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Supplementary Information for

A common polymorphism in the mechanosensitive ion channel *PIEZO1* is associated with protection from severe malaria in humans

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Table S1. Baseline demographics by study

Baseline characteristic	Overall (n=446)	Study		
		Kremsner **(n=195)**	***Kun*** **(n=195)**	***Kalmbach*** **(n=56)**
Sex, n (%)				
Male	201 (45%)	104 (53%)	74 (38%)	23 (41%)
Female	245 (55%)	91 (47%)	121 (62%)	33 (59%)
Age in months, median (range)	35 (4, 140)	25 (12, 103)	41 (8, 140)	36 (4, 120)
Parasite density (parasite/μL)[1], geometric mean (95% confidence interval)	34,664.9 (34,649.5, 34,680.3)	26,552.0 (26,536, 26,568.1)	42,643.6 (42,626.9, 42,660.3)	34,581.9 (34,572.7, 34,591.0)
Severe malaria, n (%)	253 (57%)	146 (75%)	97 (50%)	10 (18%)

Continuous and categorical variables were compared across malaria status using the Wilcoxon rank sum test and Fisher's exact test, respectively. [1]n=44 excluded due to zero values.

Table S2: Hardy-Weinberg equilibrium (HWE) for *PIEZO1* E756del polymorphism in case and control groups according to expected and observed values.

	WT/WT	WT/DEL	DEL/DEL	Chi-Square	HWE p-value
Cases					
Observed	180	58	15	10.34	0.0013
Expected	172.7	72.7	7.7		
Controls					
Observed	118	69	6	1.18	0.28
Expected	120.5	64	8.5		

If $P < 0.05$ - not consistent with HWE.

Table S3. Associations with malaria severity – multiple imputation (n=534)[†]

Characteristic	Mild malaria	Severe malaria	Odds ratio (95% CI)
PIEZO1 E756Del			
WT/WT	63%	71%	reference
WT/DEL	34%	23%	0.54** (0.35, 0.84)
DEL/DEL	3%	6%	2.11 (0.82, 5.42)
Hemoglobin			
AA	80%	95%	reference
AS	20%	5%	0.22*** (0.11, 0.42)
Age	40 (4, 140)	28 (4, 133)	0.98*** (0.97, 0.99)
Male	48%	44%	0.69 (0.44, 1.09)

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Model also adjusted for study in which the data was collected.
[†]Multiple imputation resulted in 5 imputed data sets, thus only percentages by malaria status are shown for the pooled data.

Table S4. Associations with malaria severity including hemoglobin type: multiple imputation (n=534) †

Characteristic	Odds ratio (95% CI)
PIEZO1 E756Del (ref=WT/WT)	
WT/DEL	0.51** (0.33, 0.80)
DEL/DEL	1.38 (0.53, 3.59)
Hemoglobin AS (ref=AA)	0.15*** (0.07, 0.34)
PIEZO1 and Hemoglobin interaction	
WT/DEL * AS	2.26 (0.57, 8.92)
DEL/DEL * AS	19.73 (0.82, 476.59)
Age	0.98*** (0.97, 0.99)
Male	0.70 (0.45, 1.10)

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Bayesian logistic model also adjusted for study in which the data was collected. Interaction $p = 0.11$.
†Multiple imputation resulted in 5 imputed data sets, thus only percentages by malaria status are shown for the pooled data.

Table S5. Associations with malaria severity among other PIEZO1 variants (n=532[1])

PIEZO1 variant	Malaria status		Odds ratio (95% CI)
	Mild (n=252)	Severe (n=280)	
E756EE			
WT/WT	248 (98.4%)	277 (98.9%)	1.49 (0.33, 7.62)
WT/DEL	4 (1.6%)	3 (1.1%)	reference
E755-E756Del[2]			
WT/WT	252 (100%)	274 (97.9%)	0.07 (0.00, 1.23)
WT/DEL	0	6 (2.1%)	reference
E750Q			
WT/WT	236 (93.7%)	264 (94.3%)	1.12 (0.54, 2.30)
WT/DEL	16 (6.3%)	16 (5.7%)	reference
Q749-E750Del[2]			
WT/WT	252 (100%)	279 (99.6%)	0.32 (0.01, 8.07)
WT/DEL	0	1 (0.4%)	reference
Q749Del			
WT/WT	240 (95.2%)	255 (91.1%)	0.51 (0.24, 1.02)
WT/DEL	12 (4.8%)	22 (7.9%)	reference
DEL/DEL	0	3 (1.1%)	
Q749E			
WT/WT	251 (99.6%)	275 (98.2%)	0.22 (0.01, 1.37)
WT/DEL	1 (0.4%)	5 (1.8%)	reference
E739Q[2]			
WT/WT	250 (99.2%)	280 (100%)	6.19 (0.31, 124.48)
WT/DEL	2 (0.8%)	0	reference
At least 1 non-WT			
WT/WT	217 (86.1%)	227 (81.1%)	1.45 (0.91, 2.32)
WT/DEL	35 (13.9%)	53 (18.9%)	reference

