

CLINICAL RESEARCH

Metabolism of Arsenic in Human by AS3MT Gene

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ABSTRACT

The AS3MT may be the most significant protein for the methylation of chemical elements species. The transfer of methyl radical from S-adenosyl-L-methionine (AdoMet) to powerfulness arsenical (AsIII) is catalysed by the AS3MT that is critical for arsenic metabolism in humans. Since the AS3MT genetic polymorphism is linked to arsenic resistance, the association between the single ester polymorphism (SNP) and AS3MT inorganic arsenic (iAs) metabolism is being studied. Additionally, we tend to compared chemical action properties of recombinant human AS3MT and AS3MT/M287T. In reaction S-adenosylmethionine, arsenite, or methylarsonous acid (MAsIII) as substrates and endogenous reductants, together with glutathione (GSH), a thioredoxin enzyme (TR) system and tris(2-carboxyethyl) pesticide complex (TCEP). By victimization of either TR or Trx or NADPH or TCEP, AS3MT catalyzes the conversion of iAsIII to MAsIII then to methyl radical sonic acid (MAsV), dimethylarsinous acid (DMAsIII), and diethylarsinic acid (DMAsV). The Cys156 and Cys206 gift in similarity model forms the binding website for AsIII. Cys32 and Cys61 are linked by disulphide bond. The most important product in the initiative of methylation is MAsIII which remains sure to protein until it gets methylated. The product is a lot of hepatotoxic and more malignant neoplastic disease powerfulness methyl arsenicals; however, arsenic undergoes oxidation and reduction as enzyme-bound intermediates.

KEYWORDS

Genetic mutation; Arsenic metabolism; AS3MT; MAs III; DMAs III

INTRODUCTION

As humans get evaluated, they get custom-made to the surrounding environment. The adaption happens i.e., mutation [1,2]. Mutation allows organisms to metabolize toxic things [3]. The peoples can still metabolize low level. Scientists found that due to more consumption of arsenic water, the body has developed a genetic ability to metabolize arsenic. Scientists found that peoples of Andes can do metabolism arsenic [4].

Arsenic can come in body from drinking water sources such as Inorganic Arsenic (iAs) in As^{III} or As^V i.e. arsenite or arsenate [5,6]. The As^{III} (Arsenite) species are AsO₃⁻, HAsO₃²⁻, H₂AsO₃⁻ and H₃AsO₃, while As^V (Arsenate) species are: AsO₄^{III-}, HAsO₄²⁻, H₂AsO₄⁻ and H₃AsO₄. Group I type compounds i.e. inorganic arsenic [7]. The safe level of arsenic in drinking water is 10 µg/L as per WHO [8]. High arsenic exposure can show effects like skin pigmentation, hyperkeratosis, and cancer of bladder, liver, and kidney which may cause deaths [9,10].

The arsenic metabolises by dual pathways, which are oxidative methylation and reductive methylation. During metabolism arsenic transformed to Methylarsenite (MAs^{III}), Dimethylarsenite (DMAs^{III}), and sometimes may be to Trimethylarsine (TMAs^{III}) by the enzyme As^{III} S-adenosylmethionine methyltransferase (SAM) [11,12]. In the gastrointestinal tract the Methylarsenite (MAs^{III}), Dimethylarsenite (DMAs^{III}) are get methylated to form Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). MMA and DMA are less toxic than both MAs^{III} and DMAs^{III} so they are readily excreted through urine, where they get oxidized abiotically to MAs^V and DMAs^V [13]. When the MAs^V and DMAs^V levels increase in the urine is an indication of Arsenic related diseases [14]. The distribution of arsenic metabolites in urine is 10%-30% iAs, 0%-11% MAs and 26%-30% DMAs, but this distribution can vary from individual to individual [15]. Single Nucleotide Polymorphisms in the hAS3MT gene are linked. Most SNPs have little effect on health, however the M287T SNP in hAS3MT can cause cancer and skin issues [16,17]. When methylation of inorganic arsenic occurs, it produces toxic compound than previous [18]. For example, one protective The AS3MT haplotype is prevalent in indigenous tribes in Argentina they consumed arsenic toxic water for long period. The concentration of arsenic in water is 0.8 mg/L and little urine excretion of MAs (7.5 %) and a greater percentage of DMAs (78%).

