IDENTIFICATION AND FUNCTIONAL GENOMIC ANALYSIS OF GENETIC VARIANTS OF hENT1

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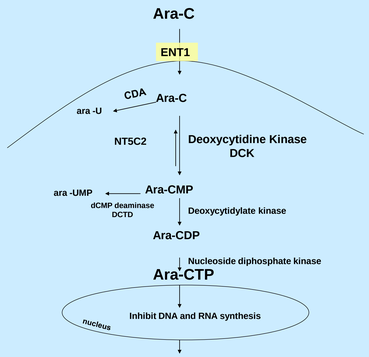
BACKGROUND

- 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).
- An uptake transporter known as human equilibrative nucleoside transporter 1 (hENT1) is responsible for ~80% of ara-C uptake into the cell.
- Several studies have shown increased hENT1 mRNA expression levels to be correlated with greater chemotherapeutic drug uptake efficacy in multiple patient populations.



OBJECTIVE

To identify and determine functional and clinical significance of genetic variants in hENT1.

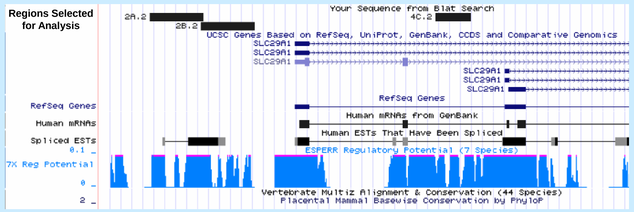
METHODS

Study Population

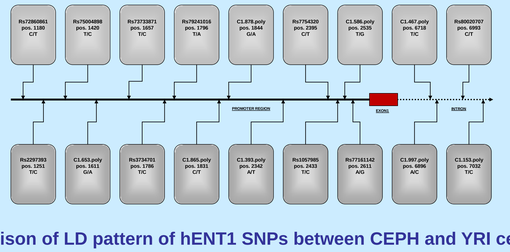
- Epstein-Barr virus-transformed B-lymphoblastoid HAPMAP cell lines derived from 30 Centre d' Etude du Polymorphisme Humain (CEPH) trios (two parents and a child) (n = 90, European descent) and 30 Yoruba trios (n = 90, African descent, referred as YRI).
- SNPs in hENT1 were identified by sequencing selected 5'UTR and intron 1 regions of regulatory importance in the genomic DNA from 180 HAPMAP samples from two different ethnicities.
- Regions 2A, 2B, and 4C were selected to be sequenced and analyzed due to their high transcription factor binding potential.
- 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.

RESULTS

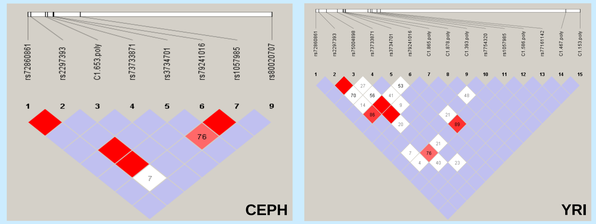
Snapshot of hENT1 (SLC29A1) Promoter Showing Regions selected for Sequencing based on High 7X Regulatory Potential



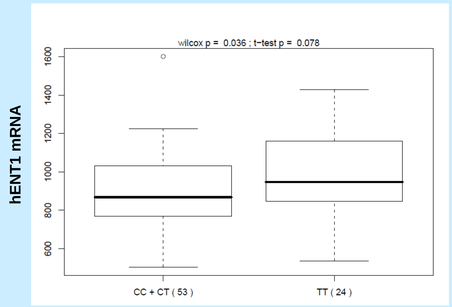
hENT1 SNP MAP



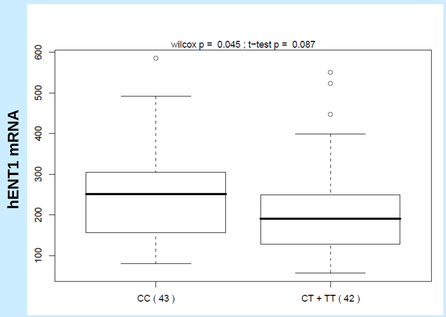
Comparison of LD pattern of hENT1 SNPs between CEPH and YRI cell lines

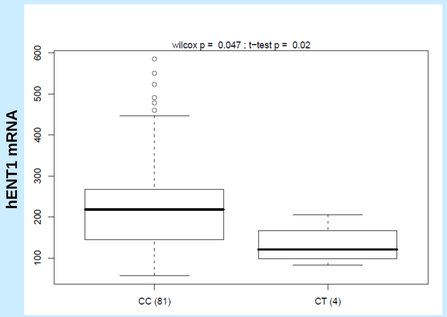


Association of hENT1 SNPs with mRNA expression in HAPMAP CEPH cell line



Association of hENT1 SNPs with mRNA expression in HAPMAP YRI cell line





CONCLUSIONS

- Acute myeloid leukemia (AML) is the second most common pediatric leukemia and has the worst prognosis of all major childhood cancers.
- hENT1 is a broadly expressed nucleoside cell transporter that actively pumps anti-cancer nucleoside analogs such as ara-C into cells. Once inside the cell, ara-C can be activated to its 5'-triphosphate derivative, ara-CTP, which is incorporated into nascent DNA and RNA strands to cause chain termination and block further replication.
- As hENT1 is essential for ara-C entry into the cell, individual differences in its expression and activity could contribute to variation in ara-C pharmacology and clinical response.
- We have identified 8 novel polymorphisms located in the promoter and intron regions of the hENT1 gene. Three polymorphisms in these regions were shown to substantially affect hENT1 mRNA expression levels observed in these patients.
- A different distribution of SNPs between CEPH and YRI was also shown in LD plots which might be contributing to observed ethnic differences in clinical outcome of ara-C treatment.
- Transfac Data for CEPH and YRI showed no deviations in Transcription Factor Binding from normal.
- Future studies are planned to determine the functional significance of hENT1 SNPs in AML patients receiving ara-C based chemotherapy.

ACKNOWLEDGEMENTS

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