A gain of function variant in PIEZO1 (E756del) and sickle cell disease

The novel PIEZO1 E756del variant was recently identified in about 30% of Africans, and associated with significant red cell dehydration and protection against malaria.[1] Erythrocytes from people with the E756del variant were found to have reduced infection by *Plasmodium falciparum* in *in vitro* studies, corresponding with equivalent findings in a mouse model.[1] As such, it might be expected to be an important determinant of severity in sickle cell disease (SCD). We have studied the prevalence and significance of this PIEZO1 variant in 788 patients with SCD. Surprisingly, we found that it had little or no influence on red cell phenotype.

All the complications of SCD arise from the polymerization of hemoglobin molecules incorporating a mutated β globin chain (HbS), βS (HBB, c.A20T, p.Glu7Val).[2] The rate of HbS polymerization is very dependent on the intracellular concentration of HbS, with the lag time before the onset of polymerization increasing with the concentration of HbS raised to the power of 30.[3] Small differences in red cell hydration, causing changes in HbS concentration therefore have a marked effect on the rate of HbS polymerization and consequent hematologic and clinical complications.[4]

In SCD, red cell cation loss and dehydration is thought to occur through three major transport pathways: K-Cl cotransport (KCC), the Gardos channel, and P_{sickle}.[4] The interaction of these channels is complicated but results in a net loss of cations from the red cell, with subsequent loss of water, and increased hemoglobin concentration.[5] Whereas the molecular basis of the Gardos channel (KCNN4) and KCC (KCC1, KCC3, KCC4) are known, P_{sickle} is a pathophysiological entity, whose molecular basis is unknown.[6]

P_{sickle} is a channel present only in sickle cell erythrocytes, which is activated by deoxygenation. It is non-selective, allowing the passage of ions and other small molecules; in particular, it is responsible for the entry of calcium into the deoxygenated sickle erythrocytes which causes the dehydration cascade.[7] Various proteins have been proposed as the basis for P_{sickle}, although recently it has been suggested that PIEZO1 is the likely origin of the P_{sickle} channel.[4,6]

PIEZO1 codes for a mechanically activated ion channel, and is widely expressed throughout the body, including in the red cell membrane. It is a large protein with 36 transmembrane domains.[8] Gain of function variants in PIEZO1 have been shown to cause dehydrated hereditary stomatocytosis,[9] whereas loss of function variants cause congenital lymphedema.[10] The properties of PIEZO1 also fit well with what is known about P_{sickle} including that it is a mechanosensitive channel and a non-specific cation conductance pathway.[6]

A recent study identified a gain of function variant in PIEZO1 (E756del), which is associated with malaria resistance and increased red cell dehydration, and which was present in about 30% of Africans.[1] It might be expected that this polymorphic variant would be an important determinant of severity in SCD. We have, therefore, investigated the hypothesis that the E756del PIEZO1 allele causes increased red cell dehydration in SCD, and is, therefore, associated with a more severe form of the disease, including increased anemia and hemolysis.

Adults and children with either sickle cell anemia (HbSS) or HbSC disease were recruited from specialist clinics at King's College Hospital. Data used in this study came from patients in two separate studies: a study of cation transport in SCD, and a study of genetic determinants of severity in SCD. Many patients were recruited into both studies allowing the data to be combined. The studies were approved by the UK National Research Ethics Committee (11/LO/0065, 13/NW/0141, 07/H0606/165, 12/LO/1610), and all patients gave written consent in accordance with the 1975 Declaration of Helsinki, as revised in 2008.

Blood samples for DNA extraction and cation transport measurements were collected when patients attended routine clinic appointments. Clinical and laboratory data were averaged from steady-state measurements recorded in the electronic patient record over an approximately 10-year period (2004-2013).[11,12] Mean hospitalization rates were calculated over ten years (2004-2013), dividing an individual's number of hematology admissions by the number of observed years. This was used as a surro-

Table 1. Hematologic parameters compared across different Piezo1 genotypes in patients with sickle cell anemia. *P* values from ANOVA, adjusted for age and sex.

