MAC (As-U > 35 μg/g CREA)[b]	19/28.4	9/28.1	7/63.6	CC-AA, AC-AA
	Locus rs 11191439 gene As3MT			
	Homozygote TT	Heterozygote CT	Homozygote CC	$p < 0.05$
As-U (μg/g CREA)[a]	32.09 ± 28.49	29.26 ± 21.30	11.70 ± 0.00	—
As-U ≥ Q1 (As-U ≥ 13.30 μg/g CREA)[b]	75/77.3	10/71.4	0/0.0	—
As-U ≥ Me (As-U ≥ 25.30 μg/g CREA)[b]	49/50.5	8/57.1	0/0.0	—
As-U ≥ Q3 (As-U ≥ 41.94 μg/g CREA)[b]	26/26.8	3/21.4	0/0.0	—
As-U > MAC (As-U > 35 μg/g CREA)[b]	32/33.0	5/35.7	0/0.0	—
	Locus rs4994 gene ADRB3			
	Homozygote AA	Heterozygote AG	Homozygote GG	$p < 0.05$
As-U (μg/g CREA)[a]	31.20 ± 26.86	39.65 ± 34.68	13.79 ± 8.37	—
As-U ≥ Q1 (As-U ≥ 13.30 μg/g CREA)[b]	69/75.8	11/73.3	2/50.0	—
As-U ≥ Me (As-U ≥ 25.30 μg/g CREA)[b]	47/51.6	9/60.0	1/25.0	—
As-U ≥ Q3 (As-U ≥ 41.94 μg/g CREA)[b]	23/25.3	6/40.0	0/0.0	—
As-U > MAC (As-U > 35 μg/g CREA)[b]	30/33.0	7/46.7	0/0.0	—

AS3MT: arsenic (+3 oxidation state)-methyltransferase; PNP: purine nucleoside phosphorylase; GSTO1: glutathione S-transferase omega 1; As-U: urinary total arsenic concentration; ADRB3: beta-3 adrenergic receptor.
[a]Values are represented as mean ± standard deviation.
[b]Values are represented as number of persons/percentage.

GSTO1 gene on the level of DMA excreted in urine in a study on 155 Chinese people exposed to high-dose inorganic arsenic in drinking water.[19]

Our study suggests that AA homozygosity in locus rs 4925 of GSTO1 gene is related to higher, exceeding maximum allowable concentration, urinary arsenic, hence individuals with such constellation should be thoroughly monitored for distant negative impact of arsenic exposure.

Genes encoding AS3MT and GSTO1 are located on the same region of chromosome 10 (10q25). Sampayo-Reyes et al. and Hernandez et al. studied polymorphisms of these genes in Mexicans exposed to arsenic in drinking water. They discovered a straight relationship between arsenic concentration in urine and level of DNA damage. This relation was modified by Met287Thr polymorphism in AS3MT gene.[15,28]

Schlawicke Egstrom et al. also confirmed a strong influence of polymorphism of AS3MT in locus rs 3740400 on level of arsenic methylation and its detoxification.[17] Hwang et al. proved that polymorphism

Table 6. Basic toxicological parameters other than urine arsenic concentration in subgroups divided according to selected polymorphisms of genes.

	Locus rs 1130650 gene PNP			
	Homozygote CC	Heterozygote CT	Homozygote TT	$p < 0.05$
Cu-S (mg/dL)[a]	105.11 ± 15.83	102.21 ± 14.41	98.80 ± 11.02	—
Zn-S (mg/dL)[a]	86.14 ± 10.76	86.96 ± 12.47	88.85 ± 14.21	—
Cd-B (μg/L)[a]	2.07 ± 2.35	1.29 ± 1.08	1.08 ± 1.11	—
Pb-B (μg/L)[a]	281.98 ± 113.55	284.23 ± 126.24	338.61 ± 87.33	—
ZnPP (μg/dL)[a]	41.32 ± 28.49	40.17 ± 16.13	56.89 ± 42.45	—
	Locus rs 4925 gene GSTO-1			
	Homozygote CC	Heterozygote AC	Homozygote AA	$p < 0.05$
Cu-S (mg/dL)[a]	103.52 ± 15.41	106.17 ± 15.76	98.06 ± 8.69	—
Zn-S (mg/dL)[a]	87.52 ± 11.86	86.17 ± 10.91	88.40 ± 8.25	—
Cd-B (μg/L)[a]	1.60 ± 2.11	2.18 ± 2.16	1.55 ± 1.50	—
Pb-B (μg/L)[a]	274.94 ± 105.58	320.56 ± 111.70	312.18 ± 122.13	—
ZnPP (μg/dL)[a]	42.18 ± 22.63	46.28 ± 29.06	35.09 ± 12.01	—
	Locus rs 11191439 gene As3MT			
	Homozygote TT	Heterozygote CT	Homozygote CC	$p < 0.05$
Cu-S (mg/dL)[a]	103.67 ± 15.58	103.21 ± 9.45	N/A	—
Zn-S (mg/dL)[a]	85.69 ± 11.53	94.47 ± 10.61	N/A	—
Cd-B (μg/L)[a]	1.85 ± 2.11	1.21 ± 1.47	N/A	—
Pb-B (μg/L)[a]	288.57 ± 111.96	323.50 ± 110.52	107.00 ± 0.00	—
ZnPP (μg/dL)[a]	44.19 ± 30.85	45.14 ± 22.90	35.00 ± 0.00	—
	Locus rs4994 gene ADRB3			
	Homozygote AA	Heterozygote AG	Homozygote GG	$p < 0.05$
Cu-S (mg/dL)[a]	103.25 ± 14.72	101.95 ± 13.25	120.00 ± 19.80	—
Zn-S (mg/dL)[a]	87.57 ± 12.25	84.45 ± 8.13	77.00 ± 0.57	—
Cd-B (μg/L)[a]	1.73 ± 1.93	2.00 ± 2.76	2.38 ± 1.04	—
Pb-B (μg/L)[a]	291.57 ± 108.91	304.40 ± 150.46	289.25 ± 69.23	—
ZnPP (μg/dL)[a]	45.27 ± 31.89	39.93 ± 13.94	48.25 ± 32.46	—