In this study, we reviewed the human genetic related to AS3MT to find out ability of enzymes to metabolise arsenic and the effect of amino acid substitution on it. Methylation of arsenic helps to prevent death and severe conditions. For this study, we synthesized the hAS3MT gene by bacterial synthesis, which helps us to get pure AS3MT for further study. Then we compare the properties of enzymes in between hAS3MT and AS3MT by using As^{III} ionized molecule, which allows us to relate the structural and enzymatic property of both. In this, we show that AS3MT is essential for arsenic methylation capacity and present evidence that HGTs (Horizontal Gene Transformation) from prokaryotes to eukaryotes underlie adaptations to arsenic which is also known as mutation. In this study we discussed about how the animals get evaluated from last centuries [19].

MATERIALS AND METHODS

We declare that, all methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by a Pravara Rural College of Pharmacy, Pravaranagar. All experiments were performed in accordance with relevant guidelines and regulations.

Reagents

Tris-(2-carboxyethyl)-phosphine (0.5 M, pH 7) was prepared. MAs^V was reduced to trivalent MAs^{III} using Na₂S₂O₃, Na₂S₂O₅, and H₂SO₄ and adjusted to pH 6.5 with NaOH [20]. High-Performance Liquid Chromatography (HPLC) coupled to inductively coupled mass spectroscopy validated the identities of the reduction products (ICP-MS). The methylation substrates were the Glutathione (GSH) conjugates As(GS) and MAs(GS), which were made by incubating 1 mM As^{III} or MAs^V with a four-fold molar excess of GSH in degassed buffers under argon for 5 hours at 23°C. [21].

Strains & Growth Conditions

For plasmid *E. coli* was used by Dheeman DS and Packianathan C. Bacterial growth was monitored by measuring the optical density at 600 nm (A600nm) [19].

Human AS3MT Gene Cloning of hAS3MT cDNA

Chemically synthesized hAS3MT gene matching to the sequence of the cDNA clone, which lacks the final nine residues of the hAS3MT sequence, with codon optimization for expression in *E. coli* and sub-cloned into the EcoRV site of pUC57-Kan-hAS3MT. The synthetic hAS3MT gene was cloned into expression vector pMAL-c2x as an EcoRI/SalI digest from pUC57-Kan-hAS3MT, resulting in a fusion with the maltose-binding protein gene at the 5' end and eight histidine residues at the 3' end of the genomic sequence. The forward primer 5'-CCAGCCATGGCTGCACTTCGTGACGCTGAGA-3' (NcoI site highlighted) and reverse primer 5'-CCTAGTCGACTCCAGCAGCATCAGGGACACATC-3' were used to amplify the 1.1 kb fragment using PCR (SalI site underlined) [19,22].

Constructing Mutation

Site-directed mutagenesis used to create mutations in the AS3MT gene. The conserved Cys32, Cys61, Cys156, and Cys206 residues were altered to serine codons, resulting in seven single-cysteine mutants of the synthetic hAS3MT. Commercial DNA sequencing verified each hAS3MT mutation.

