PKLR Variants Associated with Acute Pain in Sickle Cell Disease Influence ATP Concentrations in Red Blood Cells

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Abstract #393

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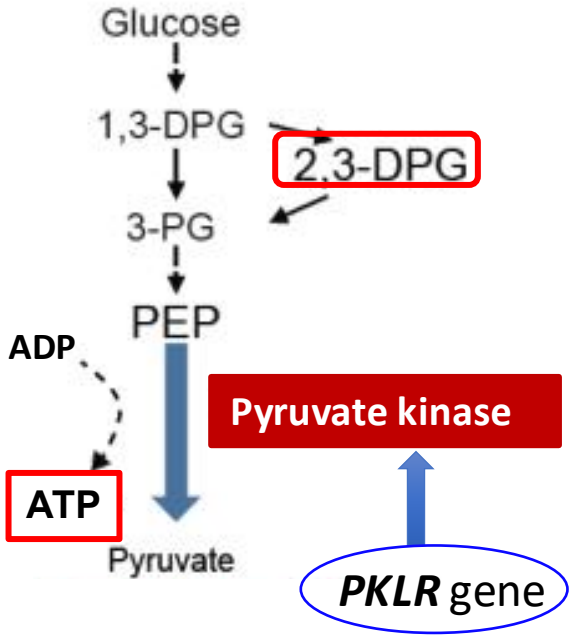
NIH National Heart, Lung, and Blood Institute

Sickle Cell Disease (SCD) and Acute Vaso-occlusive Pain

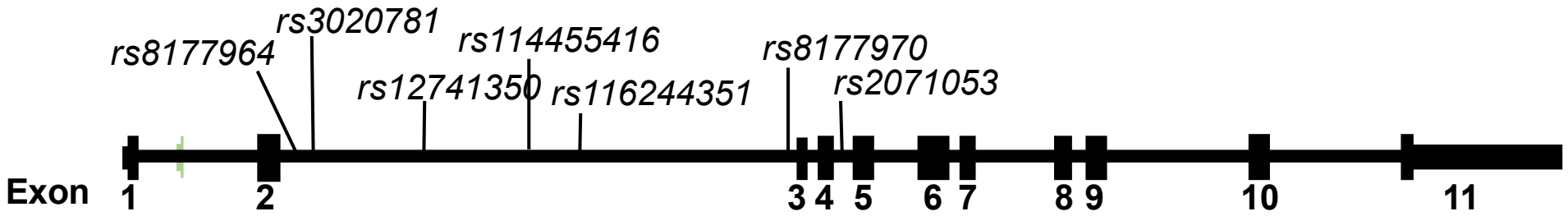
- Acute sickle pain results from vaso-occlusion triggered by sickling of deoxygenated red blood cells (RBCs)
- The frequency of acute sickle pain varies widely, influenced by both genetic and environmental factors
- 2 key factors that influence red cell sickling are 2,3-DPG and ATP red cell concentrations
  - 2,3-DPG stabilizes HbS in polymerizing T form and promotes deoxy-HbS polymerization
  - Reduced ATP promotes RBC dehydration and sickling
- Both parameters are affected by the glycolytic cycle, in which pyruvate kinase is a key enzyme

Glycolytic Pathway within RBC



PKLR intronic variants are associated with acute pain in SCD

PKLR gene (chr1q22) with associated SNPs



SNP ID	coordinates chr:position (hg19)	Location in *PKLR* gene	A1 (minor)	A2 (major)	HbSS (King's) N=242			HbSS (SIT) N=977			Weighted Fisher's meta-analysis
					Freq	Beta	p value	Freq	Beta	p value	Combined p-value
rs2071053	1:155265177	intron 4	A	G	0.37	-0.088	0.00097	0.42	-0.087	0.0814	0.0009918
rs8177970	1:155265661	intron 2	C	T	0.16	0.1299	0.00036	0.13	0.028	0.6866	0.0042704
rs116244351	1:155266935		A	G	0.16	0.1247	0.00064	0.13	0.028	0.6866	0.0068498
rs114455416	1:155267389		A	G	0.16	0.1247	0.00064	0.13	0.0281	0.686	0.006843
rs12741350	1:155268425		C	T	0.38	-0.086	0.00115	0.42	-0.097	0.0516	0.0007171
rs3020781	1:155269776		A	G	0.38	-0.086	0.00115	0.43	-0.097	0.0508	0.0007057
rs8177964	1:155269780		A	G	0.16	0.1241	0.00071	0.12	0.0486	0.4895	0.0050984

