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Developmental and Genetic Modulation of Arsenic Biotransformation: A Gene by Environment Interaction?

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Abstract

The complexity of arsenic toxicology has confounded the identification of specific pathways of disease causation. One focal point of arsenic research is aimed at fully characterizing arsenic biotransformation in humans, a process that appears to be quite variable, producing a mixture of several arsenic species with greatly differing toxic potencies. In an effort to characterize genetic determinants of variability in arsenic biotransformation, a genetic association study of 135 subjects in western Sonora, Mexico was performed by testing 23 polymorphic sites in three arsenic biotransformation candidate genes. One gene, arsenic 3 methyltransferase (AS3MT), was strongly associated with the ratio of urinary dimethylarsinic acid to monomethylarsonic acid (D/M) in children (7-11 years) but not in adults (18-79 years). Subsequent analyses revealed that the high D/M values associated with variant AS3MT alleles were primarily due to lower levels of monomethylarsonic acid as percent of total urinary arsenic (%MMA5). In light of several reports of arsenic-induced disease being associated with relatively high %MMA5 levels, these findings raise the possibility that variant AS3MT individuals may suffer less risk from arsenic exposure than non-variant individuals. These analyses also provide evidence that in this population, regardless of AS3MT variant status, children tend to have lower %MMA5 values than adults, suggesting that the global developmental regulation of arsenic biotransformation may interact with genetic variants in metabolic genes to result in novel genetic effects such as those in this report.

Keywords

Arsenic; Genetic Association; Biotransformation; AS3MT; MMA; DMA

INTRODUCTION

Our understanding of the human toxicology resulting from arsenic exposure draws upon two rich networks of data, but is limited by a lack of inter-connections that can harmonize them. At one level, there is an extensive epidemiology-based literature describing a diverse array of disease phenotypes that is associated with human arsenic exposure. Consider for a moment

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All authors state that no conflicts of interest regarding this manuscript exist.

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only the organ systems involved in the syndrome of human arsenicism, including the central nervous system, peripheral nervous system, integumentary system, urinary system, gastrointestinal system, respiratory system, hematopoietic system and the circulatory system (Guha Mazumder, 2001; Hall, 2002; Tseng, 2002; Wang et al., 2002; Rodriguez et al., 2003; Tsai et al., 2003; De et al., 2004; Wasserman et al., 2004; Baker et al., 2005; Chen et al., 2005; Hsueh et al., 2005; Mazumder et al., 2005; Yang et al., 2005; Zhou et al., 2005; Otto et al., 2006). This diversity in target organs is not an artifact of a single disease process affecting multiple sites, as environmental exposure to arsenic is also associated with a plethora of pathologic processes, including keratosis, cancer, skin pigmentation alterations (both increased and decreased), neuropathy, pulmonary bronchiectasis, peripheral vascular disease, hypertension and atherosclerosis. At another level arsenic is capable of producing a myriad of perturbations in experimental models, including cytotoxicity, cellular proliferation, inhibition of cellular proliferation, enzyme activity inhibition, enzyme activity induction, clastogenesis and malignant transformation, to in vivo experimental effects including transplacental carcinogenesis and endocrine disruption (Gonsebatt et al. {clastogenesis in human lymphocytes and epithelial cells}, 1997; Klimecki et al. {proliferative changes in a human skin model}, 1997; Hernandez-Zavala et al. {induction of heme biosynthesis enzymes}, 1999; Walter et al. {inhibition of PARP-1 activity in human cervical cell line}, 2006; Graham-Evans et al. {cytotoxicity, DNA damage in human cell lines}, 2004; Bornstein et al. {growth inhibition of human ovarian cell line}, 2005; Bredfeldt et al. {malignant transformation of human bladder epithelial cell line}, 2006; Eblin et al. {oxidative stress, DNA damage in human bladder epithelial cell line}, 2006; Liu *et al* {transplacental carcinogenesis in mice}., 2006; Sakurai and Himeno, 2006 {review of arsenic as an endocrine disruptor}). In fact, the spectrum of molecular targets, altered biochemical pathways and disease phenotypes characterized in experimental models is no less impressive in its scope than is the collective human dataset. A specific mechanism or mechanisms of action are at the crux of an explanation that links these two datasets.

