



# IDENTIFICATION AND FUNCTIONAL GENOMIC ANALYSIS OF GENETIC VARIANTS OF NT5C2

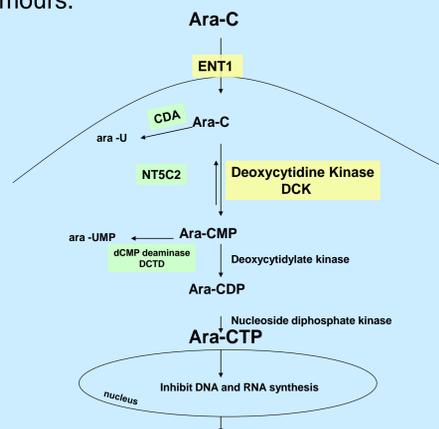
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## BACKGROUND

- Cytarabine (ara-C) is the most effective chemotherapeutic agent in the treatment of acute myeloid leukemia (AML).
- Ara-C is a prodrug that requires extensive intracellular phosphorylation for activation to ara-C 5'triphosphate (ara-CTP).
- Although deoxycytidine kinase (DCK) is the rate limiting enzyme in the activation of ara-C, cytosolic 5'-nucleotidase II (NT5C2 or purine 5'-nucleotidase, IMP-GMP specific nucleotidase or high Km 5'-nucleotidase) is involved in the inactivation of ara-C by reversal of phosphorylation by DCK through the dephosphorylation of active metabolites by its hydrolytic activity.
- Several studies have suggested that NT5C2 is involved in resistance to nucleoside analogues used in treatment of haematological malignancies and some solid tumours.



## OBJECTIVE

To identify and determine functional and clinical significance of genetic variants in NT5C2.

## METHODS

### Study Population

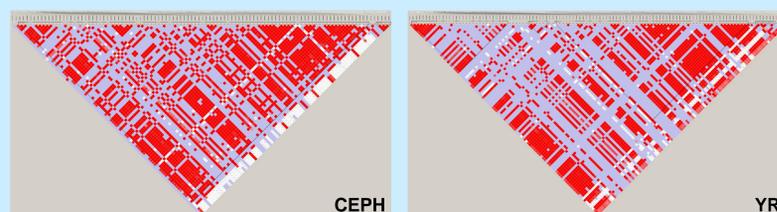
- Epstein-Barr virus-transformed B-lymphoblastoid HAPMAP cell lines derived from 30 Centre d' Etude du Polymorphisme Humain (CEPH) trios (two parents and a child) (n = 90, European descent) and 30 Yoruba trios (n = 90, African descent, referred as YRI)
- SNPs in NT5C2 were identified by sequencing all exons, 5' and 3'UTRs in the genomic DNA from 180 HAPMAP samples indicated above.
- Ara-C sensitivity (ara-C AUC) was measured by treating CEPH and YRI cell lines with varying concentrations of ara-C followed by measuring cell survival after 48 hrs (Hartford et al, Blood 2009).
- mRNA expression levels were extracted from Exon-array data.
- Genotype-phenotype association analysis was performed to identify SNPs associated with mRNA expression and ara-C sensitivity

### Patient Population

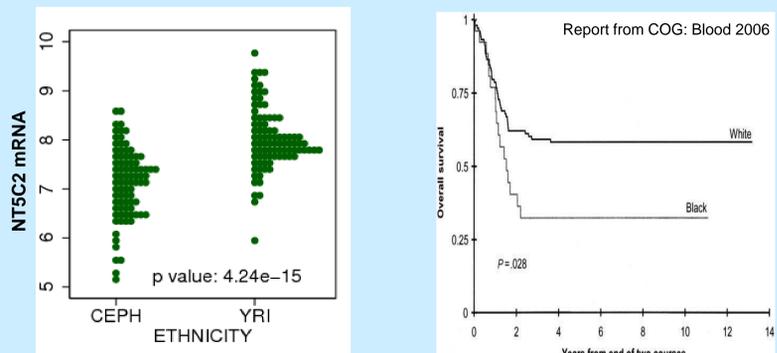
- Pediatric AML patients treated with ara-C containing chemotherapeutic regimes under St. Jude AML97 and St. Jude AML02 clinical trials were included in this study.
- NT5C2 SNPs were genotyped in the genomic DNA from AML patients.
- mRNA expression levels in the leukemia blasts were obtained from the microarray data in the diagnostic bone marrow samples.
- In AML02 clinical trial, ara-C LC50 was determined by treating leukemia blast obtained at diagnosis with varying concentrations of ara-C.