Expression and Purification of Protein

By using the Ni-NTA chromatography Wild-type AS₃MT (87 837 Da) and variant enzymes are purified by using *E. coli* [19]. Cells carrying the plasmid pET41a-hAS3MT were grown at 37°C in 1 L of Luria Broth medium with 10 gm of tryptone, 5 gm of yeast extract, and 10 gm of NaCl per litre containing 50 g/mL Kanamycin for 3 hours before induction with 0.3 mM isopropyl-D-1-thiogalactopyranoside (IPTG) After centrifuging the induced culture at 5000 rpm for 15 minutes at 4°C, it was suspended in 20 mL of buffer A containing 50 mM NaH₂PO₄ (pH 8.0), 1 mM TCEP, and 0.3 M NaCl, to which 10 mM imidazole was added. The cells were lysed in the presence of Diisopropyl fluorophosphate using a press before being centrifuged at 35000 rpm for 1 hour. Then, apply the aforementioned solution (0.7 mL/min) to a Ni-NTA agarose column that has already been loaded with 5 column volumes of buffer. hAS₃MT was then eluted (0.7 mL/min) with 8 column buffer containing 0.25 M Imidazole after being column washed (1 mL/min) with 10 of buffer containing 20 mM Imidazole. The Imidazole is then removed. As previously disclosed, natural hAS₃MT was purified. Purify thioredoxin (Trx) and thioredoxin reductase (TR) from *E. coli* BL21(DE3) bearing either pET14b-trxA or pET14b-taxi using Ni-NTA chromatography as stated above. Before use, all buffers were degassed (aliquoted) by bubbling with argon for 30 minutes [19].

Metabolism of Arsenic (AS₃MT)

There are two pathways by which the human body metabolize the arsenic compound i.e., Methylation by Oxidation and Reduction type of reaction

The activity of AS3MTs was checked at 37°C in a buffer of 50 mM NaH₂PO₄ pH 8 & 0.3 M NaCl. The chemicals in the assay are 5 mM GSH, 1 mM SAM, 10 µM Trx, 3 µM TR, and 0.3 mM NADPH, and the reactions were terminated by adding 10% (v/v) H₂O₂ to oxidize all arsenic species. Centrifugation using a 3 kDa cut-off Amicon ultra-filter was used to remove denatured protein. Then analysed by using HPLC.

Oxidative Methylation

This process is also called as bioactive process. This pathway is given by Cullen and Reimer. By the combination of Oxidative methylation, arsenate (As^V) is changed to Dimethylarsinous acid (DMA^{III}). Then Arsenate (As^V) gets converted into Arsenite (As^{III}) following to Monomethylarsonic acid (MMA^V) then it gets converted into Mono-methylarsonous acid (MMA^{III}). Then MMA^{III} converts into Dimethylarsinic acid (DMA^V) and finally it forms Dimethylarsinous acid (DMA^{III}). We can't explain the complete metabolism process because of the detection of DMVV arsenic which occurs in a major amount in human urine. Because the toxicity of MMA^V and DMA^V is substantially lower than that of iAs, methylation is thought to be a detoxification step for iAs. According to several recent investigations, MMA^{III} or DMA^{III} are more cytotoxic and genotoxic than iAs [24,25]. We noticed that if we cannot add H₂O₂ it allows us to determine trivalent arsenicals.

Reductive Methylation

This pathway of arsenic was proposed by Hayakawa et al. [26]. In this pathway, trivalent arsenicals are conjugated with glutathione (GSH) and then they get methylated. In the first step, As^{III} changes to As^{III}GS₃ then MMA+3GS₂ is formed then later it gets reduced to Dimethylarsenoglutathione (DMA^{III}GS) [Figure 1B]. Then correspondingly the MMA+3GS₂ and DMA^{III}GS are get oxidized to MMA^V and DMA^V. We investigated the renal metabolites and hepatic metabolites after giving the arsenic intravenously to the mice (0.5 mg/kg body weight), then we observed that when a trivalent species (As^{III}) of arsenic binds to a thiol group (R-SH) present in proteins. Then the protein-arsenical complex detaches from a parent protein and forms conjugation with Glutathione (GSH) to form As^{III}(GS)₃ or MMA^{III}(GS)₂ or DMA^{III}(GS). Hence it is found that during reductive methylation MMA^V and DMA^V are the end products. But in this pathway, DMA^V is in the major amount present in urine called as detoxification [27].

At the point when iAs is methylated through Oxidative and Reductive Methylation, the AS3MT quality assumes a basic part. AS3MT is an S-adenosyl-L-methionine-subordinate compound that can methylate trivalent arsenicals [28]. The human AS3MT gene is 32 kb long and has 11 exons. A variety of genetic variations SNP. A VNTR is a spot in DNA where a short nucleotide sequence is organized [28]. When AS3MT methylates inorganic arsenic, it can cause oxidative DNA damage and enhance their carcinogenicity.