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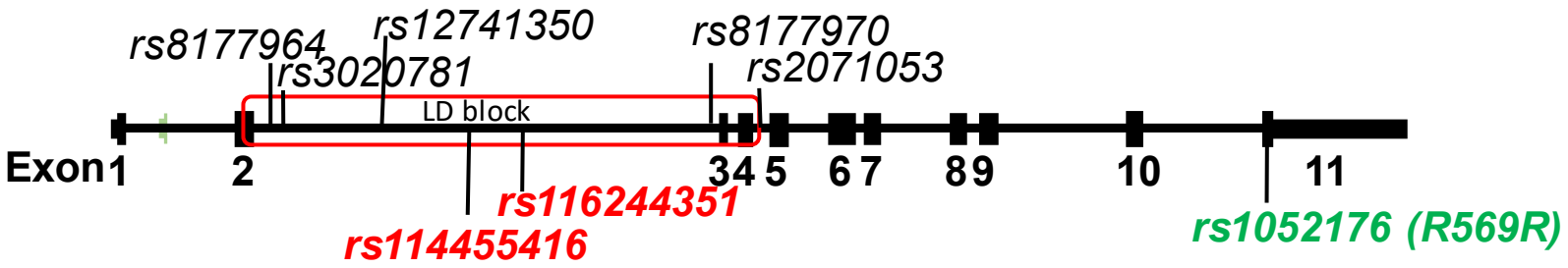
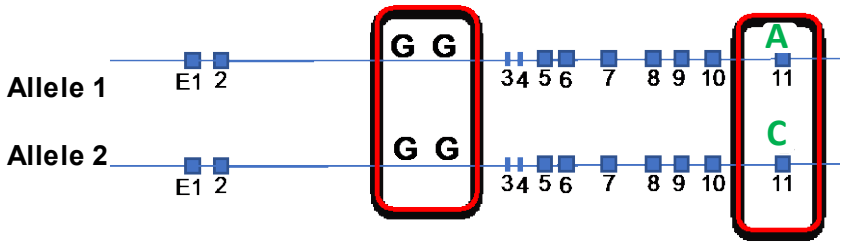
"Risk *PKLR* variants" affect *PKLR* gene expression