## RESULTS

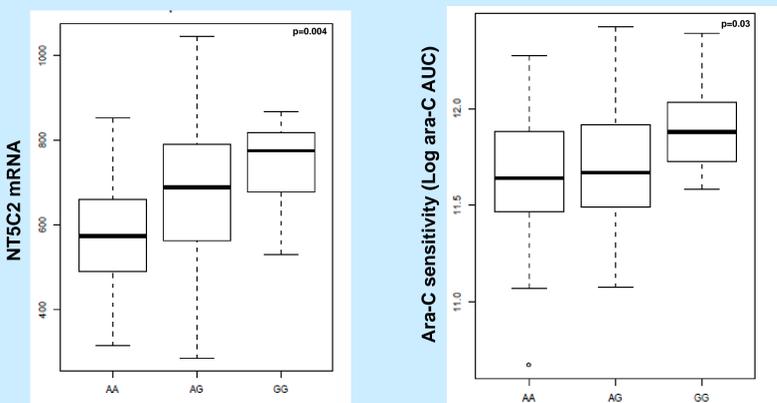
Comparison of LD pattern of NT5C2 SNPs between CEPH and YRI cell lines



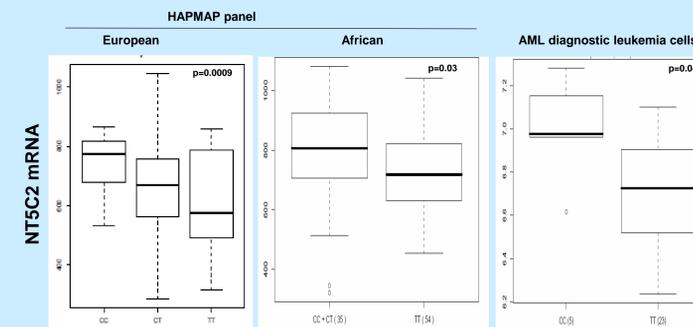
Ethnic differences in mRNA expression of NT5C2 in lymphoblast cell lines might contribute to observed differences in response



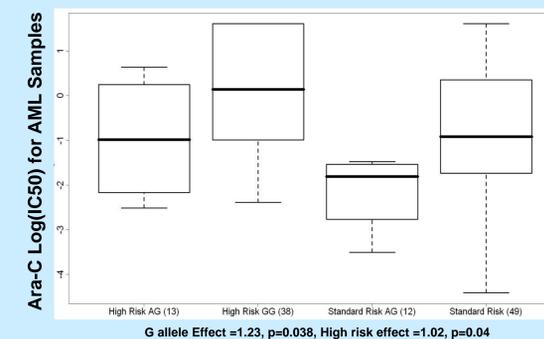
Association of NT5C2 SNPs with its mRNA expression and ara-C sensitivity in HAPMAP cell lines



A promoter SNP is associated with mRNA expression in HAPMAP cell lines and in diagnostic blast in AML patients



A promoter SNP is associated with ara-C sensitivity of diagnostic blasts from AML patients



## CONCLUSIONS

- NT5C2 is an inactivating enzyme that catalyzes conversion of ara-CMP to ara-C. Thus individual differences in its expression and activity could contribute to variation in ara-C pharmacology and clinical response.
- We have identified 38 novel polymorphisms in NT5C2 gene located in promoter, exons, introns and 3'UTR. Three coding polymorphisms were identified and one of them was predicted to be damaging by POLYPHEN.
- NT5C2 mRNA expression demonstrates ethnic differences with African ancestry population having significantly higher expression which might be contributing towards observed ethnic differences in clinical outcome.
- Two promoter polymorphisms were significantly associated with mRNA expression and ara-C cytotoxicity in HAPMAP cell lines and diagnostic leukemia blast cells from AML patients.
- Future studies are planned to determine the functional significance of NT5C2 SNPs.

## ACKNOWLEDGEMENTS

This work is supported in part by the National Institutes of Health/National Cancer Institute under grant R01CA132946-01 (Lamba); in part by the Cancer Center Support Grant CA-21765 (St. Jude Children's Research Hospital); and by the American Lebanese Syrian Associated Charities.