Assays of Arsenic Methylation

To assay measurement of conversion of SAM to S-adenosylhomocysteine (SAH) EPI generous Methyltransferase Assay kit is used where the time-resolved Förster resonance energy transfer (TR-FRET) is used [30]. The test was completed utilizing a 384-well microtiter plate in a cushion comprising of 50 mM NaH₂PO₄ (pH 8.0), containing 0.3 M NaCl, 1 μM cleaned hAS3MT, 0.5 mM GSH, 1 μM Trx, 0.3 μM TR and 0.03 mM NADPH and 10 μM of As(GS) or MAs(GS). Then we added the SAM at 10 μM. The emission was 665:620 nm for determine Homogeneous Time-Resolved Fluorescence (HTRF). The concentration calculated as given in Figure 2 [30].

For measurement, (HPLC) and for arsenic concentration Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used [19]. Then added the SAM to at 37°C. To recuperate the entirety of the arsenic, the responses were ended by the expansion of H₂O₂ at 10% (v/v) last fixation, which additionally oxidizes all arsenicals, so the items will be named MAs and DMAs. Speciation of arsenic in the still up in the air by HPLC with a C18 300A opposite

stage section with the arsenic focus estimated by ICP-MS utilizing an ELAN 9000 ICP-MS. AsIII, MAIII, DMAV, MAV, and AsV were utilized at 1 μ M as principles.

E. coli. cells expressing the genotype and mutants of the hAS3MT gene. Hence, we carried both methylation processes on them. The cells were grown for 12 hours at 37°C in a 2 mL liquid broth medium of 0.3 mM Isopropyl-D-1-thiogalactopyranoside (IPTG), 100 g/mL kanamycin, and 10 M of AsIII or 2 M MAIII or both was used. The cells were extracted, washed, and suspended in ST-1 media with 2 M MAIII before being cultured at 37°C for 3 hours. Arsenicals were speciated by HPLC using a C18 reverse phase column, and the quantity of arsenic was calculated by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (ICP-MS).

HAS3MT STRUCTURE HOMOLGY MODEL WITH POLYMORPHIC RESIDUES

Using a fully automated protein structure homology modelling system, a homology model of hAS3MT was generated from residues on the structure of PhAsIII, which is confined to CmArsM. We utilize the PATCHDOCK server to find SAM's position in the model. The AsIII bound structure of CmArsM [32] was overlaid on the found hAS3MT model using SAM. We used a visual technique to get the arsenic atom in the AsIII binding site of hAS3MT. As seen in Figure 3. The human AS3MT model structure is depicted in a cartoon diagram with a tan colour scheme.

The Relationship between Arsenic Methylation and Genotypes in Human AS3MT

In this study, we employ SNP, which stands for polymorphism identification number related to the consensus sequence site (AY817668), with the first base of the consensus number 1 and dbSNP rs# cluster id [29]. Figure 4 depicts the chromosomal positions of genetic polymorphisms in AS3MT.

Out of all SNPs, three of them has non-synonymous exon region, which is Arg173Trp, Met287Thr, and Thr306Ile. When these AS3MT-expressing cells are treated with 12.5 nM AsIII, the Met287. During our study, we discovered that repeated sequences of 5'-UTR VNTR influence the transcriptional expression of gene. According to the findings of this study, polymorphisms in AS3MT lead to individual variability in AS3MT expression and function, as well as variance in the risk of arsenic-dependent carcinogenesis.

ANIMAL CASE STUDY

This study was conducted. The selected animals (mice's) are treated with arsenic containing water in different concentrations.[34]

Collection of Different Samples

Groundwater sample collection

We collected the water from 15 random groundwater wells in the polythene bottle which is previously treated with 25% HNO₃ for 3 hours. Then the bottles are washed with water. After sample were kept under refrigerated at the laboratory.

Blood sample collection

Blood samples from subjects were collected by venous puncture using lead-free vacutainer tubes containing EDTA as an anticoagulant. The blood was collected from all subjects and transferred to laboratory for study. DNA was extracted through the HP-PCR (High Pure-Polymerase Chain Reaction).