[1] n=10 excluded due to missing values.
[2] Due to estimation issues, fit using Bayesian logistic regression.

Table S6. Prevalence of *PIEZO1* E756del genotypes among different populations from the 1000 Genomes Project Phase 3* (31)

Population	Genotype: frequency		
	Ref_homozygous	Alt_heterozygous	Alt_homozygous
AFRICAN	WT/WT: 0.660	WT/DEL: 0.286	DEL/DEL: 0.035
ACB	WT/WT: 0.677	WT/DEL: 0.281	DEL/DEL: 0.042
ASW	WT/WT: 0.721	WT/DEL: 0.213	DEL/DEL: 0.033
ESN	WT/WT: 0.596	WT/DEL: 0.343	DEL/DEL: 0.040
GWD	WT/WT: 0.566	WT/DEL: 0.345	DEL/DEL: 0.053
LWK	WT/WT: 0.798	WT/DEL: 0.172	
MSL	WT/WT: 0.647	WT/DEL: 0.306	DEL/DEL: 0.035
YRI	WT/WT: 0.648	WT/DEL: 0.306	DEL/DEL: 0.037
AMRERICAN	WT/WT: 0.960	WT/DEL: 0.037	
CLM	WT/WT: 0.926	WT/DEL: 0.064	
MXL	WT/WT: 1.000		
PEL	WT/WT: 1.000		
PUR	WT/WT: 0.933	WT/DEL: 0.067	
EAST ASIAN	WT/WT: 0.998	WT/DEL: 0.002	
CDX	WT/WT: 0.989	WT/DEL: 0.011	
CHB	WT/WT: 1.000		
CHS	WT/WT: 1.000		
JPT	WT/WT: 1.000		
KHV	WT/WT: 1.000		
EUROPEAN	WT/WT: 0.990	WT/DEL: 0.002	
CEU	WT/WT: 1.000		
FIN	WT/WT: 0.990	WT/DEL: 0.010	
GBR	WT/WT: 1.000		
IBS	WT/WT: 1.000		
TSI	WT/WT: 0.963		
SOUTH ASIAN	WT/WT: 0.982	WT/DEL: 0.010	
BEB	WT/WT: 0.953	WT/DEL: 0.023	
GIH	WT/WT: 0.990	WT/DEL: 0.010	
ITU	WT/WT: 1.000		
PJL	WT/WT: 0.979	WT/DEL: 0.010	
STU	WT/WT: 0.980	WT/DEL: 0.010	

*Ensembl GRCh37 release 99. ACB: African Caribbeans in Barbados; ASW: Americans of African Ancestry in Southwest USA; ESN: Esan in Nigeria; GWD: Gambian in Western Divisions in the Gambia; LWK: Luhya in Webuye, Kenya; MSL: Mende in Sierra Leone; YRI: Yoruba in Ibadan, Nigeria; CLM: Colombians from Medellin, Colombia; MXL: Mexican Ancestry from Los Angeles USA; PEL: Peruvians from Lima, Peru; PUR: Puerto Ricans from Puerto Rico; CDX: Chinese Dai in Xishuangbanna, China; CHB: Han Chinese in Beijing, China; CHS: Southern Han Chinese; JPT: Japanese in Tokyo, Japan; KHV: Kinh in Ho Chi Minh City, Vietnam; CEU: Utah Residents with Northern and Western European Ancestry; FIN: Finnish in Finland; GBR: British in England and Scotland; IBS: Iberian Population in Spain; TSI: Toscani in Italia; BEB: Bengali from Bangladesh; GIH: Gujarati Indian from Houston, Texas; ITU: Indian Telugu from the UK; PJL: Punjabi from Lahore, Pakistan; SRI: Sri Lankan Tamil from the UK. Ref: Reference; Alt: Alternate.