	E756del/ E756del (7/7) n=46		E756del/WT (7/8) n=161		WT/WT (8/8) n=405		*P*
	n	mean	n	mean	n	mean	
Hb (g/L)	22	83.6	61	83.3	146	86.5	0.293
MCHC (g/L)	22	337	61	335	146	333	0.593
Retics (%)	13	13.9	36	11.7	83	12.1	0.304
Neutrophils ($x10^9$/L)	22	5.4	61	4.8	143	5.1	0.448
Platelets ($x10^9$/L)	22	387	61	401	146	396	0.917
LDH	21	434	61	454	138	423	0.493
HbF%	12	7.0	30	6.3	71	7.0	0.580
P_{sickle}	8	2.0	25	1.6	50	1.7	0.191
Admissions per year	23	0.49	66	0.80	156	0.71	0.517

P-values from ANOVA, adjusted for age and sex. WT: wild type; Hb: hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Retics: reticulocytes; LDH: lactate dehydrogenase; HbF: fetal hemoglobin; n: number. P_{sickle} units: mmol K^+ (l cells.h)$^{-1}$.

Table 2. Hematologic parameters compared across different Piezo1 genotypes in patients with sickle cell anemia.

	E756del/E756del (7/7) n=11		E756del/WT (7/8) n=65		WT/WT (8/8) n=120		*P*
	n	mean	n	mean	n	mean	
Hb (g/L)	9	114	45	116	78	116	0.917
MCHC (g/L)	9	345	45	345	78	350	0.109
Retics (%)	6	5.3	34	4.6	48	4.7	0.676
Neutrophils (x10^9/L)	9	4.5	45	4.1	78	3.9	0.525
Platelets (x10^9/L)	9	229	45	265	78	237	0.297
LDH	9	274	42	248	73	244	0.428
HbF %	4	3.1	27	2.1	35	1.5	0.146
P_{sickle}	5	0.96	23	1.2	43	1.0	0.69
Admissions per year	9	0.59	40	0.26	72	0.32	0.149

P-values from ANOVA, adjusted for age and sex. WT: wild type; Hb: hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Retics: reticulocytes; LDH: lactate dehydrogenase; HbF: fetal hemoglobin; n: number. P_{sickle} units: mmol K^+ (l cells.h)$^{-1}$.

gate marker of pain frequency. Activity of P_{sickle} was defined as the deoxygenation-induced Cl^--insensitive K^+ transport in the continued presence of clotrimazole and was measured according to published protocols.[13]

DNA samples from 788 patients with SCD of African and African-Caribbean origin were tested for the presence of the E756del polymorphism, associated with increased red cell dehydration. Adequate data on the rate of hospital admissions was available for a subset of 366 adult patients. A 204bp amplicon was generated encompassing rs572934641 (-/TCC) on Chromosome 16 (UCSC Dec2013 Hg38 assembly) using published primer sequences.[1] The Reverse primer was modified with FAM fluorescent dye. Denatured PCR product was fragment sized by capillary electrophoresis on an ABI Prism 3130 Automated Sequencer (Applied Biosystems, Foster City, CA, USA) with a Genescan 500 ROX size standard (Applied Biosystems). Allele calling was performed on resulting traces in Genemarker v.1.95 (Soft Genetics, State College, PA, USA). ANOVA (IBM SPSS 24, New York, NY, USA) was used to compare the different clinical, laboratory and cation transport measurements across the different PIEZO1 E756 genotypes.

The E756 deletion occurs within a sequence of triplet repeats, with eight triplet repeats occurring in the wild type, and seven with E756del. The overall allele frequency of E756del was 20.1% in our sample of 788 patients, although phenotypic information was not available for all patients. In addition, we found small numbers of people with five, six or nine repeats, although we had no reliable phenotypic information on these.

We compared hematologic parameters in wild-type patients (8/8 repeats), E756del heterozygotes (7/8 repeats) and E756del homozygotes (7/7 repeats) in patients with HbSS (Table 1) and HbSC (Table 2). Where necessary, the laboratory parameters were logarithmically transformed to achieve a normal distribution. In one-way ANOVA, none of these parameters showed any trends associated with the presence of the PIEZO1 E756 deletion in either HbSS or HbSC disease, including the frequency of admission to hospital. In addition, P_{sickle}, a direct measurement of red cell cation leak possibly related to PIEZO1 function,[6] showed no association with the E756del variant.