Collection of a urine sample

Approximately 15 ml of urine sample is collected bottle and the bottle is cleaned as mentioned in Groundwater is also collected. The sample was freeze to prevent oxidation. Then sample was filtered to remove unwanted waste.

Risk assessment

The following calculation was used to assess human exposure to arsenic in groundwater using the Lifetime Average Daily Dose (LADD), which is the amount of daily arsenic exposed from one or more sources and is given in g of arsenic per kilogramme body weight per day (g/kg/day):

$$LADD = \frac{C \times IR \times ED \times EF}{BW \times AT}$$

Where,

- C - Arsenic concentration in water ($\mu\text{g/L}$),
- IR - The water intake rate per day (L/day),
- ED - Exposure duration (years),
- EF - The exposure frequency (days/year),
- Kg - The body weight/Kg,
- AT - The average time (day)

By the LADD value we determined the Hazard Quotient (HQ) by the following equation;

$$HQ = \frac{LADD}{RfD}$$

Where,

RfD is reference dose for arsenic is 10 $\mu\text{g/kg/day}$ as given by WHO for India to avoid non-cancerous outcomes such as hyperpigmentation, keratosis, and possible vascular complications.

Determination of groundwater quality and arsenic

It was necessary to check turbidity, pH, conductivity, temperature, dissolved oxygen, chloride, fluoride, nitrite, nitrate, magnesium, and other physiochemical parameters. In the laboratory, chloride (mg/L) and fluoride (mg/L) were measured using a Benchtop Multiparameter pH/ISE with the appropriate ion-selective electrodes.

Urinary arsenic species (UAS) determination by instrumental analysis

Using HPLC-HG-AFS, the urinary arsenic species (UAS) (AsIII, AsV, MMA, and DMA) were determined (High-Performance Liquid Chromatography-Hydride Generation-Atomic Fluorescence Spectrometry). A Hamilton PRP-X100 anion-exchange column with a diameter of 250 mm aqueous buffer KH_2PO_4 or K_2HPO_4 with a pH of 5.8 is used as the mobile phase. The flow rate was 1.0 mL/min. To find out Total Urinary Arsenic, the urine sample is subjected within HNO_3 and HClO_4 to convert arsenic to inorganic arsenic (iAs). Finally, the HG-AFS method is employed to calculate UAs (Hydride Generation-Atomic Fluorescence Spectrometry).

Quality assurance for arsenical

Limit of detection (LOD) and limit of quantification (LOQ) were employed to detect arsenic in water, yielding values of 0.7 g/L and 1.2 g/L, respectively. The concentration of arsenic in urine is measured. We reported 106.22 g/L of total iAs, which is the sum of the AsIII and AsV. We also used HPLC-HG-AFS to verify the recovery of

arsenic species, yielding a total of 107.8 2.4 g/L, which matched to 99.5 2.1 g/L of AsV and 8.3 0.3 g/L of DMA. The following were the Urinary Arsenic Species (UAS) Limits of Detection (LOD): AsIII is 0.17 g/L, AsV is 0.38 g/L, MMA is 0.30 g/L, and DMA is 0.45 g/L.

STATISTICAL ANALYSIS

Because arsenic concentrations in groundwater and urinary arsenic species do not have a normal distribution. The genotype distributions of GSTP1-rs1695, GSTO2-rs156697, and As3MT-rs3740400 were measured using the Hardy-Weinberg equilibrium (HWE). Allelic frequencies were obtained by dividing the frequencies of heterozygous and homozygous alleles by the total number of allelic variants. We split the total number of individuals into two groups: those with a low daily dosage (LADD 0.3 g/kg/day) and those with a high daily dose (LADD >0.3 g/kg/day). We do the comparison between low and high exposure doses. To understand the differences between low and high intake of toxics, an effect size test was performed [37]. The polymorphisms and LADD were used as independent factors in the study, while urine arsenic species were used as dependent variables. The study included genetic dominant models (heterozygous + homozygous genotype) as well as possible confounders (age, BMI, smoking history, and lifestyle). With a stronger biological sense, dominant models over Potential Confounders model are explored in the research population. We used various factors to analyse the multi-collinearity of independent variables (VIF) [38].