PNP: purine nucleoside phosphorylase; GSTO1: glutathione *S*-transferase omega 1; Pb-B: blood lead concentration; Cd-B: blood cadmium concentration; ZnPP: blood zinc protoporphyrins concentration; Zn-S: serum zinc concentration; Cu-S: serum copper concentration; AS3MT: arsenic (+3 oxidation state)-methyltransferase; ADRB3: beta-3 adrenergic receptor.
[a]Values are represented as mean ± standard deviation.

G > C in intron 6 and T > C in intron 10 of AS3MT gene is related to significant difference in excretion of methylated forms of arsenic, especially of DMA.[1]

Agusa et al. studied the frequency of particular AS3MT gene polymorphisms in populations of various countries (Mexico, India, Chile, Argentina, Taiwan, and others).[9] To describe this gene's polymorphic model in East-Central Europe Lindberg research studies were used. Lindberg et al. showed that allele C in locus rs 11191439 of AS3MT gene is related to higher MMA (V) and lower DMA (V) concentration in urine, especially in men.[34]

Our study yielded no statistically significant correlation between AS3MT polymorphism and urinary arsenic, nevertheless subjects with allele T in locus rs 11191439 had their total arsenic concentration more than twice as high as homozygous CC. No direct relationship does not exclude the relevance of this result. Sampayo-Reyes et al. showed that arsenic affects DNA damage and that AS3MT polymorphism

has an important impact on this damage, however, mostly in children.[15] In a research from 2017, Hsieh et al. discovered a link between AS3MT polymorphism, decrease in arsenic methylation, and child development retardation.[35]

The role of PNP polymorphism is even harder to determine and scientific data on the subject is limited. In the aforementioned Antonelli's meta-analysis, this polymorphism was not included in the study due to the lack of bibliographic data.[10] However, research from 2015 by Gaxiola-Robles et al. showed that increased PNP activity induces increase in glutathione peroxidase and reductase activity but not in aforementioned transferase.[20] Nevertheless, due to limited data, it is hard to univocally assess connections between processes of arsenic detoxification, GSTO activity, and polymorphic constellation of GSTO1 gene. Chaudhuri et al. provided unambiguous proof of dependence between arsenic toxicity and PNP gene polymorphism, namely polymorphism His20His-, Gly51Ser-, and Pro57Pro-induced skin cancer.[12]

Our study showed a correlation between homozygous T allele in locus rs 1130650 of PNP gene and lower urinary arsenic concentration which could be the result of lower absorption of arsenic among the carriers and as a consequence may contribute to lower arsenic toxicity.

The adrenergic beta-3 receptor is the protein that works as a regulator of lipolysis as well as thermogenesis. From all adrenergic receptor, ADRB3 is the most interesting to investigate in terms of skyrocketing frequency of metabolic syndrome. Until now, connection between Trp64Arg polymorphism of gene ADRB-3 (rs4994) and development of abdominal obesity, diabetes, insulin resistance, and atherogenic dyslipidemia was found.[29–31] This polymorphism was also reported to influence the effectiveness of pharmacological treatment of obesity.[36] In addition, relationship of Gln27Glu polymorphism of gene ADRB-2 with lower body mass was observed.[37] On the other hand, the role of xenobiotics, including biotoxic metals, is getting more and more significant when discussing pathogenesis of metabolic diseases and atherosclerosis.[22,25] Therefore, investigating the relationship between arsenic concentration and aforementioned ADRB-3 (rs 4994) polymorphism seems both reasonable and interesting. Our study showed no statistically important relationship, however, we observed a marked tendency to higher urinary arsenic concentration in allele A carriers in locus rs 4994 of ADRB-3 gene. Considering the fact that most (82.3%) of studied copper smelter workers were obese, 26.5% had hypertension, 6.2% had diabetes, and 4.4% had atherogenic dyslipidemia, it might be concluded that metabolic syndrome is caused not only by behavioral factors (their impact is without doubt the most crucial) but also by genetic factors and environmental pollution.

This study has significant limitations that may prompt other researchers to continue our work. In the scope of the studied group, the restrictions are no control group (people who are not professionally exposed to arsenic) and overrepresentation of men. In case of testing health consequences of arsenic exposure, a control group of unauthorized subjects would be necessary. In studies on genetic predisposition, lack of such group is a significant limitation, however, it does not exclude the possibility of valuable inference concerning the examined group of those professionally exposed to arsenic. A large overrepresentation of men among the subjects was a reflection of the gender structure of employees within the examined factory. Due to the substantially lower number of women in the examined group, the analysis of the impact of gender on observed relationships was not performed. In terms of methodology, the limitations include no determination of inorganic arsenic (AsIII and AsV) and metabolites of arsenic (MMA and DMA), as well as glutathione (GSH and GSSG). Additional laboratory analyzes were not performed due to the limited amount of biological material.