***PKLR* gene (chr1q22) with associated SNPs**



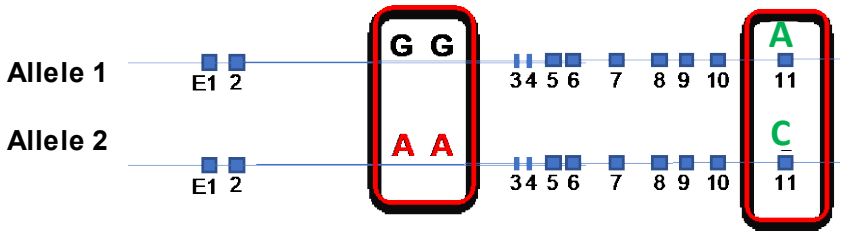
Homozygous WT intron 2



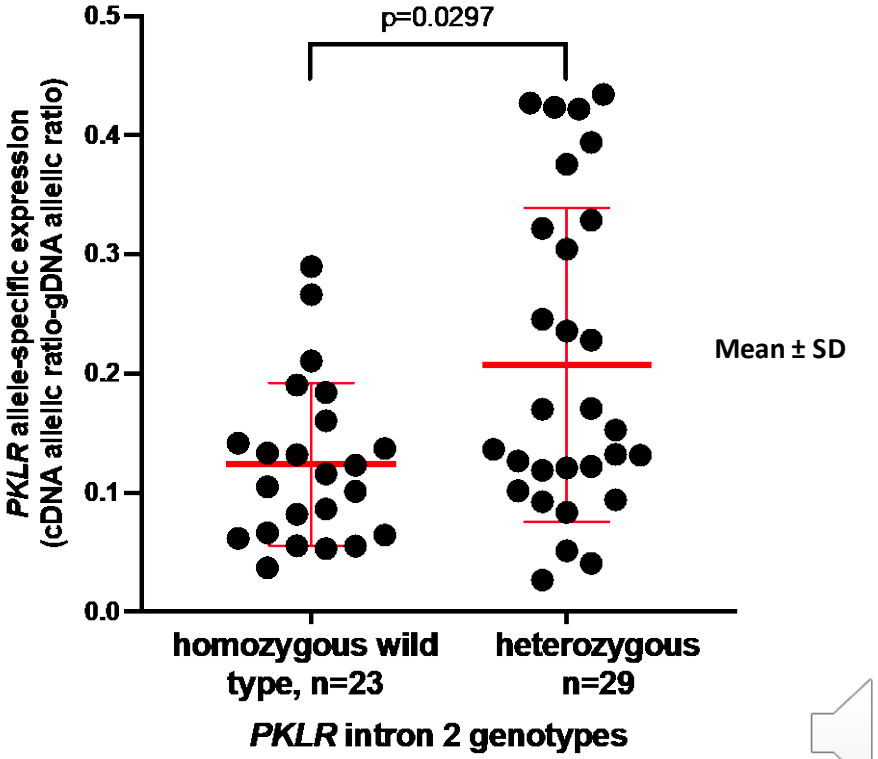
Allele-specific expression ratio

1:1

Heterozygous intron 2



1:1 or ?:?



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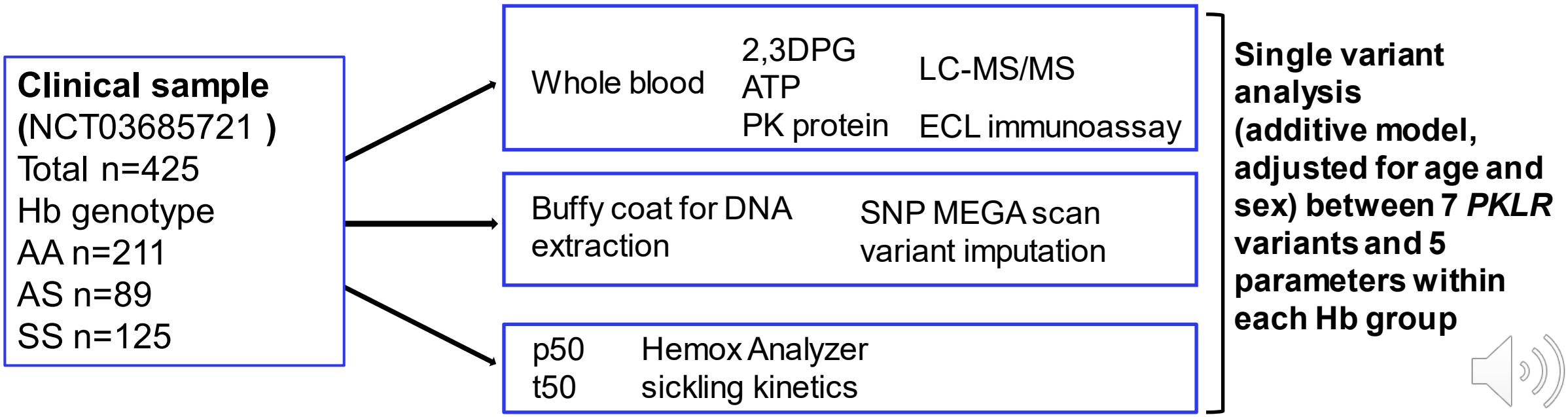
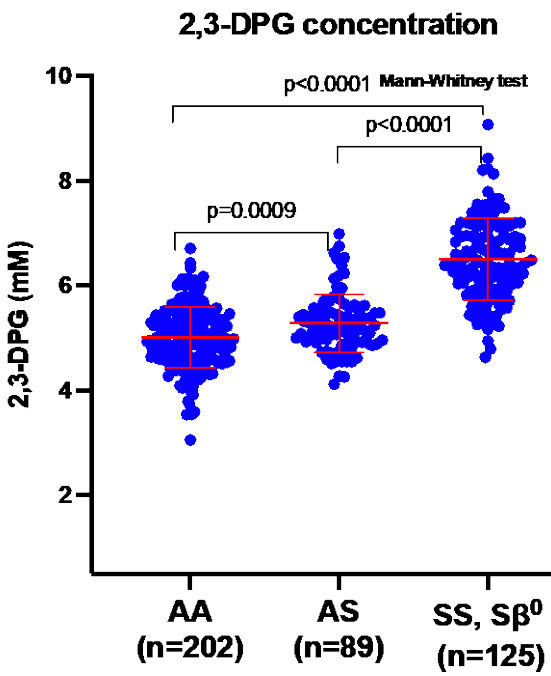
Study Design