DISCUSSION

During the transformation of arsenic, the conversion of inorganic arsenic to Methylarsonic acid and dimethyl arsenic acid is the most important step [42]. On basis of metabolism processes of arsenic, we were curious to find that it is suitable to inhibit cancer or not [43].

The main step is to give annotation of the AS3MT gene, resulting in several differences from the current “provisional” NCBI annotation. During the re-sequencing, we identified 27 polymorphisms, including three non-synonymous cSNPs and a VNTR. For allozymes, Trp173 and Ile306, levels of enzyme activity and immune-reactive protein were strikingly decreased when compared with the WT allozyme [44-46].

All of the substituted residues are on the surface of the protein except for Thr306, which is buried inside the enzyme, so a T306I substitution is disrupt the structure (Figure 3). The second methylation step is reduced when M287T SNP is occurred [47]. Met287 is located on the surface of AS3MT where the molecular inhibitors bind [48]. We also see that when M287T substitutes the binding site it retards the allosteric conformational change and reduces the rate of methylation of this variant. This property is observed in individuals with the M287T polymorphism epidemiological studies.

During the stability study in temperature, we found that the variants denature between 4 to 20-fold faster than wild-type hAS3MT. The protective AS3MT polymorphisms are located outside of the coding sequence regulatory elements.

RESULTS

Methylation of arsenic is conversion from product to substrate [39,40]. The ratio of MAs/iAs is the primary methylation index, while the ratio of DMAs/MAs is the secondary methylation index (SMI). The SMI is primarily used to assess methylation capability in persons exposed to inorganic arsenic. [41]. We evaluated the methylation

index of wild-type hAS3MT to that of eight polymorphic enzymes in this work. SMI and PMI were found to be lower in eight polymorphic enzymes. The greatest value of the DMAs/MAs ratio in wild-type enzymes is 2.3 0.3. M287T, R251H, and T306I SMI values (about 1.2) were lower than the wild-type enzyme but higher than the other SNPs, while the SMI values of H51R, I136T, and R173W enzymes were roughly 0.45. This research demonstrates that the eight non-synonymous missense variations of hAS3MT had a decreased arsenic methylation capacity when compared, implying that there is variance in arsenic methylation from individual to individual, which may raise the risk of arsenic-related disorders.

CONCLUSION

The gene-gene interactions As3MT*GSTM1 and GSTO2*GSTP1 were discovered to be possible regulators of urinary arsenic metabolites, increasing MMA and decreasing DMA, in this work. A synergistic effect of these polymorphisms and age, LADD of arsenic, and alcohol use may also alter a significant fraction of the population's arsenic individual metabolic capacity. Despite some discrepancies between genotypes and metabolism in human case studies, we discovered that two SNPs, AS3MT 12390 (rs3740393) in intron and 14458 (rs11191439, Met287Thr) in exon, vary across all nations, indicating that SNPs may be ethnically independent polymorphisms, but they can affect arsenic methylation. Argentina's population has a more proportion of DMA and a lower DM. This different distribution may have led to the findings that Argentina's population had a higher percentage of DMA and a lower percentage of MMA in the urine when compared to another research. It's worth looking into if the genotype distribution of AS3MT 12390 (rs3740393) is unique to this group (Argentinean Andes) and how this unique SNP selection happened.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.”

Ethics Statement

This is an observational study. The Pravara Research Ethics Committee has confirmed that no ethical approval is required.

Consent to Participate (Ethics)

Informed consent was obtained from all individual participants included in the study.

Consent to Publish (Ethics)

The participant has consented to the submission of the case report to the journal.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Pratik V. Malvade, Mayur S. Bhosale, and Sayli R. Chavan. The first draft of the manuscript

was written by Pratik V. Malvade and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

Availability of Data and Material

We do not wish to share our data before we have thoroughly analyzed it. All data sources described in the study are directed at the corresponding author.

Data Availability Statement

This article is a review article. So, data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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