In conclusion, our study has proved that individual tendency to absorb toxic metals (especially arsenic) in workplaces with the same standard of occupational hygiene is determined by genetic factors. Thus, based on frequency of particular polymorphisms, a group of workers mostly predisposed to extensive arsenic absorption can be extracted and their work can be planned in more rational way (for instance by avoiding high-exposure worksites). Another step would be to asses direct health influence of chronic exposure to arsenic and other metals, especially in terms of cancer morbidity, diabetes, and cardiovascular disease. Notwithstanding, it must be remembered that exposure to toxic metals is only one of the many factors involved in etiopathogenesis of these diseases, next to a number of detrimental behavioral factors such as smoking cigarettes, minimal physical activity, and improper diet. Therefore, the right prophylaxis should focus on both wide educational actions and systematic toxicological monitoring as well as, when possible, directing workers to less exposed worksites.

Conclusions

1. Correlation between polymorphisms of PNP and GSTO-1 genes and urinary arsenic concentration was found in subjects occupationally exposed to arsenic which confirms the hypothesis that tendency to absorb toxic metals is genetically determined.
2. People occupationally exposed to arsenic with homozygous allele T in locus rs 1130650 of PNP gene are predisposed to lower urinary arsenic concentration.
3. AA homozygosity in locus rs 4925 of GSTO-1 gene may result in higher urinary arsenic concentration.
4. In people occupationally exposed to arsenic, polymorphism in locus rs 11191439 of AS3MT gene and in locus rs4994 of ADRB3 gene has no statistically significant influence on urinary arsenic concentration, however, the presence of some alleles in these loci indicates the tendency toward higher absorption of arsenic.
5. In people particularly predisposed to higher arsenic absorption, that is, having certain polymorphisms in genes PNP, AS3MT, GSTO-1, and ADRB-3, a comprehensive assessment of distant health effects of arsenic exposure should be performed, including oncological screening, as well as blood pressure, lipid, and glucose control. In this group, early non-pharmacological prevention of arterial hypertension and other metabolic syndrome components, such as diabetes, dyslipidemia, and obesity should be introduced.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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References

1. IARC 2012. Arsenic and arsenic compounds (IARC monographs on the accumulation of carcinogenic risks to humans, Vol. 100c), 2012, pp. 41–93.
2. WHO. *Air quality guidelines for Europe*. 2nd ed. Copenhagen: WHO Regional Publications, European Series, 2000.
3. WHO. *Arsenic and arsenic compounds (Environmental Health Criteria 224)*. 2nd ed. Geneva: World Health Organization, International Programme on Chemical Safety, 2001.
4. Aposhian HV and Aposhian MM. Arsenic toxicology: five questions. *Chem Res Toxicol* 2006; 19: 1–15.
5. Sumi D and Himeno S. Role of arsenic (+3 oxidation state) methyltransferase in arsenic metabolism and toxicity. *Biol Pharm Bull* 2012; 35: 1870–1875.
6. Apostoli P, Bartoli D, Alessio L, et al. Biological monitoring of occupational exposure to inorganic arsenic. *Occup Environ Med* 1999; 56: 825–832.
7. Hayakawa T, Kobayashi Y, Cui X, et al. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch Toxicol* 2005; 79: 183–191.
8. Challenger F, Higgenbottom C and Ellis L.The formation of organo-metalloid compounds by microorganisms. Part I. Trimethylarsine and dimethylethylarsine. *J Chem Soc* 1933; 5: 95–101.
9. Agusa T, Fujihara J, Takeshita H, et al. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. *Int J Mol Sci* 2011; 12: 2351–2382.
10. Antonelli R, Shao K, Thomas DJ, et al. AS3MT, GSTO, and PNP polymorphisms: impact on arsenic methylation and implications for disease susceptibility. *Environ Res* 2014; 132: 156–167.
11. Chung C, Pu Y, Su C, et al. Gene polymorphisms of glutathione S-transferase omega 1 and 2, urinary arsenic methylation profile and urothelial carcinoma. *Sci Total Environ* 2011; 409: 465–470.
12. De Chaudhuri S, Ghosh P, Sarma N, et al. Genetic variants associated with arsenic susceptibility: study of purine nucleoside phosphorylase, arsenic (+3) methyltransferase, and glutathione S-transferase omega genes. *Environ Health Perspect* 2008; 116: 501–505.
13. Hwang YH, Chen YH, Su YN, et al. Genetic polymorphism of As3MT and delayed urinary DMA