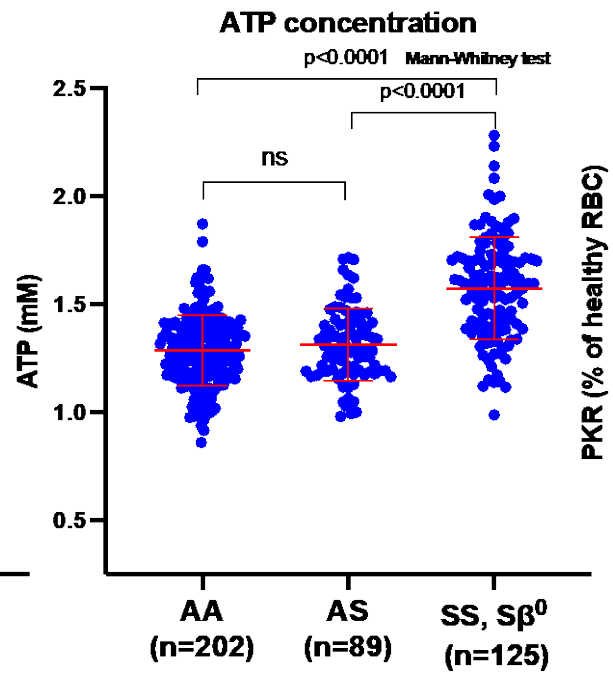
- "Risk *PKLR* variants" affect gene expression
- Do the "Risk *PKLR* variants" also affect levels of PKR protein expression, 2,3-DPG, ATP, oxygen affinity (p50, oxygen pressure at 50% saturation) and sickling kinetics (t50, time for 50% of cells to sickle)?
- We carried out association study between the "risk *PKLR* variants" and PK protein (PKR), metabolites, p50 and t50