Our data confirm that E756del is common in people of African origin, with an allele frequency of 20%, broadly similar to the heterozygote frequency of 36% (9 out of 25 individuals) reported by Ma *et al.*[1] As anticipated, we also identified a significant number of patients who were homozygous for the deletion. Based on Ma *et al.*'s paper, we were expecting to find that those patients with E756del showed evidence of increased red cell dehydration (higher MCHC, increased P_{sickle} activity) with faster HbS polymerization causing more anemia and hemolysis. This was evidently not the case in either HbSS or HbSC disease. In addition, we found more than 20 patients with HbSS who were homozygous for E756del (7/7 repeats), and hematologically identical to patients with the wild-type genotype (8/8 repeats), confirming that this PIEZO1 allele does not significantly affect red cell cation transport in SCD, and is not an important determinant of severity in SCD.

The lack of effect of E756del in SCD may suggest that this PIEZO1 deletion has a relatively small effect on cation permeability,[1] although surprisingly Ma showed marked changes in osmotic fragility and red cell morphology associated with this polymorphism. In particular, the increased cation loss caused by E756del may be insignificant in the face of the much larger cation fluxes present in abnormal sickle red cells.[13] Our study shows that the E756del is not linked to P_{sickle} activity (oxygen sensitive, non-selective ion and small molecule permeability pathway)[14] but does not necessarily disprove the hypothesis that the PIEZO1 protein mediates some or all of the physiological activity measured as P_{sickle}. Our results are broadly similar to a recent study showing that the E756del allele is not associated with clinical complications (leg ulcers, priapism) or markers of hemolysis, although this study did show an association with increased red cell density measured using phthalate density distribution.[15]

In summary, the PIEZO1 E756del variant is present in about 20% of patients with SCD of African origin, and although it may have subtle effects on red cell hydration, it is not an important determinant of laboratory or clinical parameters in SCA or HbSC disease, and is not associated with changes in P_{sickle} activity.

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References

1. Ma S, Cahalan S, LaMonte G, et al. Common PIEZO1 Allele in African Populations Causes RBC Dehydration and Attenuates Plasmodium Infection. Cell. 2018;173(2):443-455.e12.
2. Perutz MF, Mitchison JM. State of haemoglobin in sickle-cell anaemia. Nature. 1950;166(4225):677-679.
3. Hofrichter J, Ross PD, Eaton WA. Kinetics and mechanism of deoxyhemoglobin S gelation: a new approach to understanding sickle cell disease. Proc Natl Acad Sci U S A. 1974;71(12):4864-4868.
4. Brugnara C. Sickle cell dehydration: Pathophysiology and therapeutic applications. Clin Hemorheol Microcirc. 2018;68(2-3):187-204.
5. Gibson JS, Ellory JC. Membrane transport in sickle cell disease. Blood Cells Mol Dis. 2002;28(3):303-314.
6. Gallagher PG. Disorders of erythrocyte hydration. Blood. 2017;130(25):2699-2708.
7. Ranney HM. Psickle, the temporary leaky link between sickling and cellular dehydration. J Clin Invest. 1997;99(11):2559-2560.
8. Coste B, Mathur J, Schmidt M, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science. 2010;330(6000):55-60.
9. Zarychanski R, Schulz VP, Houston BL, et al. Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis. Blood. 2012;120(9):1908-1915.
10. Fotiou E, Martin-Almedina S, Simpson MA, et al. Novel mutations in PIEZO1 cause an autosomal recessive generalized lymphatic dysplasia with non-immune hydrops fetalis. Nat Commun. 2015;6:8085.
11. Rees DC, Thein SL, Osei A, et al. The clinical significance of K-Cl cotransport activity in red cells of patients with HbSC disease. Haematologica. 2015;100(5):595-600.
12. Gardner K, Fulford T, Silver N, et al. g(HbF): a genetic model of fetal hemoglobin in sickle cell disease. Blood Adv. 2018;2(3):235-239.
13. Hannemann A, Rees DC, Tewari S, Gibson JS. Cation Homeostasis in Red Cells From Patients With Sickle Cell Disease Heterologous for HbS and HbC (HbSC Genotype). EBioMedicine. 2015;2(11):1669-1676.
14. Milligan C, Rees DC, Ellory JC, et al. A non-electrolyte haemolysis assay for diagnosis and prognosis of sickle cell disease. J Physiol. 2013;591(Pt 6):1463-1474.
15. Ilboudo Y, Bartolucci P, Garrett ME, et al. A common functional PIEZO1 deletion allele associates with red blood cell density in sickle cell disease patients. Am J Hematol. 2018 Aug 18. [Epub ahead of print].