




Genomics and Therapeutics in SCD

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Pediatrics

Therapeutics: Relevant Issues

- Limited therapeutic options and indications
  - Transfusion (cerebrovascular disease)
  - Hydroxyurea (acute vaso-occlusive events)
  - Other agents typically have small sample sizes
- Few cohorts with accurate phenotypes
  - Qualitative, subjective endpoints
  - Source documentation may be lacking
  - Problem with negative phenotypes

Therapeutics: Role of Genomics

- Prediction of at-risk individuals (selection)
  - Children who are likely to develop stroke
  - Patients likely to have increased clinical severity
- Variable treatment responses (mechanisms)
  - HbF response to hydroxyurea, MTD
  - Fast versus Slow oral absorption
- Adverse effects (safety)
  - Alloimmunization, drug toxicity, genotoxicity

Cerebrovascular Disease in SCA

- 5-10% of patients with sickle cell anemia (SCA) will develop an infarctive stroke during childhood
  - Peak incidence at 6-8 years of age
  - Substantial neurocognitive deficits
  - Transfusions for primary and secondary stroke prevention
- Previous genetic studies have selected genes that are presumed to affect stroke risk
  - Candidate gene approach, biased
  - Without subsequent validation studies

Genes "Associated" with Stroke

Gene	Gene Function	Gene	Gene Function
BAI1	Angiogenesis	**IL4R**	Inflammation
ADRB2	Blood Pressure regulation	**BMP6**	Inflammation
AGT	Blood Pressure regulation	**CCL2**	Inflammation
MET	Cell Survival	**TGFBR3**	Inflammation
CSF2	Cellular Adhesion	**TNF-α**	Inflammation
ECE1	Cellular Adhesion	**LTC4S**	Inflammation
SELP	Cellular Adhesion	**MMP3**	Inflammation
TEK	Cellular Adhesion	**LPL**	Lipid Metabolism
NINJ2	Cellular Adhesion	**ADCY9**	Neuronal Signaling
AIMP3	DNA repair	**ERG**	Transcriptional regulator
ANXA2	Hypercoagulability	**GPx-3**	Vascular Tone

DNA Samples for Stroke Analysis

Study	Sample Size	Stroke	Control
HUSTLE	108	5	103
SWiTCH	130	130	-
CSSCD	176	21	155
C-DNA	105	20	85
Total	**519**	**176**	**343**

- All studies have appropriate IRB approval
- Each sample has documented phenotypes
- All DNA samples passed stringent quality controls

Validation of Candidate SNPs

- Genotyped 38 SNP's in 22 genes

  Also determined α-thalassemia, beta-globin haplotype, G6PD
- Only 4 SNP's had validated association with stroke
- α-thalassemia was protective against stroke

Gene	SNP ID	Minor Allele	Control (n=206)	Stroke (n=260)	Genetic model	Odds Ratio	95% CI	p-value
ADCY9	rs2238432	A	33.0%	22.3%	Dominant	0.47	0.28 - 0.79	**0.003**
ANXA2	rs11853426	T	34.9%	44.6%	Recessive	2.70	1.25 - 5.84	**0.007**
TEK	rs489347	G	35.9%	46.9%	Recessive	2.16	1.11 - 4.23	**0.016**
TGFBR3	rs284875	T	7.8%	13.5%	Dominant	2.53	1.28 - 4.99	**0.005**
HbA2	rs63751476	Δ3.7	17.0%	8.8%	Dominant	0.45	0.24 - 0.84	**0.009**

