# Beta-naphthylamine (BNA) induced genotoxicity in Chinese hamster ovary cells expressing different human NAT2 alleles.

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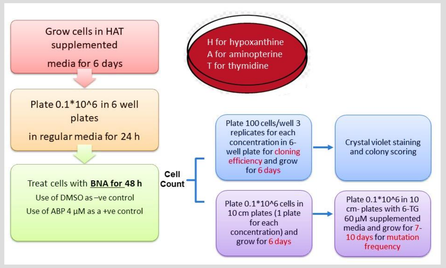
## Introduction:

Occupational and tobacco exposure to aromatic amines including beta-naphthylamine (BNA) is associated with increased bladder cancer risk, the fourth most prevalent cancer among men in the United States. BNA requires bioactivation by cytochrome P450 (CYP) 1A2 followed by *O*-acetylation by *N*-acetyltransferase 2 (NAT2). Previous studies have reported that exposure to aromatic amines leads to mutagenicity, DNA damage and increased reactive oxygen species (ROS). Variant NAT2 haplotypes confer genetic polymorphism in humans and their effect to modulate the genotoxicity associated with BNA needs to be investigated.

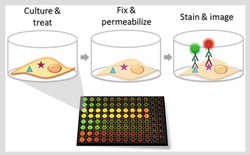
**Objective:** The purpose of this study is to investigate the role of NAT2 haplotype in the mutagenicity, genotoxicity and ROS induced by BNA.

## Methods:

- **CHO cells construction:** Chinese hamster ovary (CHO) cells were stably transfected with human CYP1A2 and either *NAT2*4* (rapid or reference), *NAT2*5B* (slow or variant common in Caucasians) or *NAT2*7B* (slow or variant common in Asians).
- **Measurement of BNA-induced mutants using HPRT mutation assay:**



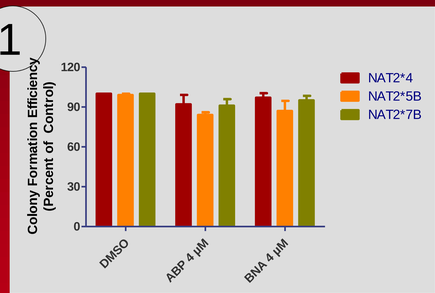

- **Measurement of BNA-induced DNA damage using γ-H2AX in cell western assay:**




- **Measurement of BNA-induced ROS using DCFDA fluorescence assay.**
- Results were tested for significance by **two-way analysis of variance (ANOVA) followed by Bonferroni test.**

## Results:

1

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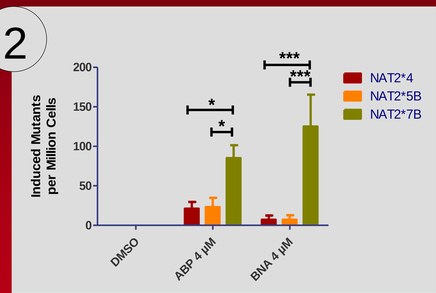
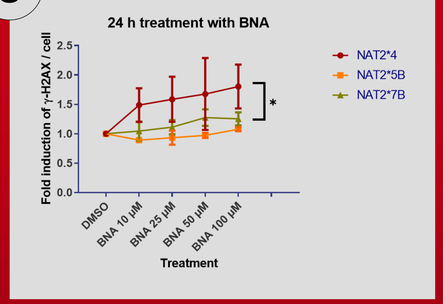



•Colony formation efficiency was not significantly different between different genotypes. HPRT mutations were higher in *NAT2*7B* compared to *NAT2*4* and *NAT2*5B* following treatment with BNA 4 µM. ABP 4 µM was used as a positive control (*p< 0.05, ***p< 0.001) **(Fig 1, 2)**.

3

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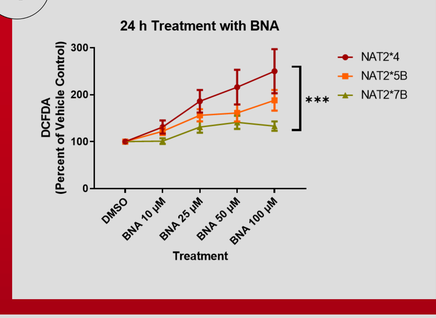
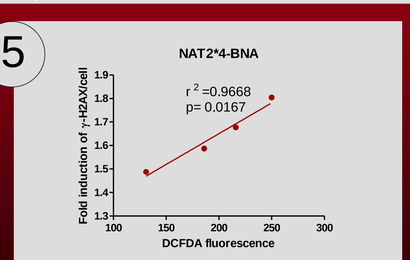



•In cell western γH2AX assay in CHO cell lines following treatment with BNA (0-100 µM) for 24 hours). Each bar represents Mean ± S.E.M. for three or four experiments. *NAT2*4* showed higher γH2AX levels reflecting more DNA damage compared to *NAT2*5B* or *NAT2*7B*. *= p< 0.05**(Fig 3).**

•DCFDA fluorescence assay in CHO cell lines following treatment with BNA (0-100 µM) for 24 hours). Each bar represents Mean ± S.E.M. for three or four experiments. *NAT2*4* showed higher DCFDA fluorescence reflecting more ROS generation compared to *NAT2*7B*. There was no significant difference between *NAT2*5B* and *NAT2*7B*. ***= p< 0.001**(Fig 4).**

5




•Pearson correlation graph shows a good correlation between ROS level measured by DCFDA fluorescence assay and DNA damage measured by In cell western γH2AX assay in CHO cells transfected with *NAT2*4* allele. $R^2$ = 0.9668, p<0.05 **(Fig 5).**

## Summary and Conclusion:

- HPRT mutations were higher in *NAT2*7B* compared to *NAT2*4* and *NAT2*5B* referring to higher risk of mutagenicity for this allele at low BNA concentrations.
- DNA damage and ROS were higher in *NAT2*4* compared to *NAT2*5B* and *NAT2*7B* referring to higher risk of genotoxicity for this allele at higher BNA concentrations.
- For *NAT2*4*, ROS level is correlated with DNA damage suggesting oxidative stress as a mechanism underlying BNA induced genotoxicity.
- The overall risk following exposure to BNA is genotype and concentration dependent.

The current study provides a **mammalian model to investigate aromatic amines induced mutagenicity, genotoxicity and ROS generation**. In addition, it suggests that heterogeneity within the "slow" NAT2 acetylator phenotype should be incorporated into cancer risk studies following aromatic amines exposure.

## Future Work:

Further studies will be done to investigate other aromatic amine carcinogens and their associated mutagenicity and genotoxicity

## Reference and Acknowledgement:

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