Table S7. Demographic characteristics of ethnicity subset versus full cohort

Characteristic	Ethnicity Subset (n=182)	Full cohort (n=446)
Sex, n (%)		
Female	109 (60%)	245 (55%)
Male	73 (40%)	201 (45%)
Age, median [range]	40 [8-140]	35 [4-140]
PIEZO1 E756del variant, n (%)		
WT/WT	113 (62%)	298 (67%)
WT/DEL	55 (30%)	127 (28%)
DEL/DEL	14 (8%)	21 (5%)
Parasite density, median [range]	39,500 [20-1,200,000]	35,000 [20-1,544,880

Table S8. Associations with malaria severity for ethnicity subset (n=182) versus original model (n=446): main effects

Characteristic	**Including ethnic group** Odds ratio (95% CI)	**Original model†** Odds ratio (95% CI)
PIEZO1 E756del	*Global p-value=0.056*	*Global p-value=0.007*
WT/DEL	0.52 (0.26, 1.02)	0.50** (0.31, 0.81)
DEL/DEL	2.62 (0.76, 10.84)	2.26 (0.82, 6.94)
Hemoglobin type AS	0.35* (0.14, 0.86)	0.27*** (0.13, 0.52)
Age	1.00 (0.98, 1.01)	0.98*** (0.97, 0.99)
Male	0.95 (0.50, 1.80)	0.70 (0.45, 1.08)
Primary Gabonese ethnic group	*Global p-value=0.11*	
Fang	0.89 (0.39, 2.01)	
Omyene	0.49 (0.14, 1.53)	
Other	1.73 (0.81, 3.75)	

† additionally included study in model; *p<0.05; **p<0.01; ***p<0.001

Table S9. Associations with malaria severity for ethnicity subset (n=182) versus original model (n=446): interaction model

Characteristic	**Including ethnic group** Odds ratio (95% CI)	**Original model†** Odds ratio (95% CI)
PIEZO1 E756del	*Global p-value=0.057*	*Global p-value=0.007*
WT/DEL	0.40* (0.19, 0.83)	0.46** (0.28, 0.75)
DEL/DEL	1.18 (0.30, 5.29)	1.34 (0.45, 4.35)
Hemoglobin type AS	0.10** (0.01, 0.40)	0.16*** (0.06, 0.38)
PIEZO1*Hemoglobin	*Global p-value=0.013*	*Global p-value=0.047*
WT/DEL:AS	8.29* (1.07, 83.03)	2.74 (0.53, 13.02)
DEL/DEL:AS	Inf (0, NA)	Inf (0, NA)
Age	1.00 (0.99, 1.01)	0.98*** (0.97, 0.99)
Male	0.94 (0.49, 1.81)	0.70 (0.45, 1.10)
Primary Gabonese ethnic group	*Global p-value: 0.11*	
Fang	0.85 (0.37, 1.96)	
Omyene	0.59 (0.17, 1.90)	
Other	1.79 (0.82, 4.00)	