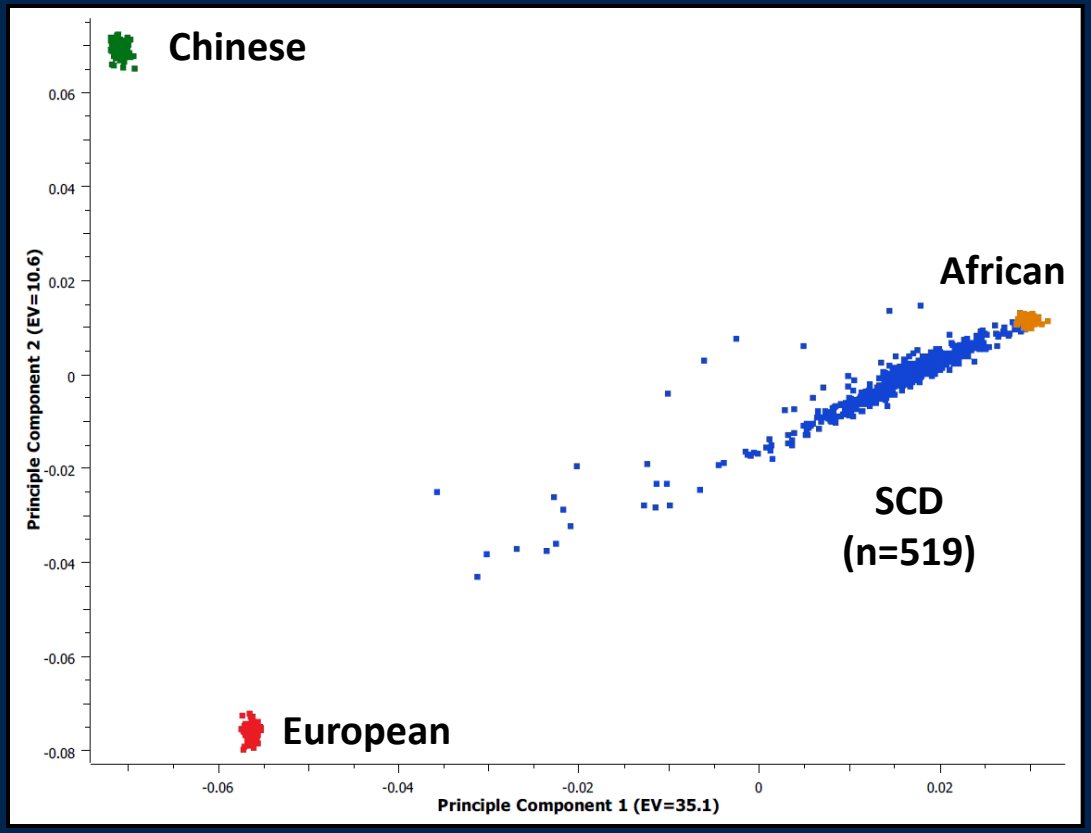
(Flanagan *et al.*, Blood 2011)

Genome Wide Association Study of Genetic Modifiers of Stroke Risk

- DNA samples: SWiTCH, HUSTLE, plus the pediatric CSSCD and C-DNA cohorts
  - 176 stroke samples
  - 343 control samples
- An unbiased genome wide search
  - Affymetrix SNP6.0 platform
  - Genotyped all 519 DNA samples
  - Golden Helix to perform quality control checks and association study