Reconciling these disparate datasets will require overcoming obstacles that include identifying the critical molecular targets of a set of xenobiotic analogs (inorganic arsenic and its metabolites) with a notorious promiscuity in their target reactivity and in their ability to perturb a multiplicity of biochemical pathways. Essential to this will be an understanding of arsenic biotransformation: the chemical species formed through arsenic biotransformation in humans, their relative abundance and how this changes over time in both acute and chronic exposure scenarios. While the exact mechanism of action for a given arsenic toxicity endpoint may remain elusive, it is clear that human arsenic biotransformation creates several chemical species with dramatically different toxic potencies, and studies of human arsenic biotransformation offer the possibility of providing algorithms that predict their timing and quantity at target tissues (Lee and Ho, 1994; Vega et al., 2001; Eblin et al., 2006). For these reasons human arsenic biotransformation has been, and continues to be, an active focus of research.

The results of over a decade of studies of human arsenic biotransformation and its association with health effects have complemented mechanistic biochemical studies in advancing an understanding of arsenic toxicology. Compiled data and conclusions exist in several excellent reviews and will not be reiterated here (Goering et al., 1999; Kitchin, 2001; Pott et al., 2001; Styblo et al., 2002; Tchounwou et al., 2003). Instead, with the body of human arsenic biotransformation research as a backdrop, this report will focus on the potential for heritable differences in arsenic biotransformation that are driven by genetic variation.

There are recurrent references in the arsenic literature to genetic determinants of human variability in arsenic biotransformation (Vahter et al., 1995; Hopenhayn-Rich et al., 1996; Concha et al., 1998; Goering et al., 1999; Vahter, 2000). Initially these hypotheses appear to have been supported by three lines of evidence. First, researchers had extensively characterized

arsenic biotransformation in a diverse array of animal species and noted marked interspecies differences in arsenic biotransformation (Vahter, 1999; Csanaky and Gregus, 2002). Second, within humans, differences were reported in arsenic biotransformation that were associated with either geographical locale or ethnicity (Hopenhayn-Rich et al., 1996; Concha et al., 1998; Loffredo et al., 2003). Finally, the existence of published examples of human polymorphisms in other metabolic biotransformation pathways suggested the possibility that the same could be true for arsenic.

Despite an initial report of a genetic association between glutathione-S-transferase M1 (GSTM1) and glutathione-S-transferase T1 (GSTT1) and urinary arsenic metabolite levels, relatively little direct support for this hypothesis existed in the literature prior to 2002 (Chiou et al., 1997). In that year a study conducted in Chile demonstrated a statistically significant correlation between genetic relatedness and the concordance of urinary arsenic metabolite levels (Chung et al., 2002). While the subject numbers, and particularly the family numbers, were modest, the data nevertheless supported a role for genetic variation in explaining at least a part of the long-observed inter-individual variation in arsenic biotransformation. Subsequent genetic association studies of arsenic biotransformation collectively suggested roles for the GSTT1 and glutathione-S-transferase omega-1 (GSTO1) (Marnett et al., 2003; Kile et al., 2005).

MATERIALS AND METHODS

Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR to quantify AS3MT and glyceraldehyde phosphate dehydrogenase (GAPDH) expression utilized pre-made assay oligonucleotide reagents from Applied Biosystems (Foster City, California). GAPDH oligonucleotides were ordered as part number 4333764T. The AS3MT assay identification number was Hs00221125_ml. Reactions were assembled in 20 µl volumes, according to manufacturer protocol for One Step PCR Master Mix (Applied Biosystems, product number 4309169), and included 60 ng of total RNA. Reactions were temperature-cycled (95°C for 10 minutes, then 40 cycles of 95°C for 15 second and 60°C for one minute), and fluorescence measured, in a sequence detection system 7900HT (Applied Biosystems) instrument. Fluorescence data was analyzed using Sequence Detection Systems software (version 2.2, Applied Biosystems) and the comparative C_T method to measure “fold” change in gene expression relative to GAPDH expression.

Samples

Total RNA from pooled, normal human tissues was obtained from Stratagene (La Jolla, California). These samples included fetal and adult kidney, liver, and lung, adult adrenal gland, adult ovary and adult testis.