excretion after organic arsenic intake from oyster ingestion. *J Environ Monit* 2010; 12: 1247–1254.
14. Chen JW, Wang SL, Wang YH, et al. Arsenic methylation, GSTO1 polymorphisms, and metabolic syndrome in an arseniasis endemic area of southwestern Taiwan. *Chemosphere* 2012; 88: 432–438.
15. Sampayo-Reyes A, Hernández A, El-Yamani N, et al. Arsenic induces DNA damage in environmentally exposed Mexican children and adults. Influence of GSTO1 and AS3MT polymorphisms. *Toxicol Sci* 2010; 117: 63–71.
16. Petrick JS, Ayala-Fierro F, Cullen WR, et al. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol Appl Pharmacol* 2000; 163: 203–207.
17. Engström K, Nermell B, Conchac G, et al. Arsenic metabolism is influenced by polymorphisms in genes involved in one-carbon metabolism and reduction reactions. *Mutat Res* 2009; 667: 4–14.
18. Carter DE, Aposhian HV and Gandolfi AJ. The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: a toxicochemical review. *Toxicol Appl Pharmacol* 2003; 193: 309–334.
19. Fu S, Wu J, Li Y, et al. Urinary arsenic metabolism in a Western Chinese population exposed to high-dose inorganic arsenic in drinking water: influence of ethnicity and genetic polymorphisms. *Toxicol Appl Pharmacol* 2014; 274: 117–23.
20. Gaxiola-Robles R, Labrada-Martagón V, Bitzer-Quintero OK, et al. Purine nucleoside phosphorylase and the enzymatic antioxidant defense system in breast milk from women with different levels of arsenic exposure. *Nutr Hosp* 2015; 31: 2289–2296.
21. Ghosh P, Banerjee M, Giri AK, et al. Toxicogenomics of arsenic: classical ideas and recent advances. *Mutat Res* 2008; 659: 293–301.
22. Gong G and O'Bryant SE.Low-level arsenic exposure, AS3MT gene polymorphism and cardiovascular diseases in rural Texas counties. *Environ Res* 2012; 113: 52–57.
23. Yu L, Kalla K, Guthrie E, et al. Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. *Environ Health Perspect* 2003; 111: 1421–1427.
24. Hsieh YC, Lien LM, Chung WT, et al. Significantly increased risk of carotid atherosclerosis with arsenic exposure and polymorphisms in arsenic metabolism genes. *Environ Res* 2011; 111: 804–810.
25. Kuo CC, Su PH, Sun CW, et al. Early-life arsenic exposure promotes atherogenic lipid metabolism in adolescence: a 15-year birth cohort follow-up study in central Taiwan. *Environ Int* 2018; 118: 97–105.
26. Gomez-Rubio P, Meza-Montenegro MM, Cantu-Sotob E, et al. Genetic association between intronic variants in AS3MT and arsenic methylation efficiency is focused on a large linkage disequilibrium cluster in chromosome 10. *J Appl Toxicol* 2010; 30: 260–270.
27. Mukherjee B, Salavaggione OE, Pelleymounter LL, et al. Glutathione S-transferase omega 1 and omega 2 pharmacogenomics. *Drug Metab Dispos* 2006; 34: 1237–1246.
28. Paiva L, Hernández A, Martínez V, et al. Association between GSTO2 polymorphism and the urinary arsenic profile in copper industry workers. *Environ Res* 2010; 110: 463–468.
29. Clément K, Vaisse C, Manning BS, et al. Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 1995; 333: 352–354.
30. Luglio HF, Sulistyoningrum DC and Susilowati R. The role of genes involved in lipolysis on weight loss program in overweight and obese individuals. *J Clin Biochem Nutr* 2015; 57: 91–97.
31. Mirrakhimov AE, Kerimkulova AS, Lunegova OS, et al. An association between TRP64ARG polymorphism of the B3 adrenoreceptor gene and some metabolic disturbances. *Cardiovasc Diabetol* 2011; 10: 89.
32. Trzcinka-Ochocka M, Jakubowski M and Raźniewska G. Asessment of occupational exposure to lead in Poland. *Med Pr*. 2005; 56: 395–404.
33. Chung JS, Kalman DA, Moore LE, et al. Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. *Environ Health Perspect* 2002; 110: 729–733.
34. Lindberg AL, Kumar R, Goessler W, et al. Metabolism of low-dose inorganic arsenic in a central European population: influence of sex and genetic polymorphisms. *Environ Health Perspect* 2007; 115: 1081–1086.
35. Hsieh RL, Huang YL, Shiue HS, et al. Arsenic methylation capacity and developmental delay in preschool children in Taiwan. *Int J Hyg Environ Health* 2014; 217: 678–686.
36. Sakane N, Sato J, Tsushita K, et al. Effects of lifestyle intervention on weight and metabolic parameters in patients with impaired glucose tolerance related to beta-3 adrenergic receptor gene polymorphism Trp64Arg(C/T): results from the Japan Diabetes Prevention Program. *J Diabetes Investig* 2016; 7: 338–342.
37. Saliba LF, Reis RS, Brownson RC, et al. Obesity-related gene ADRB2, ADRB3 and GHRL polymorphisms and the response to a weight loss diet intervention in adult women. *Genet Mol Biol* 2014; 37: 15–22.

REVIEW ARTICLE

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Genetic aspects of obesity and metabolic syndrome in people occupationally exposed to arsenic and certain heavy metals

Genetyczne aspekty otyłości i zespołu metabolicznego u osób zawodowo narażonych na arsen i niektóre metale ciężkie

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

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Abstract

Introduction. Obesity is a common health issue affecting over 650 million people worlwide. It is an integral element of the metabolic syndrome and leads to the development of arterial hypertension and type 2 diabetes, as well as many other different conditions, including numerous tumours. At the same time, exposure to arsenic, cadmium and lead is increasingly attributed to the development of the metabolic syndrome. Environmentally, this influence concerns the entire population, and is particularly visible in the occupationally exposed population.

Objective. The aim of this study is to prevent selected, best-known elements of the genetic variability and its significance in the development of the metabolic syndrome, taking into account exposure to arsenic, lead and cadmium.

State of the art. CNR1, FTO are related to an increased risk of developing metabolic syndrome, while certain variants of genes responsible for arsenic metabolism (As3MT) and lead (ALAD) are related to their higher toxicity. Knowledge about the relationship between exposure to arsenic, lead and cadmium, and the polymorphism of genes responsible for the development of the metabolic syndrome is scarce. This gap does not appear in more recent research based on the micro RNA expression. Expression of certain miRNA allows detection of both exposure to arsenic and increased risk of cardiovascular episodes in the future, as well as the existence of organ damage at present.

Conclusion. Reinforcement and use of the miRNA knowledge appears to be the right direction, but we should not forget current knowledge about polymorphism of individual nucleotides.