GWAS in Patients with SCA

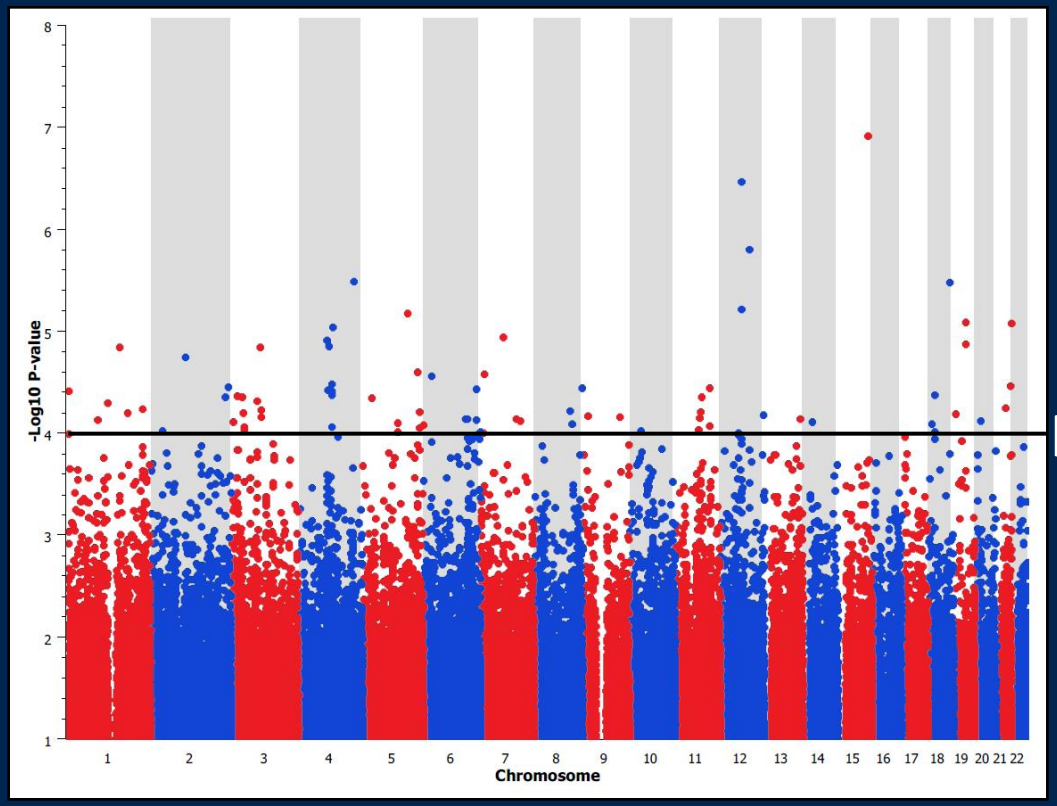
Principal Component Analysis



Outliers were removed from subsequent analyses

Stroke GWAS Results

- Tested >700,000 SNPs for association
- Compared Control 343 patients
  Stroke 155 patients



p-value cut off
$p < 1X10^{-4}$

Overall Stroke GWAS Results

Genetic Model	Pass Threshold ($p < 1x10^{-4}$)	Pass Threshold ($p < 1x10^{-5}$)	Pass Threshold ($p < 1x10^{-6}$)
Recessive	56	3	1
Dominant	83	9	4
Total	**139**	**12**	**5**

139 SNP's have p-values less than 0.0001

All represent good targets for further in depth study

Examination of the genomic location and function of related genes for all SNPs

Whole Exome Sequencing

Created four random DNA pools:

Group A	Control	n = 52 DNA samples (HUSTLE)
Group B	Control	n = 52 DNA samples (HUSTLE)
Group C	Stroke	n = 60 DNA samples (SWiTCH)
Group D	Stroke	n = 60 DNA samples (SWiTCH)

Captured and sequenced every exon in the genome using Illumina Next Generation Sequencing technology

Identified all non-synonymous and insertion/deletion mutations present in the SCA cohort

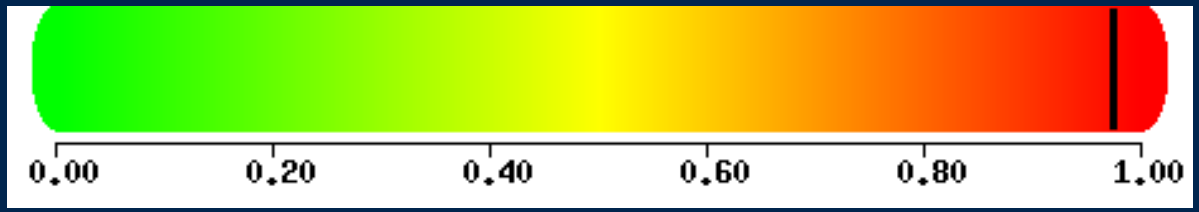
- Identify mutations near genomic areas found by GWAS
- Identify mutations found by exon sequencing only

Whole Exome Results

Identified a total of 28,562 mutations in SCA cohort

Used PolyPhen2 and SIFT algorithms to predict effects of mutation on encoded proteins

Mutation predicted to be probably damaging with score of 0.975 (sensitivity 0.68; specificity 0.94)