Statistical Analysis

Primary data measurements of urinary arsenic concentrations from a Mexican population described in a previously published study (Meza et al., 2005) were analyzed for an additional phenotype, percent MMA in the +5 valence. (%MMA5). This was calculated by the following formula $[100 * (\text{MMA5 concentration} / \text{Total Urinary Arsenic Concentration})]$. Subsequent to establishing that %MMA5 did not significantly deviate from expectations of a normal distribution, genetic association of this phenotype with AS3MT polymorphism 30585 was tested with a t-test comparing the mean %MMA5 of non-variant subjects (homozygous TT genotype) against variant subjects (combined group of heterozygous CT genotype and homozygous CC genotype subjects).

RESULTS

Prior to 2005 there had been no comprehensive genetic association study incorporating candidate genes with published evidence of direct involvement in arsenic biotransformation. Our 2005 study of arsenic metabolism in Mexican children and adults was aimed at exploring genetic determinants of variability in arsenic biotransformation in populations of local relevance exposed to arsenic at concentrations of local relevance.

The subjects of the study were from the Yaqui Valley area of western Sonora state in Mexico. In total complete data was available for 135 participants, who lived in five towns with tube well arsenic concentrations ranging from 5.5 $\mu\text{g/l}$ to 43.3 $\mu\text{g/l}$ (Meza et al., 2005). Health status and demographic information, first morning void urine samples and blood or buccal samples for DNA isolation were collected from the participants. At the time the study began, the literature-validated genes involved in the biotransformation of arsenic were glutathione-S-transferase omega 1 (GSTO1), purine nucleoside phosphorylase (NP) and arsenic (3) methyltransferase (AS3MT, formerly abbreviated Cyt19). The first phase of the project involved completing the resequencing of the genes in DNA from 46 immortalized lymphoblastoid cell lines from 22 normal subjects of European ancestry and 24 normal subjects of indigenous American ancestry. The choice of these samples was dictated by the goal of modeling the possible polymorphisms that would be expected in a Mexican population that was likely to be an admixture of these ancestries. This resequencing effort produced high resolution catalogs of 33, 48 and 58 polymorphic sites in GSTO, NP and AS3MT, respectively. This included location, allele frequency and linkage disequilibrium (LD) data. Table 1 lists the location and gene context of these polymorphisms.

The strategy used to filter the 139 polymorphisms that were discovered in the three candidate genes to the final set of 23 polymorphisms tested in the genetic association study was a combination of choosing polymorphisms that tagged bins of linkage disequilibrium (Carlson et al., 2004), together with selected potentially functional polymorphisms, such as non-synonymous single nucleotide polymorphisms. Using this approach, a tractable number of genetic tests can be used to directly or indirectly test all high frequency polymorphisms in a gene, together with those polymorphisms with functional potential. As opposed to typical genetic association studies of one or a few polymorphisms, this approach represents a genetic test of an entire gene that maximizes the chance of identifying functional polymorphic sites, while at the same time enhancing the meaning of any negative finding to more convincingly rule out the entire tested gene as a determinant of a phenotype of interest.

Two phenotypes were tested in the Mexican population, the ratio of urinary arsenite to arsenate and the ratio of urinary dimethylarsinic acid (DMA5) to monomethylarsonic acid (MMA5), dubbed "D/M". Screening the entire population of 135 individuals for differences in mean value of arsenite to arsenate and D/M between the genotype groups identified three linked SNPs in AS3MT that were associated with D/M ratio, shown in Table 2. Variant alleles in this cluster of linked SNPs were associated with higher D/M values at a level of significance that remained high ($P=0.003$) after a Bonferroni correction for 46 tests.

A subsequent analysis of known covariates of D/M revealed that participant age was also strongly associated with D/M. Age in this population was not continuously represented. Rather, there was a discreet group of children aged 7-11 years and an adult group ranging from 18 to 79 years of age. A plot of mean D/M value by genotype for one representative SNP of the linked cluster, stratified by child or adult status illustrates the dramatic effect of age in modulating the genetic effect of AS3MT on D/M (Fig. 1). Children carrying AS3MT variants of this cluster have substantially higher D/M ratios than do AS3MT wild-type children. In contrast to this, there is an absence of this effect in adults. We have subsequently performed

additional analyses to try to understand the relationship between AS3MT polymorphisms, DMA5, MMA5 and D/M. Similar to D/M, there is a clear age-restricted genetic effect of AS3MT on the %MMA5 (Fig. 2). No such effect was observed for DMA (data not shown), suggesting that the lower %MMA5 values that are strongly associated with AS3MT variants are likely to have driven the genetic effect on the D/M phenotype. This effect of AS3MT on %MMA5 in children is also significant in terms of potential toxicological consequences. Of all arsenic metabolic phenotypes measured in human studies of association between biotransformation and disease, a relatively high urinary MMA5 level among affected individuals is arguably the most consistently replicated finding (Steinmaus et al., 2005; Steinmaus et al., 2006).