Key words

metabolic syndrome, obesity, arsenic, cadmium, lead, genetic variability

Streszczenie

Wprowadzenie. Otyłość jest powszechnym problemem zdrowotnym, dotyczy ponad 650 mln osób na świecie. Jest integralnym elementem zespołu metabolicznego, prowadzi do rozwoju nadciśnienia tętniczego i cukrzycy typu 2, ale też wielu różnych innych schorzeń, w tym licznych nowotworów. Jednocześnie coraz większą wagę przywiązuje się do wpływu narażenia na arsen, ołów i kadm na rozwój zespołu metabolicznego. Wpływ ten środowiskowo dotyczy całej populacji, jednak jest szczególnie widoczny u osób narażonych zawodowo na działanie tych pierwiastków.

Cel pracy. Celem tej pracy jest przedstawienie wybranych najlepiej poznanych elementów zmienności genetycznej i jej znaczenia w rozwoju zespołu metabolicznego z uwzględnieniem narażenia na arsen, ołów i kadm.

Aktualny stan badań. Polimorfizm pojedynczych nukleotydów jest dość dobrze poznany. Poszczególne warianty genów leptyny, CNR1, FTO związane są z większym ryzykiem rozwoju zespołu metabolicznego, a pewnie warianty genów odpowiedzialnych za metabolizm arsenu (As3MT) i ołowiu (ALAD) związane są z większą toksycznością tych pierwiastków. Natomiast wiedza o związku narażenia na arsen, ołów i kadm a polimorfizmami genów odpowiedzialnych za rozwój zespołu metabolicznego jest niewielka. Luki pozbawione są nowsze badania, bazujące na ocenie ekspresji mikroRNA. Ekspresja pewnych mikroRNA pozwala wykryć zarówno narażenie na arsen, jak i zwiększone ryzyko epizodów sercowo-naczyniowych w przyszłości, a także istnienie uszkodzeń narządowych już w chwili obecnej.

Wnioski. Ugruntowanie i wykorzystanie wiedzy o mikroRNA wydaje się słusznym kierunkiem, jednak nie powinniśmy zapominać o posiadanej już wiedzy o polimorfizmie pojedynczych nukletotydów.

Słowa kluczowe

otyłość, zespół metaboliczny, kadm, arsen, ołów, zmienność genetyczna

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INTRODUCTION

Obesity is a common medical and economic problem. According to the World Health Organization (WHO), globally there are over 650 million obese and 1.9 billion overweight people [1]. These data are all the more impressive as they keep growing, although this illness is potentially avoidable. The classic definition of obesity is BMI over 30 [2]. A new definition of obesity was formulated by the American endocrinological associations, which defines stage 1 obesity as BMI over 25 and the occurrence of complications of obesity [3]. This definition attempts to draw attention to the fact that obesity is a prelude tor other diseases, particularly from the spectrum of the metabolic complex.

Obesity is an integral part of the classically defined metabolic complex, accompanied by hyperglycaemia, arterial hypertension and dyslipiaemia [4]. Subsequently, there were numerous attempts to expand this definition, yet the changes never gained general acceptance.

Numerous studies were conducted in an attempt to explain the causes of the metabolic complex and obesity. They take into account both the genetic diversity and the effect of the outside environment.

Arsenic, as well as certain heavy metals, such as lead and cadmium, contribute to the onset of metabolic syndrome [5, 6, 7]. Although they are common in nature, there is a particular group of occupationally exposed individuals in which their harmful effects can be particularly evident. One of these groups consists of several thousand employees of the copper smelting plants in the Lower Silesian province of south-west Poland. Apart from the exposed population, heavy metals are also present in tap water. Numerous studies conducted in different sites in Poland have confirmed the presence of metals (especially lead), and in almost every study concentration above acceptable drinking standards were observed. However, over the years tendency of metals concentrations seems positive.

On the other hand, one study showed that the standard monitoring of heavy metals concentrations in Poland is not reliable. Arsenic is a known carcinogen, but its harmful effects, particularly during chronic exposure, are much broader – there were observations of increased cardiovascular risk, peripheral vessel diseases, respiratory system diseases, and neutropaenia [8]. The effect of the arsenic on the development of insulin resistance and diabetes [9] is particularly noticeable. The effects of cadmium exposure mostly include not only thickening of the arterial intima-media, but also renal damage and bone demineralization [9]. The first symptom of chronic lead poisoning is usually anaemia and iron deficiency. These are followed by damage to the kidneys, immune system, or reproductive system [10].

The effect of arsenic, lead and cadmium on the development of the metabolic syndrome is well known. Nevertheless, numerous observations indicate a high individual variability – individuals with the same exposure and similar environmental conditions were observed to have various concentrations of arsenic or lead [11]. This is the effect of a considerable genetic variability of the genes responsible for the broadly-defined metabolism of arsenic, lead and cadmium. Hence, it is difficult to assess the effect of metals on the development of the metabolic syndrome in isolation from individual genetic variability – both responsible for the metabolism of arsenic or lead, and the genes responsible for the development of metabolic syndrome.

The most common well-documented genetic mutations are changes to the genetic polymorphism and micro-RNA variability.

OBJECTVE

The aim of this study is to present selected, best-known elements of the genetic variability and its significance in the development of the metabolic syndrome, taking into account exposure to arsenic, lead and cadmium.