† additionally included study in model; *p<0.05; **p<0.01; ***p<0.001

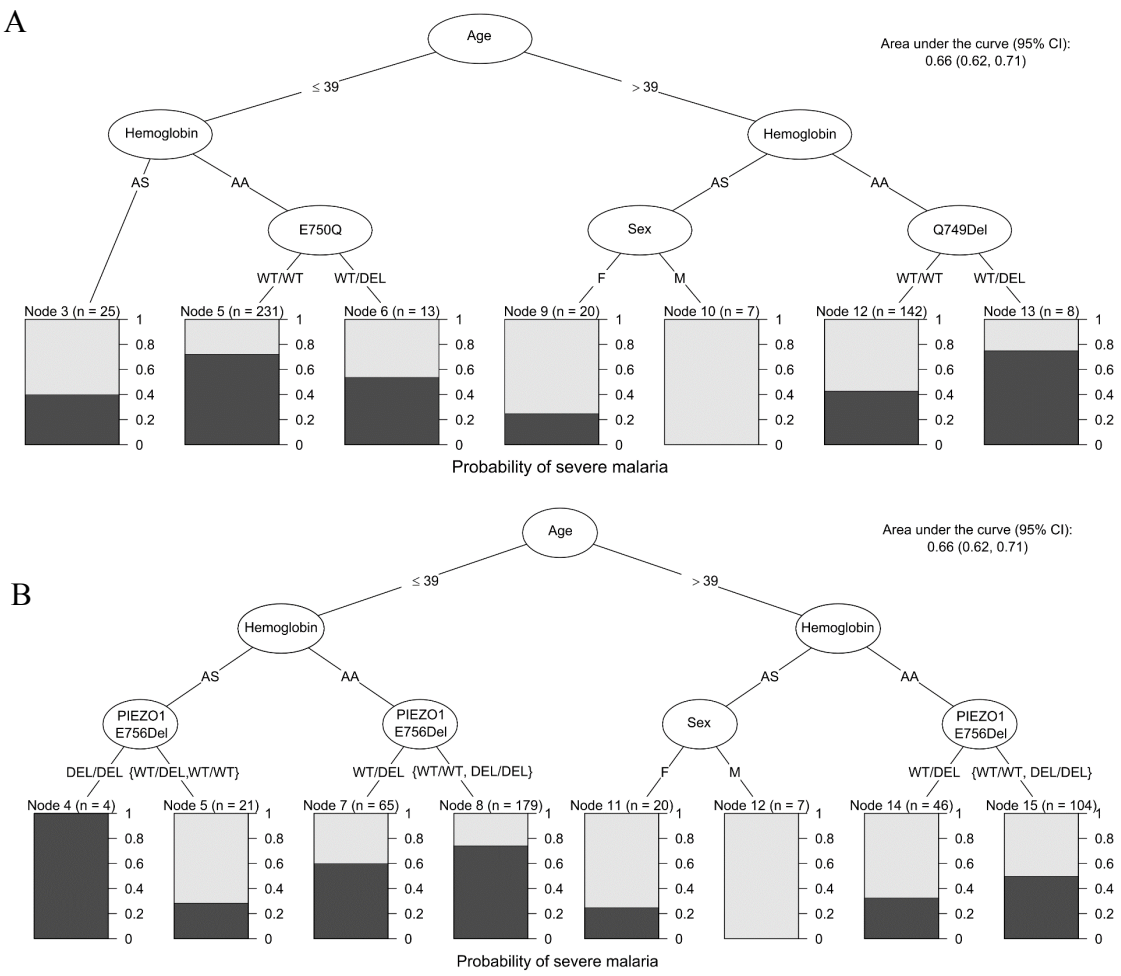

Figure S1. Classification trees predicting severe malaria with various possible predictors included. (A) Possible predictors: all PIEZO1 variants except for E756del, hemoglobin type, sex, and age. (B) Possible predictors: all PIEZO1 variants, hemoglobin type, sex, and age. The higher the black bars, the higher the likelihood of severe malaria.