One limitation in our understanding of arsenic induced disease is the definition of the critical exposure period. Some light has been shed on this in a recent study of arsenic associated lung disease, suggesting that a discrete, childhood arsenic exposure may be sufficient for an increased risk of a disease that manifests in adulthood (Smith et al., 2006). While speculative, it is at least possible that the AS3MT variant children may be relatively protected from arsenic-associated diseases, including those that appear after a long latent period. Ongoing work in our group is aimed at identifying the entire gamut of linked polymorphisms in the AS3MT variant Mexican children in order to develop a subset of potentially functionally relevant polymorphisms that could explain this effect. Preliminary data from these studies suggests that the effect is not associated with any non-synonymous AS3MT polymorphisms.

While there are many examples of phenotypes that are influenced by age, and by extension genetic associations that are influenced by age, there are few, if any, reports that describe genetic associations that are so dramatically age-restricted as this AS3MT association. As such, possible explanations for this effect are conjectural. One hypothesis that could account for our observations is that the phenotype of arsenic biotransformation itself has a developmentally regulated component, likely through modulation of gene expression mediated by a cis-acting element. In that setting a regulatory polymorphism located within the cis-acting element could exert an effect during that developmental time window, but have no apparent effect during the developmental stages in which that cis-acting element was not involved. There is, in fact, data to support the central idea of developmental regulation of arsenic biotransformation. Studying 756 arsenic-exposed subjects from Chile, Caceres *et al.* (2005) reported a strong effect size of age upon total urinary arsenic levels, although conclusions from this study are somewhat limited by a lack of arsenic chemical speciation. Chowdhury *et al.* (2003) studied a Bangladesh population exposed to drinking water arsenic levels averaging 376 µg/l. When subjects' urinary D/M values were plotted against their age, a prominent rise in D/M levels was observed within a discreet age range similar to the Yaqui Valley children. The possibility that the same global age-related developmental effect on arsenic biotransformation could be present in the Yaqui Valley population was of obvious interest. Studying this, however, is complicated by prior knowledge of the low %MMA5 values in AS3MT variant children which would bias an assessment of an overall age effect. To explore this we compared the mean %MMA5 of all adults with that of only the AS3MT wild-type children. Mean %MMA5 was significantly higher in adults analyzed as a single group of combined genotypes, compared to AS3MT wild-type children ($10.5\% \pm 4.8$ in adults Vs. $8.5\% \pm 3.4$ in children, $P=0.04$), supporting the idea that there may be a global developmental regulation of arsenic biotransformation. Noteworthy in this regard is data describing the expression level of AS3MT in a panel of normal human tissues, publicly hosted by the Genomics Institute of the Novartis Research Foundation (Su et al., 2002). Within the panel of human tissues studied, AS3MT expression is highest in the adrenal gland (Fig. 3). Complementing those microarray-derived data, we used quantitative real-time polymerase chain reaction to measure AS3MT RNA expression in a more limited panel of normal human tissues (Fig. 4). Two particularly interesting points emerge from this data. First, at least in some tissues from this limited survey, there appears to be a developmental

regulation of AS3MT expression, with fetal tissue such as lung demonstrating differential expression relative to its adult counterpart. Second, AS3MT appears to be expressed at levels comparable to, or greater than, the liver in two organs that share a role in male steroid hormone biosynthesis, the adrenal gland and testis. Thus, AS3MT, a gene with a developmentally restricted genetic association to arsenic biotransformation, is capable of developmentally regulated gene expression and is expressed in organs with well-known global developmentally associated physiological roles, such as the control of puberty.