GENETIC VARIABILITY OF SELECTED GENES RELATED TO THE DEVELOPMENT OF THE METABOLIC SYNDROME – STATE OF THE ART

Leptin. Leptin is one of the more important and best-known compounds participating in regulating the sense of hunger. It is a hormone produced mostly by adipocytes. It triggers a sense of satiety, stops the sense of hunger, and stimulates the sympathetic nervous system through the interaction with leptin receptors expressed mostly in the hypothalamus. The gene responsible for its production is located on the 7th chromosome. Both leptin gene polymorphism rs7799039 or leptin receptor gene polymorphism rs1137101 have clinically relevant single nucleotide polymorphisms. Despite numerous studies, there is no explicit proof of any relationship between the leptine gene polymorphism and obesity [12]. Various genetic models have been studied, including models with dominant and co-dominant expression. On the other hand, new studies have shown a relationship between the leptine gene polymorphism and arterial hypertension, coronary heart disease, or LDL cholesterol concentration [13, 14].

Cadmium exposure has the most evident effect on leptine concentration – cadmium causes the emergence of abnormal, small adipocytes and reduces secretion of leptine, which leads to an increase in insulin resistance, obesity and the development of metabolic syndrome [15]. However, the role of genetic variability in the context of cadmium exposure remains unknown and the authors were unsuccessful in finding studies which consider both genetic variability and cadmium exposure.

CB1. Endogenous cannabinoids, which affect the cannabinoid receptors type 1 (CB1), have similar activity compared to leptine in the context of metabolic syndrome. They are located mostly in different parts of the brain and spine, with little expression in other organs. Their expression prevents development of undue neurological activity. As a result, they are responsible for the feelings of satiety and hunger. Genetic variability of several locations of the gene responsible for the CB1 creation have been described. The best known example is the transformation of adenosine into locus rs 1049353, which causes higher BMI and bigger waist circumference [16, 17]. There is no obesity without excessive calories in the food. Hence, it is likely that individuals with a specific genetic configuration who eat more food also consume more arsenic and are exposed to its toxic effects. As mentioned before, arsenic directly leads to the development of insulin resistance, and the main route of exposure in the general population

is food. As commonly known, exogenous cannabinoids stimulate appetite and, as a result, arsenic intake.

Another problem is the accumulation of arsenic and other heavy metals in cannabis plants. These plants accumulate significant amounts of heavy metals, and using its products, either legally or illegally, is connected with heavy metal exposure. In cannabis sativa this accumulation is so great that it is used in the process of phytoremediation – the process of purifying soil from heavy metals by small herbs and plants. An adverse synergy is very likely – a disadvantageous genetic constellation – a genetic predisposition to excessive calorie intake and increased exposure to arsenic, which jointly lead to the development of insulin resistance. However, there are no studies to prove this explicitly.

As3MT. Upon entering the organism, arsenic undergoes methylation and partial reduction, and is then expelled with urine. This process also creates oxygen free radicals and more toxic forms of arsenic, such as monomethylarsonic acid. The main participating enzymes are arsenite methyltransferase (As3MT), purine nucleoside phosphorylase (PNP), or glutathione S-transferase omega-1 (GSTO1) [18]. The genetic variability of the genes responsible for these enzymes leads to various concentration of arsenic in the exposed population, as well as changes in the ratios of individual arsenic forms excreted with urine. It has been demonstrated that certain polymorphic forms of the As3MT gene are related to higher concentrations of dimethylarsinates [19]. In the Mexican population, As3MT gene polymorphisms – in locus rs12767543, rs3740393 and rs11191453 of chromosom 10, were all associated with a high uDMA/uMMA ratio. Similar dependences are observed in the Polish population. In turn, higher concentration of the dimethylarsinates increases the insulin resistance by blocking the insulin-dependent transported GLUT4 [20].

Increased insulin resistance is the onset of metabolic syndrome which, after several years, leads to complications and vascular disease, particularly coronary heart disease. As demonstrated by Gong, even a minor, chronic exposure to arsenic, such as drinking ground water, increases the risk of coronary heart disease, the same as the adverse variant of polymorphism As3MT [21]. This risk appears to increase even more when both these factors are combined; however, there is no hard evidence for this.

ALAD. Lead is an inhibitor of several enzymes which participate in the haem synthesis. Its inhibiting effect is the strongest for the delta-aminolevulinic acid dehydratase, displacing zinc from the active enzyme location. Therefore, polymorphism of the gene responsible for production of the delta-aminolevulinic acid is a factor determining the concentration of lead in the serum and its potential toxic effect [22]. Higher lead concentration is also related to the development of metabolic syndrome [23]. It was also found that mixed exposure to heavy metals, including lead, is also related to the development of obesity, arterial hypertension and type II diabetes [7]. The authors, however, failed to find any research investigating a direct connection between the ALAD gene polymorphism and the metabolic syndrome, although this connection appears to be likely.

FTO. The FTO gene (fat mass and obesity-associated gene), commonly known as the obesity gene, is probably the most examined genetic mutation which contributes to the development of obesity. It codes the enzyme alpha-ketoglutarate dioxygenase responsible for the demethylation of numerous nucleic acid molecules. The latest meta-analyses confirm that the adverse variants of the FTO gene polymorphism are significantly related to obesity [24]. On the other hand, dietary interventions has the same effect, irrespective of one's polymorphic variants, which suggests that environmental interventions and lifestyle prevail over the innate genetic variability [25]. At the same time, there is no known connection between the FTO gene polymorphism and exposure to metals, although, again, one may expect an adverse synergy in the context of metabolic syndrome development.

MICRO-RNA. Apart from polymorphism of individual nucleotides, the literature also broadly discusses the participation of the micro RNA (miRNA) in pathogenesis and development of numerous diseases, e.g. tumours, cardiac and vascular diseases, diabetes and insulin resistance, or immunologic disorders.

MicroRNA is a group of small, non-coding RNA which, in mature form, regulate the gene expression at the post-transcription level. They play an important role in the processes crucial for the proper functioning of cells, and may affect the signal-transferring process and be a part of the mechanism defending the organism against viruses [26].