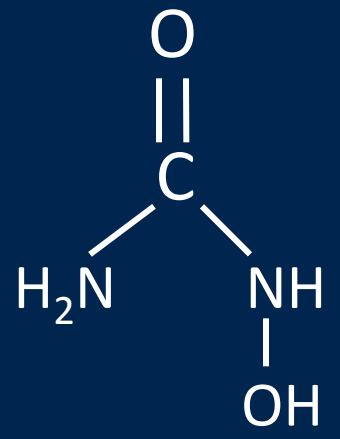
294 mutations are significantly associated with stroke [using Fishers exact testing and corrected for FDR ($p<0.0001$)]

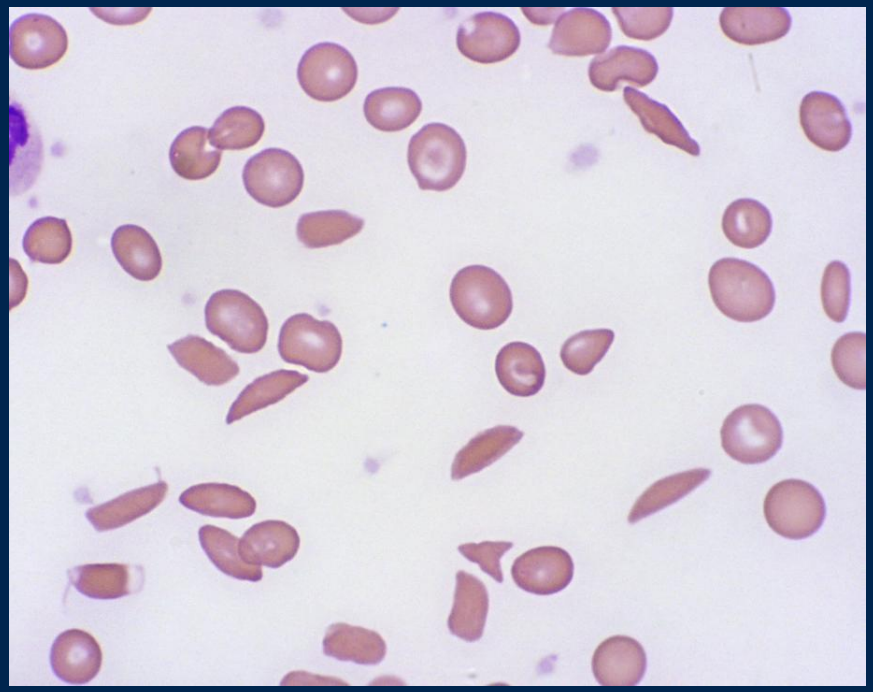
Compared to the GWAS results (139 SNPs), there are 135 mutations that are located within 500kb of a GWAS SNP and have association with stroke ($p<0.05$)

Genomics: Cerebrovascular Disease

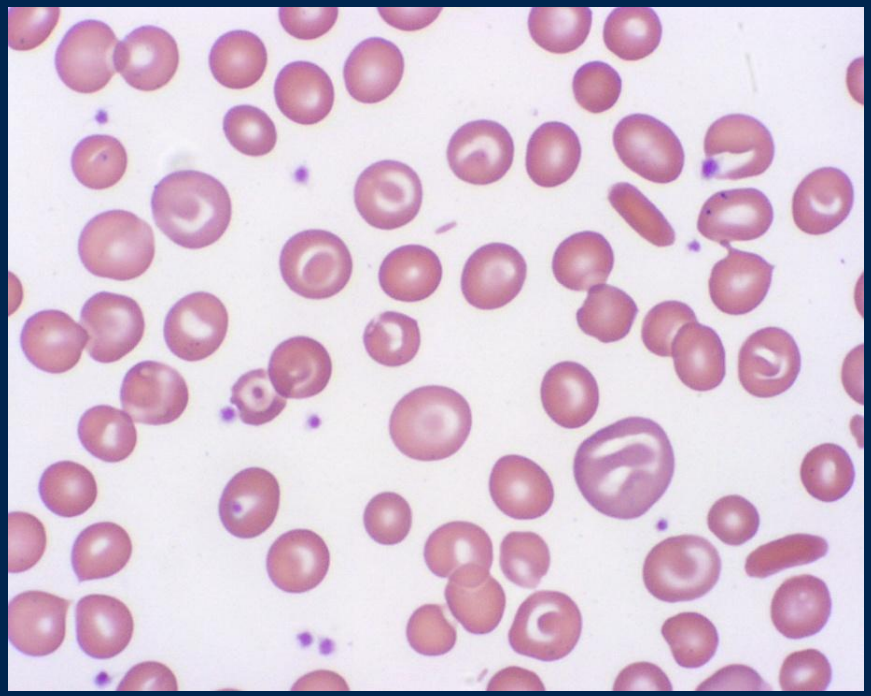
- GWAS for stroke may be feasible
  - Cohort size, phenotypes, quality, validation
- Selection criteria (at-risk patients)
  - Stroke, silent infarcts, abnormal TCD
- Mechanisms and Safety
  - Pathogenesis
  - Treatment responses, adverse events