Several important caveats exist in the interpretation of this data. The genetic association between AS3MT sequence variants and arsenic metabolism has not yet been replicated in an independent population. Second, genetic admixture of the Yaqui valley study subjects needs to be considered as a factor that could influence the genetic association. In fact, the higher variant allele frequency at AS3MT site 30585 that was observed in children compared to adults suggests that they may not have shared a similar degree of admixture. The likely consequence of an admixture effect in this study is not the significance of the genetic association per se, but rather the location of the causative polymorphism or polymorphisms, because of the recognized effect of admixture on linkage disequilibrium. Finally, drinking water arsenic level should be considered as a potential factor in the genetic association. Within the spectrum of globally studied exposure levels, the Yaqui valley exposure levels were modest. It is possible that in populations exposed to higher levels of drinking water arsenic the genetic effect of AS3MT may have different characteristics, such as the appearance of an effect in adults that was not apparent at lower exposure levels.

CONCLUSIONS: DEVELOPMENTAL AND GENETIC MODULATION OF ARSENIC BIOTRANSFORMATION: A GENE BY ENVIRONMENT INTERACTION?

Arsenic's ability to affect diverse molecular targets, and to associate with a broad array of disease phenotypes that do not necessarily extrapolate across species, underscores the complexity of human arsenic toxicology. Continued progress toward an understanding of the relevant mechanism or mechanisms of action in human arsenicism will rely upon attacking the problem from complementary approaches that use information from, and in turn inform, each other. The genetic association study of Sonoran participants suggests that the genetic determinants of arsenic biotransformation may be conditional in their effect, namely conditional on developmental stage. While the AS3MT genetic association is unusual, studies of the human genetics of complex phenotypes are replete with examples of genetic effects that are modified by environmental influences, such as the interaction between genetic determinants of allergy and asthma, and environmental exposure to microbial ligands (Kabesch, 2006). In light of those “gene by environment” interactions, from the standpoint of the cell, the developmental progression of the organism may simply represent yet another environmental modulation.

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Fig. 1. Relationship between D/M ratio and AS3MT genetic variants in Mexican adults and children. 95% confidence intervals for mean urinary D/M ratio for wild-type (homozygous wild-type) and variant (heterozygous or homozygous variant) genotypes at AS3MT 30585 polymorphism, stratified by children and adults. * $P = 1.4 \times 10^{-6}$ between AS3MT 30585 non-variants and variants within children (calculated in log-transformed data).

Fig. 2. Relationship between %MMA5 and AS3MT genetic variants in Mexican adults and children. 95% confidence intervals for urinary percent MMA5 for wild-type (homozygous wild-type) and variant (heterozygous or homozygous variant) genotypes at AS3MT 30585 polymorphism, stratified by children and adults. * $P = 8.1 \times 10^{-6}$ between AS3MT 30585 non-variants and variants within children.

Fig. 3. Microarray-based gene expression data for AS3MT in a panel of normal human tissues. (Data from Novartis Research Foundation)

Fig. 4. AS3MT expression levels in normal human adult and fetal tissues. Comparative C_T ratio of AS3MT to GAPDH was determined in three independent experiments, four replicates per experiment. These values were compared to adult liver, with mean liver value arbitrarily set to 1.0. Bars represent mean from all experiments, error bars represent 95% confidence limits of the mean.