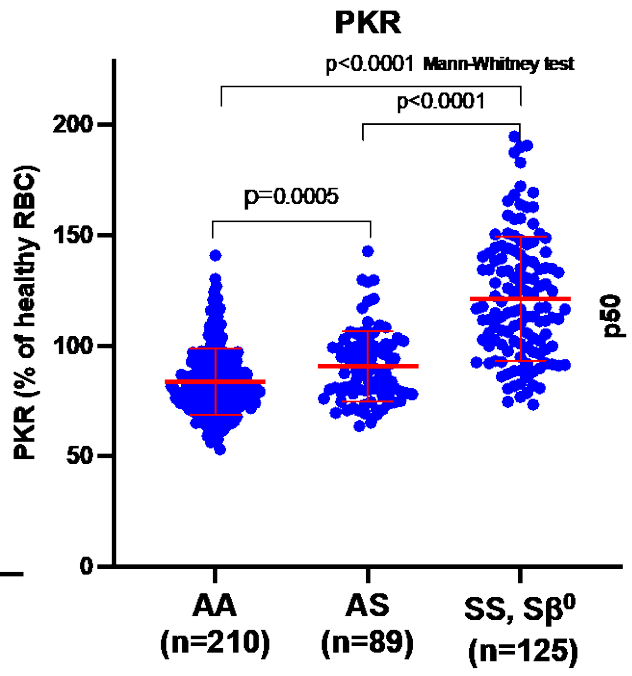


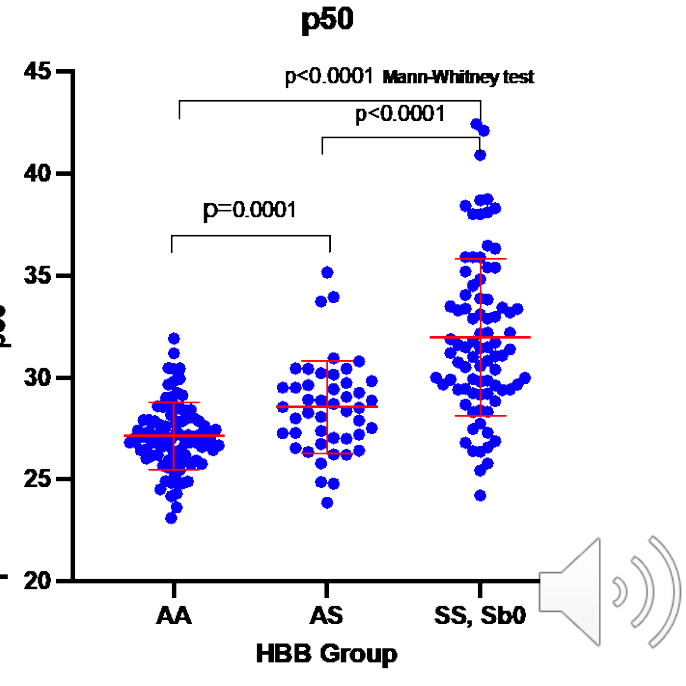
Characterization of parameters in AA, AS and SS groups

Total (n=425)	AA (n=211)		AS (n=89)		SS (n=125)		p value (one way ANOVA)
	n	mean ± SD	n	mean ± SD	n	mean ± SD	
ATP (mM)	202	1.287 ± 0.162	89	1.315 ± 0.166	125	1.575 ± 0.237	3.09E-35
2,3-DPG (mM)	202	5.013 ± 0.589	89	5.283 ± 0.561	125	6.504 ± 0.786	7.46E-64
PKR (% of healthy RBC)	210	83.78 ± 14.90	89	90.68 ± 16.03	125	121.45 ± 27.96	8.76E-49
p50	88	27.14 ± 1.65	46	28.56 ± 2.27	85	31.96 ± 3.85	6.37E-23
t50 (final O2: AS 0%, SS 5%)	N/A	--	65	120.17 ± 68.82	113	218.06 ± 109.75	--