Hydroxyurea Treatment





Before Hydroxyurea



Taking Hydroxyurea

Clinical Outcomes: Infants compared to Adults

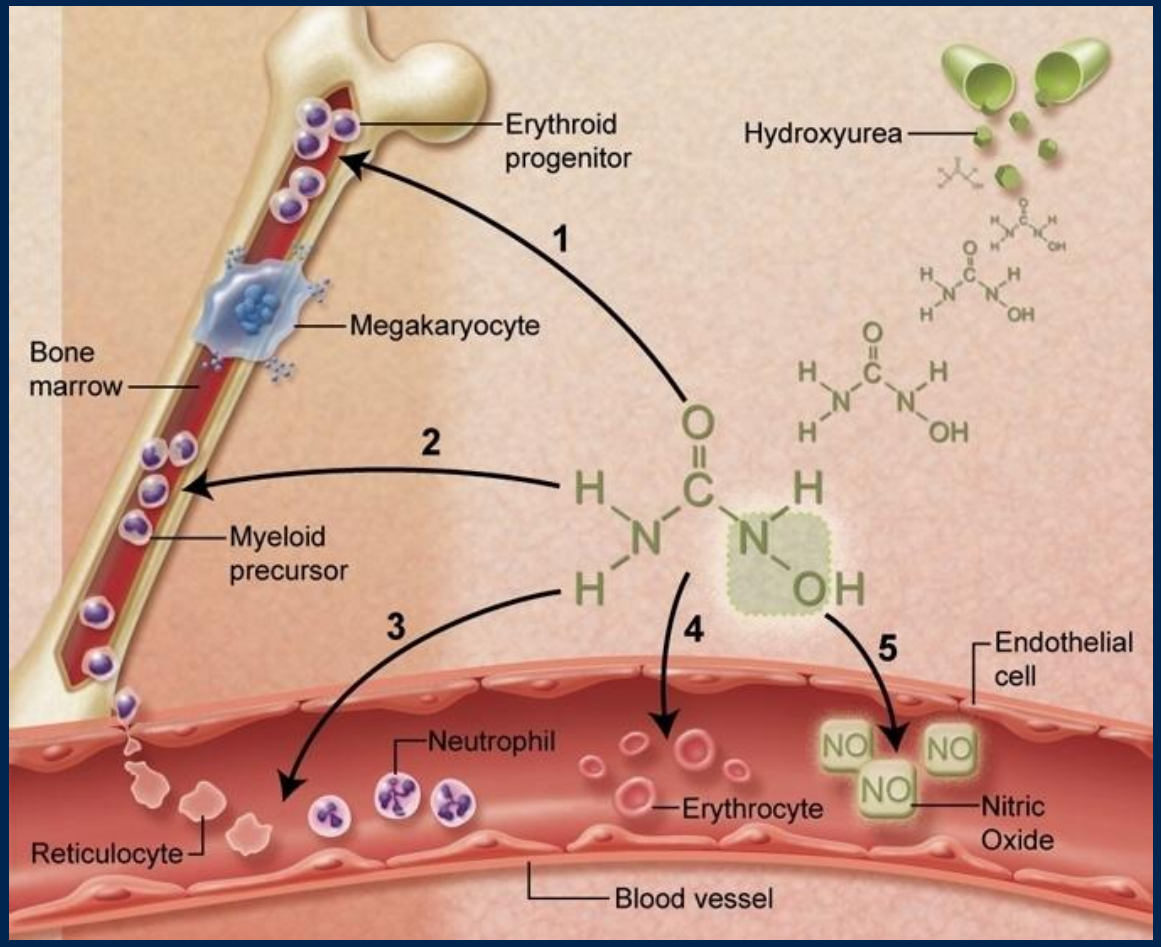
	BABY HUG			MSH		
	HU	PL	p-value	HU	PL	p-value
Patients	96	97		152	147	
Pain	177	372	0.002	2.5/y	4.5/y	<0.001
Acute Chest	8	27	0.017	25	51	<0.001
Dactylitis	24	123	<0.001	—	—	—
Hospitalization	232	321	0.050	1.0/y	2.4/y	—
Transfusion	35	60	0.033	48	73	0.001