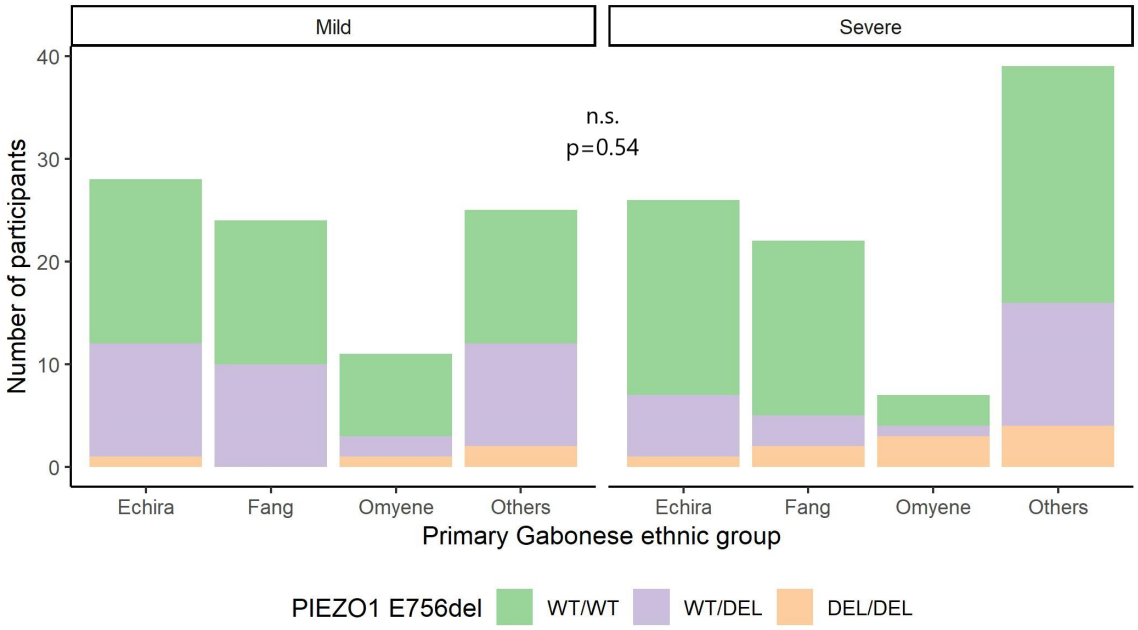

Figure S2. Bar plots depicting the distribution of *PIEZO1* E756del within each primary Gabonese ethnic group category by malaria status (mild versus severe). A logistic model for malaria severity as a function of E756del, ethnic group, and their interaction was fit; ns= not significant, p=0.54.

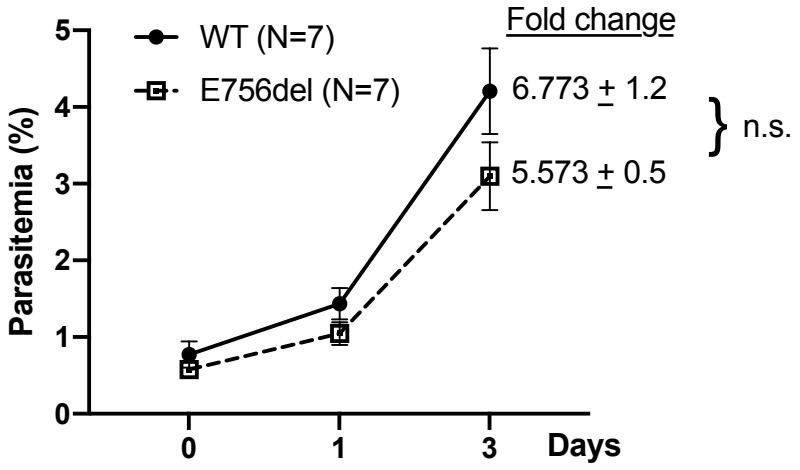

Figure S3. Growth of *P. falciparum* strain 3D7 growth in previously cryopreserved erythrocytes from wild-type or PIEZO1 E756del donors. Each point represents mean raw parasitemia of N=7 donors for each genetic background, and error bars represent SEM. Each sample was run with two technical replicates. "Fold change" indicates the average fold difference (±SEM) in parasitemia on day 3 relative to day 0. n.s., not significant, p-value=0.3725. SEM=Standard error of mean.

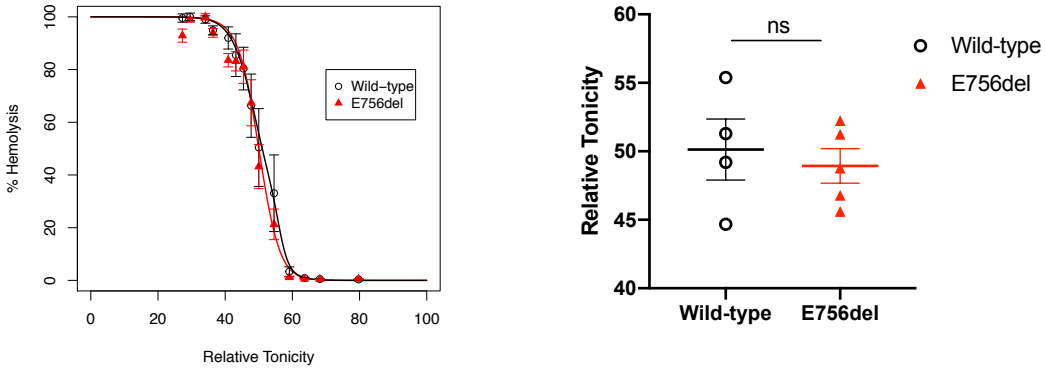

Figure S4. Osmotic fragility assays using samples stored overnight at 4°C. (A) RBCs from wild-type donors (N=4) had similar osmotic fragility curves to those from donors with the PIEZO1 E756del mutation (N=5). (B) Quantification of osmotic fragility based on relative tonicity at 50% hemolysis. ns= not significant, p=0.635.