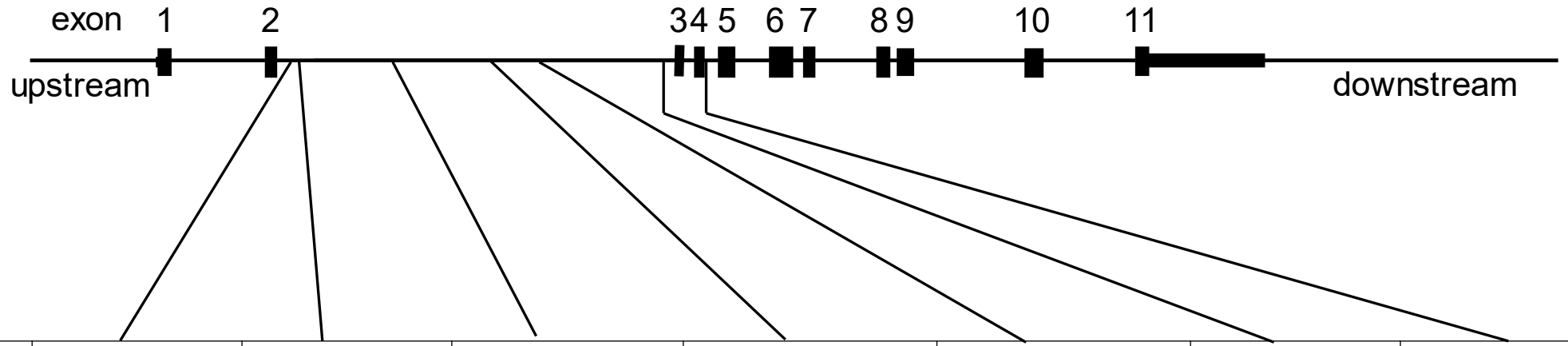






Association of "Risk *PKLR* variants" with ATP, 2,3-DPG and t50

***PKLR* – chr1q22**



SNP ID	*rs8177964*	***rs3020781***	***rs12741350***	*rs114455416*	*rs116244351*	*rs8177970*	***rs2071053***
Hb AA							
Hb AS	ATP	ATP, t50	ATP, t50	ATP	ATP	ATP	ATP, t50
HB SS		2,3-DPG	2,3-DPG				2,3-DPG

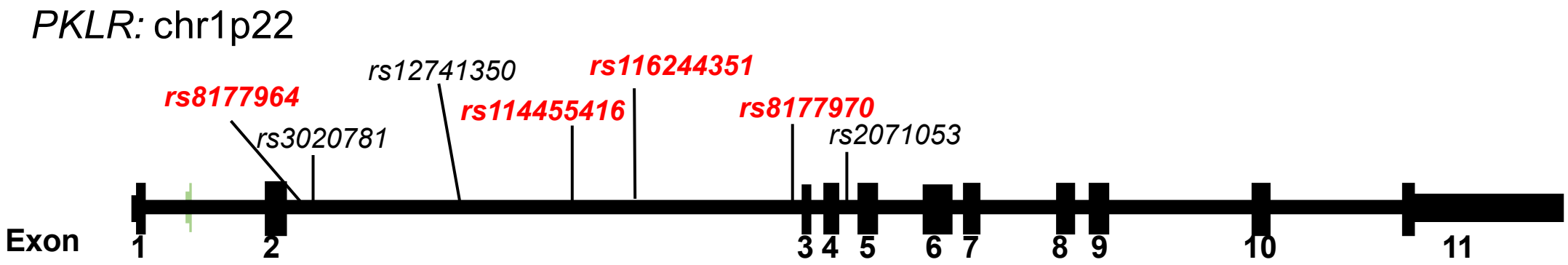
- All 7 pain-associated SNPs are associated with ATP concentration in AS group, 3 are associated with 2,3-DPG in HbSS (not HbAS) and t50 in HbAS (not HbSS)