Table 1

Polymorphisms discovered in AS3MT, NP, and GSTO1

AS3MT		NP		GSTO1	
ATG Offset	Gene Context	ATG Offset	Gene Context	ATG Offset	Gene Context
-1039	5' Genomic	-2432	5' Genomic	-1859	5' Genomic
-788	5' Genomic	-1960	5' Genomic	-1637	5' Genomic
-649	5' Genomic	-1626	5' Genomic	-1309	5' Genomic
-599	5' Genomic	-1552	5' Genomic	-1242	5' Genomic
-400	5' Genomic	-1545	5' Genomic	-803	5' Genomic
-262	5' Genomic	-969	5' Genomic	-710	5' Genomic
-37	Exon1: 5' UTR	-892	5' Genomic	-452	5' Genomic
16	Exon1: 5' UTR	-272	5' Genomic	-320	5' Genomic
17	Exon1: 5' UTR	-96	Exon1: 5' UTR	-220	5' Genomic
49	Exon1: 5' UTR	567	Intron 1	82	Intron 1
192	Intron 1	613	Intron 1	3671	Intron 2
524	Intron 2	1039	Intron 1	3871	Intron 2
561	Intron 2	1368	Intron 1	4535	Intron 2
911	Intron 3	1663	Intron 1	5161	Intron 3
1045	Intron 3	1917	Intron 1	5437	Intron 3
1138	Intron 3	1954	Intron 1	5711	Intron 3
1142	Intron 3	2688	Intron 1	6065	Intron 3
2393	Intron 3	2843	Exon 2: Syn	6436	Intron 3
3499	Intron 4	2934	Exon 2: G51S	6646	Intron 3
3786	Intron 5	2954	Exon 2: Syn	7436	Intron 3
3847	Intron 5	3063	Intron 2	7465	Intron 3
3977	Intron 5	3126	Intron 2	7504	Intron 3
4023	Intron 5	3162	Intron 2	8102	Exon 4: A140D
4024	Intron 5	3397	Intron 2	8147	Exon 4: E155del
4070	Intron 5	3632	Intron 2	8176	Intron 5
4747	Intron 5	3746	Intron 2	8182	Intron 5
5207	Intron 6	3923	Intron 2	8700	Intron 5
7388	Intron 6	3946	Intron 2	10180	Intron 5
7444	Intron 6	3991	Intron 2	10703	Intron 5
7588	Intron 7	4048	Intron 2	12375	Exon 6: E208K
7897	Intron 7	4298	Intron 2	12460	Exon 6: A236V
8492	Intron 7	4346	Intron 2	12502	Exon 6: 3' UTR
8597	Intron 7	4396	Intron 2	12508	Exon 6: 3' UTR
8725	Intron 7	4784	Intron 2		
9154	Intron 7	5077	Intron 2		
9213	Intron 7	5614	Exon 5: Syn		
9456	Exon8: M287T	5837	Intron 5		
20984	Intron 8	5866	Intron 5		
21363	Intron 9	5898	Intron 5		
21788	Intron 9	6205	Intron 5		
22213	Intron 9	6243	Intron 5		
30415	Intron 9	6272	Intron 5		
30585	Intron 9	6653	Intron 5		
30678	Intron 9	6760	Intron 5		
30737	Intron 9	7347	Exon 6: 3' UTR		
30801	Intron 9	7688	3' Genomic		
30989	Intron 9	7821	3' Genomic		
31207	Exon 10: 3' UTR	7904	3' Genomic		
31421	Exon 10: 3' UTR				
31454	Exon 10: 3' UTR				
31978	3' Genomic				
32217	3' Genomic				
32441	3' Genomic				

AS3MT		NP		GSTO1	
ATG Offset	Gene Context	ATG Offset	Gene Context	ATG Offset	Gene Context
32614	3' Genomic				
32730	3' Genomic				
32803	3' Genomic				
32851	3' Genomic				
32948	3' Genomic				

ATG offset positions in reference to the following GenBank submissions: AS3MT-AY817668, NP-AY817667, GSTO1-AY817669

Abbreviations: UTR-untranslated RNA, Syn-Synonymous Non-Synonymous polymorphisms denoted by amino acid residue location, with wild-type and substituted amino acid, eg M287T for threonine substitution for methionine at residue 287

Table 2

Genetic association testing for 23 polymorphic sites in AS3MT, NP and GSTO1

GENE	SITE	Phenotypes	
		3/5 Ratio	D/M Ratio
GST-O	-1859	N.S.	N.S.
GST-O	-1242	N.S.	N.S.
GST-O	5711	N.S.	N.S.
GST-O	8102	N.S.	N.S.
GST-O	8147	N.S.	N.S.
NP	-1626	N.S.	N.S.
NP	-1545	N.S.	N.S.
NP	567	N.S.	N.S.
NP	2934	N.S.	N.S.
NP	3746	N.S.	N.S.
NP	5837	N.S.	N.S.
NP	6760	N.S.	N.S.
NP	7821	N.S.	N.S.
AS3MT	-400	N.S.	N.S.
AS3MT	-262	N.S.	N.S.
AS3MT	49	N.S.	N.S.
AS3MT	2393	N.S.	0.024
AS3MT	5207	N.S.	N.S.
AS3MT	7388	N.S.	0.008
AS3MT	7588	N.S.	N.S.
AS3MT	8597	N.S.	N.S.
AS3MT	20984	N.S.	N.S.
AS3MT	30585	N.S.	0.003

N.S. – not significant at $P \leq 0.05$

Values reported are significance values for two-tailed t-tests under dominant genetic model, comparing mean phenotype value between two genetic groups.
 All values adjusted for 46 test Bonferroni correction.