Hydroxyurea Laboratory Effects
(N=111, therapy for 3.2 ± 2.7 years)

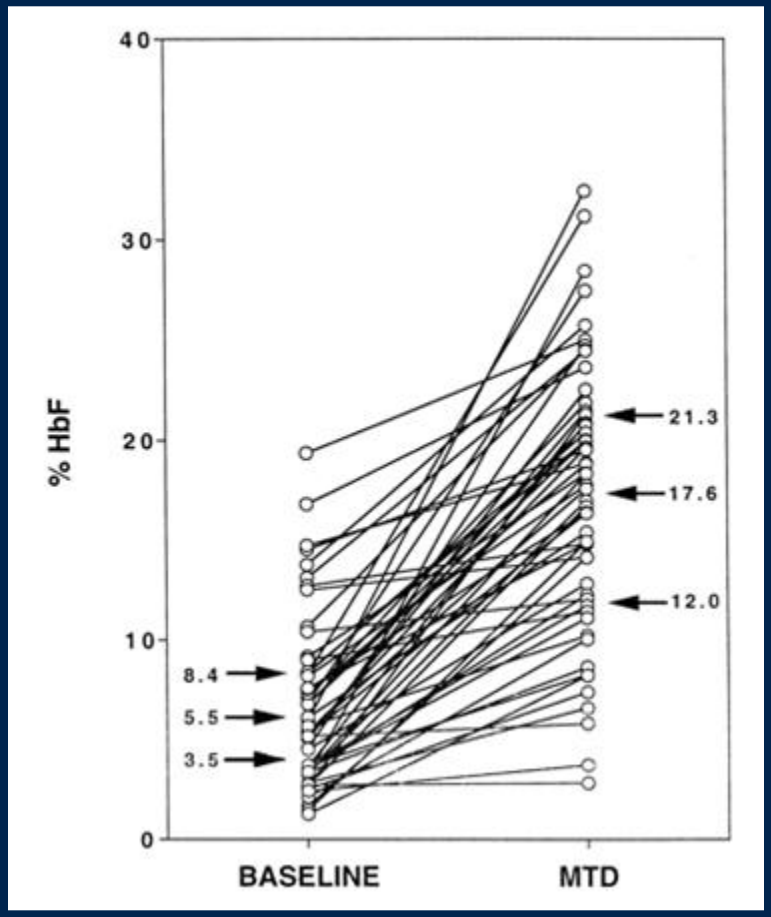
	Baseline Values	Hydroxyurea MTD	p value
Age (years)	7.6 ± 4.6	10.7 ± 4.3	--
Hb (gm/dL)	8.6 ± 1.3	9.7 ± 1.1	<.001
MCV (fL)	85 ± 6	107 ± 13	<.001
HbF (%)	10.6 ± 6.6	23.2 ± 7.8	<.001
ARC (x 10^9/L)	264 ± 123	130 ± 52	<.001
WBC (x 10^9/L)	13.6 ± 4.3	7.5 ± 3.0	<.001
ANC (x 10^9/L)	6.7 ± 2.9	3.8 ± 2.2	<.001
T Bili (mg/dL)	3.0 ± 1.7	1.8 ± 1.4	<.001
LDH (IU/L)	662 ± 326	453 ± 220	<.001