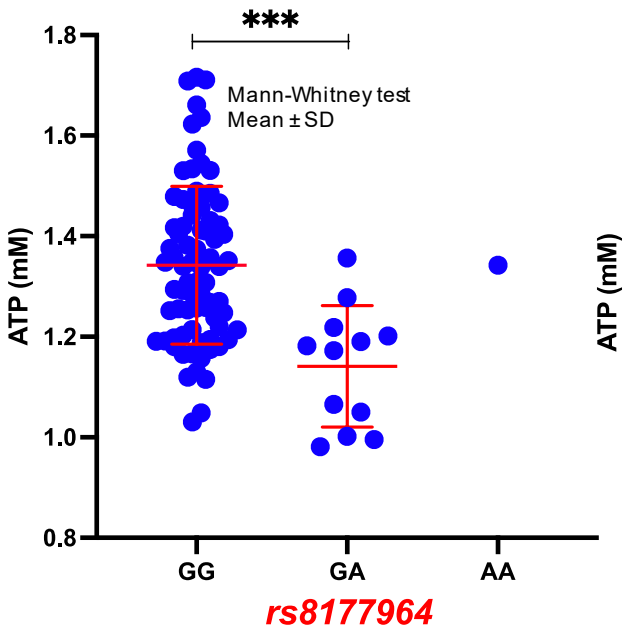
PKLR genetic association analysis

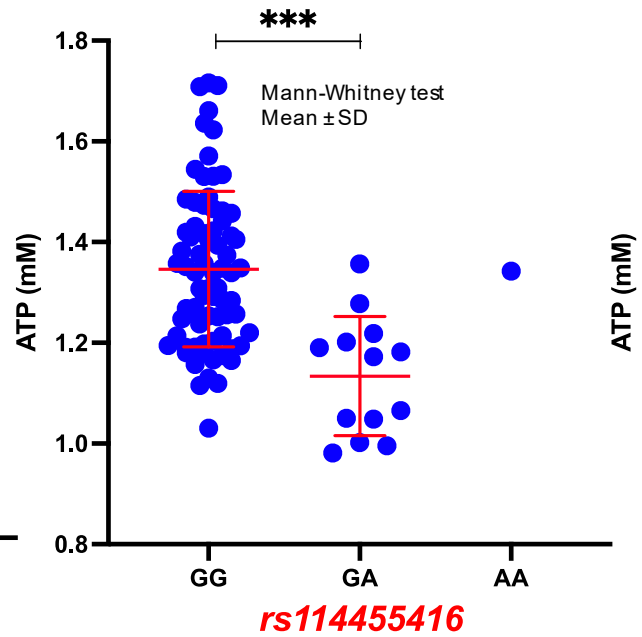
Var	rs ID	POS (chr:position, hg19)	A			AA, N=211			AS, N=89			SS, N=126		
			REF	ALT		MAF	Original_p	FDR_p	MAF	Original_p	FDR_p	MAF	Original_p	FDR_p
ATP (mM)	***rs8177964***	1:155269780	G	A		0.1	0.690194	0.825203	0.079	0.001065	0.00932	0.123	0.334752	0.924453
	rs3020781	1:155269776	G	A		0.405	0.12314	0.489116	0.41	0.018752	0.09376	0.389	0.702845	0.924453
	rs12741350	1:155268425	T	C		0.405	0.12314	0.489116	0.41	0.018752	0.09376	0.381	0.672846	0.924453
	rs114455416	1:155267389	G	A	intron 2	0.102	0.478208	0.669491	0.084	0.000181	0.00212	0.127	0.242472	0.848652
	rs116244351	1:155266935	G	A		0.102	0.478208	0.669491	0.084	0.000181	0.00212	0.127	0.242472	0.848652
	rs8177970	1:155265661	T	C		0.102	0.478208	0.669491	0.084	0.000181	0.00212	0.127	0.242472	0.848652
	rs2071053	1:155265177	G	A	intron 4	0.402	0.174684	0.489116	0.404	0.008361	0.05852	0.377	0.781526	0.924453

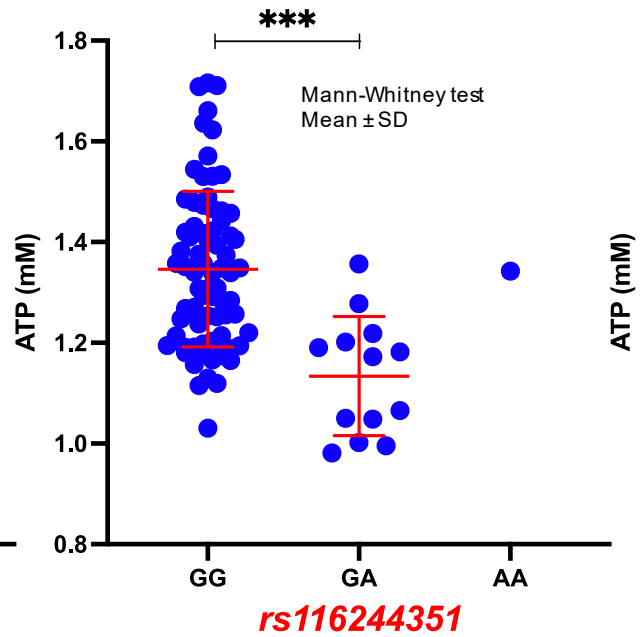
- **4 variants remain significantly associated with ATP concentration in AS group after multiple testing.**