Numerous miRNA have been identified, both with pro-adipogenic and anti-adipogenic effect. Currently, it is believed that distortion of the miRNA balance leads to the development of obesity and increased insulin resistance, and individual miRNAs are a potential therapeutic target in obesity treatment [27].

The effect of arsenic, lead and cadmium exposure on the miRNA variability is currently widely examined. The number of new reports increases every month, unlike the dependence between polymorphisms – in this case, original and contributing studies are scarce. For this reason, a detailed description of all the miRNAs which number several dozen, exceeds the scope of this study. Explicit examples have been selected which demonstrate the significance of miRNA in the context of environmental and occupational exposure.

In potable water there is a clearly visible effect of arsenic exposure on the expression of miRNA 155 and 126 – the higher the arsenic concentration, the greater the expression of the aforesaid miRNAs. At the same time, there is a reported relationship between the expression of the aforesaid miRNAs and the increase in cardiovascular risk [28]. The discovery of this relationship shows how arsenic directly contributes to an increase in cardiovascular risk. Designation of the expression of these miRNAs allows identification of individuals who are both potentially exposed to arsenic and bear an increased cardiovascular risk.

Moreover, the designation of the miRNA expression allows not only assessment of the cardiovascular risk, or foresee the possibility of incidents in the future, but also to detect current organ damage caused by arsenic. An increased expression of miRNA 155 is of both predictive and diagnostic value for skin damage caused by arsenic exposure. An increased expression of miRNA 21 and 145 has both predictive and diagnostic value for liver damage caused by arsenic, and increased miRNA 181 expression for renal damage [27]. Also, arsenic exposure through drinking contaminated water was found

to have a potential impact – by an increased expression of individual miRNAs – on the development of large intestine cancer, type 2 diabetes, and damage of the signal pathways in the immune system [29]. Studies on cellular cultures confirmed that it is arsenic, particularly in mixed exposure to arsenic, lead and cadmium, that induces an increased miRNA expression, not any other factors [30].

CONCLUSIONS

The designation of micro-RNA expression seems to be a promising direction not only in the diagnostics of exposure to arsenic, but also to other metals, such as lead and cadmium. It not only permits the isolation of risk groups, but also an almost direct diagnosing of organ damages caused, for instance, by exposure to arsenic. Further studies of miRNA diversity are still needed which would confirm their significance and isolate specific changes with the highest potential for clinical use. At the same time, one should not forget about the polymorphism of individual nucleotides of the genes responsible both for the development of the metabolic syndrome, and for arsenic metabolism. Although these changes have been known for many years, to-date there has been little success in connecting the relationship between polymorphism of the genes responsible for the development of the metabolic syndrome, and exposure to arsenic and certain heavy metals.