Hydroxyurea in Sickle Cell Anemia
Multiple Potential Mechanisms of Action

Ware, Blood 2010; 115:5300-5311



Hydroxyurea: Variable HbF Response



Multi-Center HUG-KIDS Trial
Kinney, et al. Blood 1999; Ware, et al. Blood 2002

Median HbF values

- Pre-treatment: 5.5%
- At hydroxyurea MTD: 17.6%

Predictors of MTD HbF

- Baseline %HbF
- MTD dose
- WBC, reticulocytes
- Medication adherence

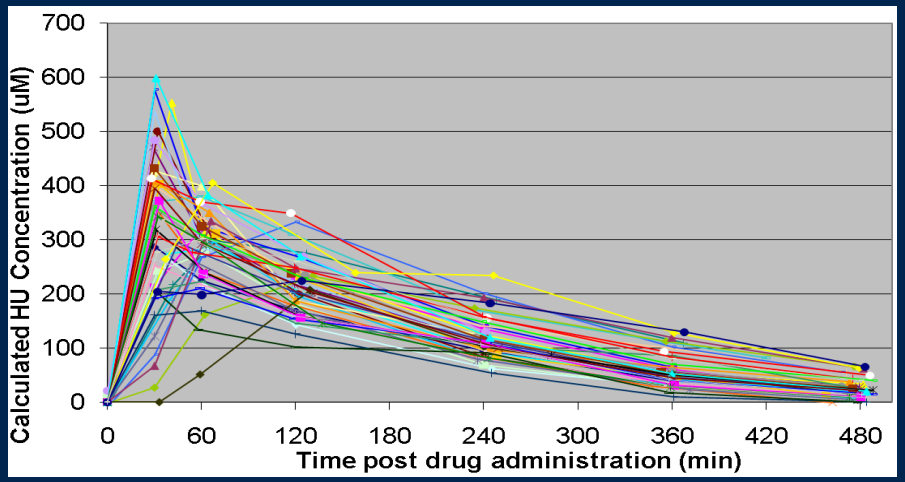
Hydroxyurea PK, PD, PGx
HUSTLE: NCTC00305175

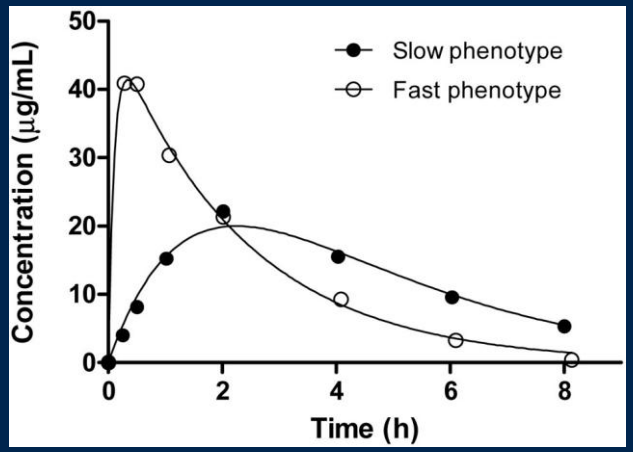
New Cohort
Old Cohort

First-dose PK
MTD PK

Predictors of Response
%HbF, MTD

Pharmacogenetics
Candidate genes





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HbF GWAS in SCA

Study sample size (HUSTLE)

	Baseline %HbF	Delta %HbF (MTD)
Patients	174	155
%HbF	7.9 ± 5.2	19.7 ± 6.9

%HbF levels as a quantitative trait

- Testing SNPs for any association
- Additive genetic model: [AA] > [Ab] > [bb] vs HbF
- 252 SNPs associated with any phenotype ($p<1x10^{-4}$)
- 35 SNPs are below 1 $X10^{-5}$

Genomics: Hydroxyurea Response

- GWAS for hydroxyurea may be feasible
  - Cohort size, phenotypes, quality, validation
- Whole exome sequencing is feasible
  - Correlation of exonic mutations with GWAS
- Selection, Mechanisms, and Safety
  - Responses – high versus low responders
  - Adverse events – genotoxicity

Hydroxyurea: Global Setting

Vast global burden must be addressed

Only available disease-modifying therapy

Can impact both morbidity and mortality

Pilot data will be forthcoming

Enormous opportunity for genomics analyses