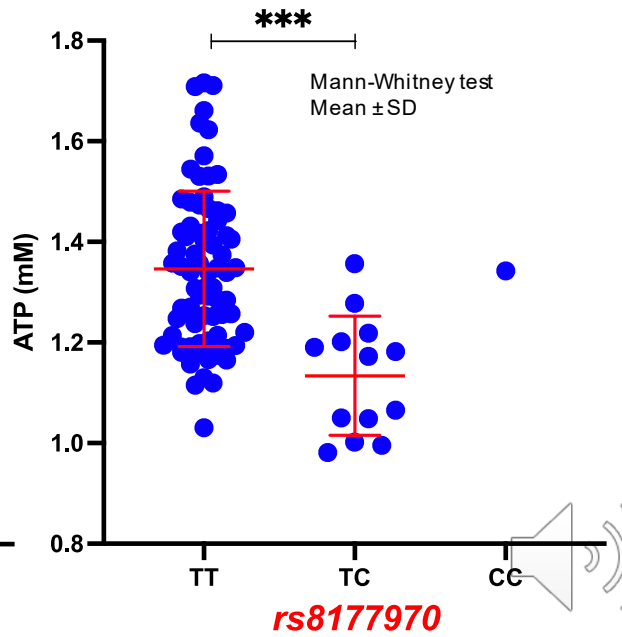
SNPs associated with ATP in HbAS group









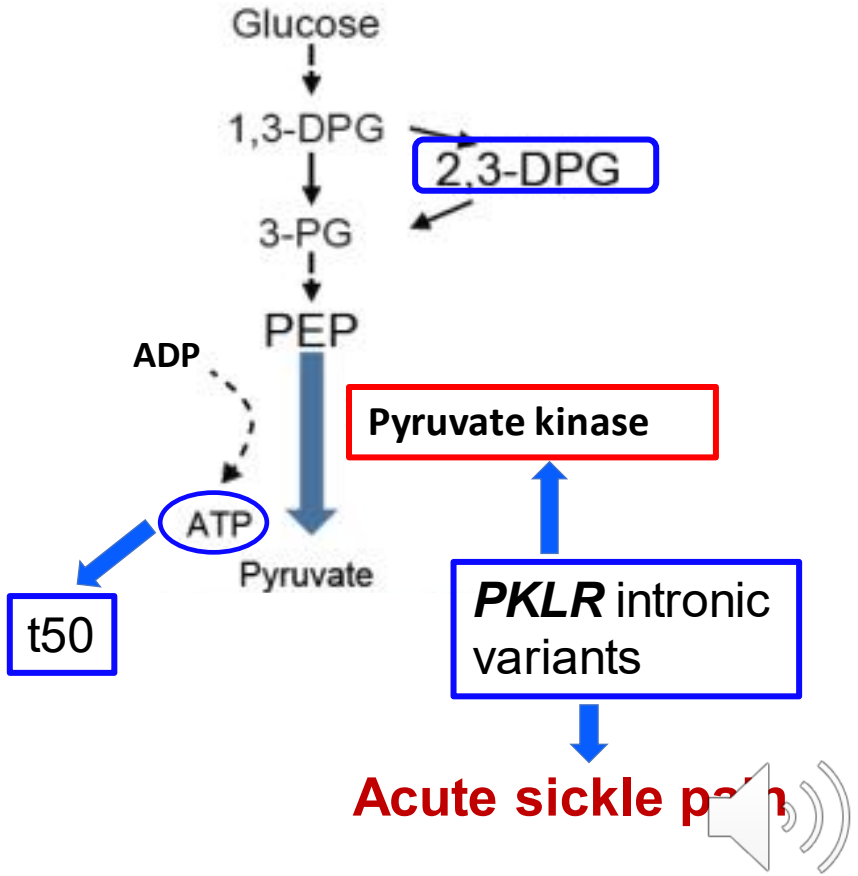


***: p<0.001

Summary

- A *PKLR* intron 2 'high-risk" haplotype was previously implicated in acute sickle pain by affecting *PKLR* expression
- The same "Risk *PKLR* variants" are associated with reduced ATP levels providing a biological basis for the genetic association with acute sickle pain
- 3 variants are also associated with elevated 2,3-DPG levels in HbSS (not observed in HbAS)
- The same 3 variants are associated with t50 in HbAS (not HbSS)
- The variant associations are observed more clearly in HbAS but not in HbSS, possibly because of the much more homogeneous cell population in HbAS.
- *PKLR* intron variants may directly contribute to the severity and frequency of acute pain episodes in SCD, but additional studies with larger sample sizes are warranted.

Glycolytic Pathway within RBC



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