REFERENCES

1. WHO. Obesity and overweight – key facts. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed: 2020.01.14).
2. Uzunlulu M, Telci Caklili O, Oguz A. Association between Metabolic Syndrome and Cancer. Ann Nutr Metab. 2016; 68(3): 173–179.
3. Garvey WT, Mechanick JI, Brett EM, Garber AJ, Hurley DL, Jastreboff AM, et al. American Association of Clinical Endocrinologists and American College of Endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity. Endocr Pract. 2016; 22 Suppl 3: 1–203.
4. Tatoń J, Bernas M. Metabolic syndrome – controversies between academic debate and real world medical practice. Endokrynol Otyłość i Zaburzenia Przemiany Mater. 2008; 5(1): 13–26.
5. Bulka CM, Persky VW, Daviglus ML, Durazo-Arvizu RA, Argos M. Multiple metal exposures and metabolic syndrome: A cross-sectional analysis of the National Health and Nutrition Examination Survey 2011–2014. Environ Res. 2019; 168: 397–405.
6. Rhee SY, Hwang YC, Woo JT, Sinn DH, Chin SO, Chon S, et al. Blood lead is significantly associated with metabolic syndrome in Korean adults: An analysis based on the Korea National Health and Nutrition Examination Survey (KNHANES), 2008. Cardiovasc Diabetol. 2013; 12: 9.
7. Wang X, Mukherjee B, Park SK. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003–2014. Environ Int. 2018; 121(Pt 1): 683–694.
8. Ratnaike RN. Acute and chronic arsenic toxicity. Postgrad Med J. 2003; 79(933): 391–396.
9. Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 2017; 8(3): 135–145.
10. Gidlow DA. Lead toxicity. Occup Med (Lond). 2015; 65(5): 348–356.
11. Yang Y, Wu J, Sun P. Effects of delta-aminolevulinic acid dehydratase polymorphisms on susceptibility to lead in han subjects from southwestern China. Int J Environ Res Public Health. 2012; 9(7): 2326–2338.
12. Yan J, Wang X, Tao H, Yang W, Luo M, Lin F. Lack of association between leptin G-2548A polymorphisms and obesity risk: Evidence based on a meta-analysis. Obes Res Clin Pract. 2015; 9(4): 389–97.
13. Manriquez V, Aviles J, Salazar L, Saavedra N, Seron P, Lanas F, et al. Polymorphisms in Genes Involved in the Leptin-Melanocortin Pathway are Associated with Obesity-Related Cardiometabolic Alterations in a Southern Chilean Population. Mol Diagn Ther. 2018; 22(1): 101–113.
14. Nowzari Z, Masoumi M, Nazari-Robati M, Akbari H, Shahrokhi N, Asadikaram G. Association of polymorphisms of leptin, leptin receptor and apelin receptor genes with susceptibility to coronary artery disease and hypertension. Life Sci. 2018; 207: 166–171.
15. Kawakami T, Nishiyama K, Kadota Y, Sato M, Inoue M, Suzuki S. Cadmium modulates adipocyte functions in metallothionein-null mice. Toxicol Appl Pharmacol. 2013; 272(3): 625–636.
16. Pawlak M, Milewicz A. Rola układu endokannabinoidowego i polimorfizmów genu CNR1 w powstawaniu otyłości. Endokrynol Otyłość i Zaburzenia Przemiany Mater. 2011: 192–196.
17. de Luis DA, Izaola O, Aller R, Lopez JJ, Torres B, Diaz G, et al. Association of G1359A polymorphism of the cannabinoid receptor gene (CNR1) with macronutrient intakes in obese females. J Hum Nutr Diet. 2016; 29(1): 118–123.
18. Agusa T, Fujihara J, Takeshita H, Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. Int J Mol Sci. 2011; 12(4): 2351–2382.
19. Hwang YH, Chen YH, Su YN, Hsu CC, Chen YH, Yuan TH. Genetic polymorphism of As3MT and delayed urinary DMA excretion after organic arsenic intake from oyster ingestion. J Environ Monit. 2010; 12(6): 1247–1254.
20. Martin EM, Stýblo M, Fry RC. Genetic and epigenetic mechanisms underlying arsenic-associated diabetes mellitus: a perspective of the current evidence. Epigenomics. 2017; 9(5): 701–710.
21. Gong G, O'Bryant SE. Low-level arsenic exposure, AS3MT gene polymorphism and cardiovascular diseases in rural Texas counties. Environ Res. 2012; 113: 52–57.
22. Onalaja AO, Claudio L. Genetic susceptibility to lead poisoning. Environ Health Perspect. 2000; 108 Suppl 1: 23–8.
23. Park SK, Schwartz J, Weisskopf M, Sparrow D, Vokonas PS, Wright RO, et al. Low-level lead exposure, metabolic syndrome, and heart rate variability: The VA Normative Aging Study. Environ Health Perspect. 2006; 114(11): 1718–1724.
24. Doaei S, Mosavi Jarrahi SA, Sanjari Moghadam A, Akbari ME, Javadi Kooshesh S, Badeli M, et al. The effect of rs9930506 FTO gene polymorphism on obesity risk: a meta-analysis. Biomol Concepts. 2019; 10(1): 237–242.
25. Lappalainen TJ, Tolppanen A-M, Kolehmainen M, Schwab U, Lindström J, Tuomilehto J, et al. The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the Finnish Diabetes Prevention Study. Obesity (Silver Spring). 2009; 17(4): 832–836.
26. Zaiou M, El Amri H, Bakillah A. The clinical potential of adipogenesis and obesity-related microRNAs. Nutr Metab Cardiovasc Dis. 2018; 28(2): 91–111.
27. Zeng Q, Zou Z, Wang Q, Sun B, Liu Y, Liang B, et al. Association and risk of five miRNAs with arsenic-induced multiorgan damage. Sci Total Environ. 2019; 680: 1–9.
28. Ruiz-Vera T, Ochoa-Martinez AC, Zarazua S, Carrizales-Yanez L, Perez-Maldonado IN. Circulating miRNA-126, -145 and -155 levels in Mexican women exposed to inorganic arsenic via drinking water. Environ Toxicol Pharmacol. 2019; 67: 79–86.
29. Banerjee N, Das S, Tripathy S, Bandyopadhyay AK, Sarma N, Bandyopadhyay A, et al. MicroRNAs play an important role in contributing to arsenic susceptibility in the chronically exposed individuals of West Bengal, India. Environ Sci Pollut Res Int. 2019; 26(27): 28052–28061.
30. Rojas E, Martinez-Pacheco M, Rodriguez-Sastre MA, Valverde M. As-Cd-Pb mixture induces cellular transformation via post-transcriptional regulation of Rad51c by miR-222. Cell Physiol Biochem. 2019; 53(6): 910–920.

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OŚWIADCZENIE O WSPÓŁAUTORSTWIE

Oświadczam, że w pracy:

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Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA CHORÓB WEWNĘTRZNYCH
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Dr hab. Łukasz Łaczmański, prof. IITD PAN.

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  Mój udział polegał na wykonaniu analiz polimorfizmów badanych genów.

Łukasz Łaczmański

Wrocław, 24.02.2021r.

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  mój udział polegał na nadzorowaniu badań immunogenetycznych, wykonaniu analiz polimorfizmów badanych genów oraz współredagowaniu merytorycznym publikacji.

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prof. dr hab. Katarzyna Bogunia-Kubik

Wrocław, 24.02.2021r.

mgr Małgorzata Kamińska

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  Mój udział polegał na oznaczeniu polimorfizmów badanych genów.

Małgorzata Kamińska

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Lek. Karolina Czerwińska

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Karolina Czerwińska

Wrocław, 24.02.2021r.

Dr n. med. Agnieszka Janus

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Mój udział polegał na współredagowaniu merytorycznym publikacji.

Agnieszka Janus

ZGODA KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 398/2018

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

dr hab. Jacek Daroszewski (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód (duchowny)
mgr prawa Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.:

„Genetyczne aspekty otyłości i zespołu metabolicznego u osób zawodowo narażonych na arsen i niektóre metale ciężkie"

zgłoszonym przez **lek. Tomasza Matysa**, uczestnika studiów doktoranckich w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej pod nadzorem dr hab. Rafała Poręby, prof. nadzw. p**od warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy projektu badawczego będącego podstawą rozprawy doktorskiej.

Wrocław, dnia 21 czerwca 2018 r.

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel