Novel mutations in *SPTA1* and *SPTB* identified by whole exome sequencing in eight Thai families with hereditary pyropoikilocytosis presenting with severe fetal and neonatal anaemia

Hereditary pyropoikilocytosis (HPP) is a severe congenital red blood cell (RBC) membrane disorder, characterized by marked RBC fragmentation, poikilocytes and microspherocytes (Gallagher, 2004; Da Costa *et al*, 2013). The condition has an autosomal recessive inheritance. Patients with HPP have biallelic mutations in genes encoding cytoskeletal proteins α-spectrin (*SPTA1*), β-spectrin (*SPTB*) or protein 4.1R (*EPB41*). The parents, with a monoallelic mutation in one of the three genes, typically have hereditary elliptocytosis (HE), a milder form of RBC membrane disorder. The spectrins and protein 4.1R form the horizontal protein cytoskeleton of the RBC membrane by the αβ spectrin heterodimer association and the spectrin-actin-protein 4.1R junctional complex (Gallagher, 2004; Da Costa *et al*, 2013). Mutant proteins cause a weakened mechanical stability of the protein connections and result in abnormal RBC morphology and haemolysis.

Hereditary elliptocytosis is common worldwide with a higher prevalence in malaria endemic areas, including Southeast Asia, and similar to thalassaemia. However, there is little information on the clinical and molecular characterization in the region. The large underlying genes pose a major limitation for identification of the causative mutations.

Herein we report the clinical, haematological and molecular characteristics of HPP in eight unrelated Thai patients who presented with severe fetal or neonatal anaemia at Chiang Mai University Hospital in Northern Thailand (Table I). The probands and their parents were subjected to trio whole exome sequencing (WES), the detailed methods for this can be found in Appendix S1. WES identified mutations in all eight patients, with seven having mutations in *SPTB* and one in *SPTA1*. It successfully found two mutant alleles in seven patients, while only one mutant allele was found in Patient 4. The 15 identified mutant alleles comprise five different mutations in seven alleles of spectrin Providence (*SPTB* c.6055T>C, p.S2019P), four spectrin Chiang Mai (*SPTB* c.6224A>G, p.E2075G), two spectrin Om Koi (*SPTA1* c.5476C>T, p.Q1826X), one spectrin Suan Dok (*SPTB* c.1041C>A, p.Y347X) and one spectrin Buffalo (*SPTB* c.6074T>G, p.L2025R). The two most common mutations, Spectrins Providence and Chiang Mai comprise 68·8% (11/16) of the mutations. The co-inherited α-LELY and thalassaemia are also shown in Table I. The details of the *SPTB* and *SPTA1* mutations detected in this study, the zygozity status and WES read depths of the reference and alternative alleles are summarized in Tables SI and SII.

Notably, three novel mutations were identified. The novel mutation in *SPTA1*, spectrin Om Koi (c.5476C>T, p.Q1826X), found in Patient 8 was predicted to result in a premature stop codon leading to protein truncation. The first novel *SPTB* mutation, spectrin Suan Dok (*SPTB* c.1041C>A, p.Y347X), seen in Patient 2, was expected to be paternally-inherited, but could not be confirmed because the paternal blood sample was not available. The c.1041C>A was predicted to result in a premature stop codon at residue 347 from the total of 2308 amino acids. These two mutations were not found in our WES database of 1064 Thai individuals.

The second novel *SPTB* mutation, spectrin Chiang Mai (c.6224A>G, p.E2075G) was identified in four of eight families. A comparative analysis of amino acids showed that the amino acid is conserved among species (Fig 1). From our Thai population WES database, the allele was found in six from 1064 unrelated individuals, suggesting a carrier frequency of 0·56%.

Two previously reported *SPTB* mutations, spectrin Providence (*SPTB* c.6055T>C, p.S2019P) and spectrin Buffalo (*SPTB* c.6074T>G, p.L2025R) associated with hydrops fetalis in Laotian families were identified (Gallagher *et al*, 1995, 1997). Spectrin Providence was found in five of our eight patients. Remarkably, it was found in the heterozygous state in 16 of 1064 unrelated individuals of our in-house WES database, indicating a carrier frequency of 1·5%. Spectrin Buffalo, identified in one of our patients, was found heterozygously in 3 of 1064 unrelated individuals, suggesting a carrier frequency of 0·28%. The previously reported *SPTB* and *SPTA1* mutations in the database for HE and HPP and the novel mutations identified from this study are summarized in Fig 1.

The α-LELY is a combination of three variants (c.5572C>G, c.6531-12C>T, c.6549-12G>A) on the *SPTA1* gene resulting in a low expression of spectrin. Heterozygous α-LELY is clinically insignificant but a compound heterozygosity with another *SPTA1* mutation may be associated with

doi: 10.1111/bjh.15559

Table I. Clinical, haematological and molecular characteristics of the patients and their families.

				Red blood cell parameters							
Family	Case	Clinical presentation	Age at presentation	Hb (g/l)	RBC ($\times 10^{12}$/l)	MCV (fl)	MCH (pg)	MCHC (g%)	RDW (%)	Thalassaemia genotype	WES genotype
1.	Patient (Female, 16 years)	Fetal anaemia and hydrops, transfusion-dependent, occasional transfusion after splenectomy at 7 years	25 weeks GA	60 (at 16 years)	2·10	86·7	28·6	33·0	33·9	α^0-thalassaemia (SEA del); HET	*SPTB* c.6055T>C; HOM
	Mother	Asymptomatic		120	4·40	85·0	27·3	32·1	16·0	Normal	*SPTB* c.6055T>C; HET
	Father	Asymptomatic		137	4·83	82·6	28·4	34·3	14·3	α^0-thalassaemia (SEA del); HET	*SPTB* c.6055T>C; HET
2.	Patient (Female, 9 years)	Fetal anaemia and hydrops, remained transfusion-dependent after splenectomy at 7 years	24 weeks GA	29	1·01	81	28·5	35·1	36·9	Normal	*SPTB* c.6055T>C; HET/*SPTB* c.1041C>A; HET
	Mother	Asymptomatic		118	5·19	67·1	22·5	33·4	N/A	α^0-thalassaemia (SEA del); HET	*SPTB* c.6055T>C; HET
	Father	Asymptomatic		145	4·65	87·0	30·6	35·2	N/A	Normal	N/A
3.	Patient (Male, 9 years)	Fetal anaemia and hydrops, remained transfusion-dependent after splenectomy at 7 years	24 weeks GA	28	1·01	82·0	31·4	38·4	27·9	Normal	*SPTB* c.6055T>C; HOM *SPTA1* α-LELY; HET
	Mother	Asymptomatic		138	4·38	87·7	31·6	36·0	12·0	Normal	*SPTB* c.6055T>C; HET
	Father	Asymptomatic		156	5·53	85	28	33	12	α^+-thalassaemia (3·7 kb del); HET	*SPTB* c.6055T>C; HET *SPTA1* α-LELY; HET
4.	Patient (Female, 7 years)	Severe anaemia from birth (Hb 83 g/l), transfusion-free after splenectomy at 5 years	37 weeks GA	116 (at 7 years)	4·73	71·7	24·5	34·2	25·1	α^+-thalassaemia (3·7 kb del); HET	*SPTB* c.6224A>G; HET/allele N/D
	Mother	Asymptomatic		143	4·75	89·3	30·1	33·7	13·3	Normal	*SPTB* c.6224A>G; HET *SPTA1* α-LELY; HET
	Father	Asymptomatic		136	6·00	68·2	22·7	33·3	18·1	α^+-thalassaemia (3·7 kb del); HOM	allele N/D
5.	Patient (Male, 4 years)	Severe anaemia from birth, transfusion dependent	37 weeks GA	71	2·06	97·7	34·6	35·4	N/A	Normal	*SPTB* c.6074T>G; HET/*SPTB* c.6224A>G; HET *SPTA1* α-LELY; HET
	Mother	Asymptomatic		108	4·10	82·0	26·4	32·3	12·0	Normal	*SPTB* c.6224A>G; HET *SPTA1* α-LELY; HET
	Father	Asymptomatic		153	5·21	87·6	29·3	33·5	14·6	Normal	*SPTB* c.6074T>G; HET

Table I. (*Continued*)

Family	Case	Clinical presentation	Age at presentation	Red blood cell parameters						Thalassaemia genotype	WES genotype
				Hb (g/l)	RBC ($\times 10^{12}$/l)	MCV (fl)	MCH (pg)	MCHC (g%)	RDW (%)		
6.	Patient (Male)	Fetal anaemia and hydrops, fetal death *in utero*	22 weeks GA	65	2·26	99·6	28·8	28·9	31·9	Normal	*SPTB* c.6055T>C; HET/ *SPTB* c.6224A>G; HET
	Mother	Asymptomatic Hypertriglyceridaemia		131	4·25	90·1	30·8	34·2	13·3	Normal	*SPTB* c.6055T>C; HET
	Father	Asymptomatic		150	5·57	79·2	26·9	34·0	12·6	Normal	*SPTB* c.6224A>G; HET
7.	Patient (Male, 1 year)	Fetal anaemia and hydrops, transfusion-dependent	30 weeks GA	36	1·37	84·7	26·3	31·0	35·0	Normal	*SPTB* c.6055T>C; HET/*SPTB* c.6224A>G; HET
	Mother	Asymptomatic		104	4·98	63·9	20·9	32·7	16·9	α^0-thalassaemia (SEA del); HET	*SPTB* c.6224A>G; HET
	Father	Asymptomatic		166	5·66	85·3	29·3	34·4	12·7	Normal	*SPTB* c.6055T>C; HET
8.	Patient (Male)	Fetal anaemia and hydrops, fetal death *in utero*	27 weeks GA	32	0·98	124·5	32·7	26·2	43·9	β-thalassaemia (*HBB* c.52A>T); HET	*SPTA1* c.5476C>T; HOM
	Mother	Asymptomatic Diabetes mellitus type I		91	5·00	56·8	18·2	32·0	17·9	β-thalassaemia (*HBB* c.52A>T); HET	*SPTA1* c.5476C>T; HET
	Father	Asymptomatic		142	4·73	88·8	30·0	33·8	12·6	Normal	*SPTA1* c.5476C>T; HET

GA, gestational age; HE, hereditary elliptocytosis; HET, heterozygous; HOM, homozygous; HPP, hereditary pyropoikilocytosis; IUT, intrauterine transfusion; N/D, not detected; SEA del, Southeast Asian deletion.

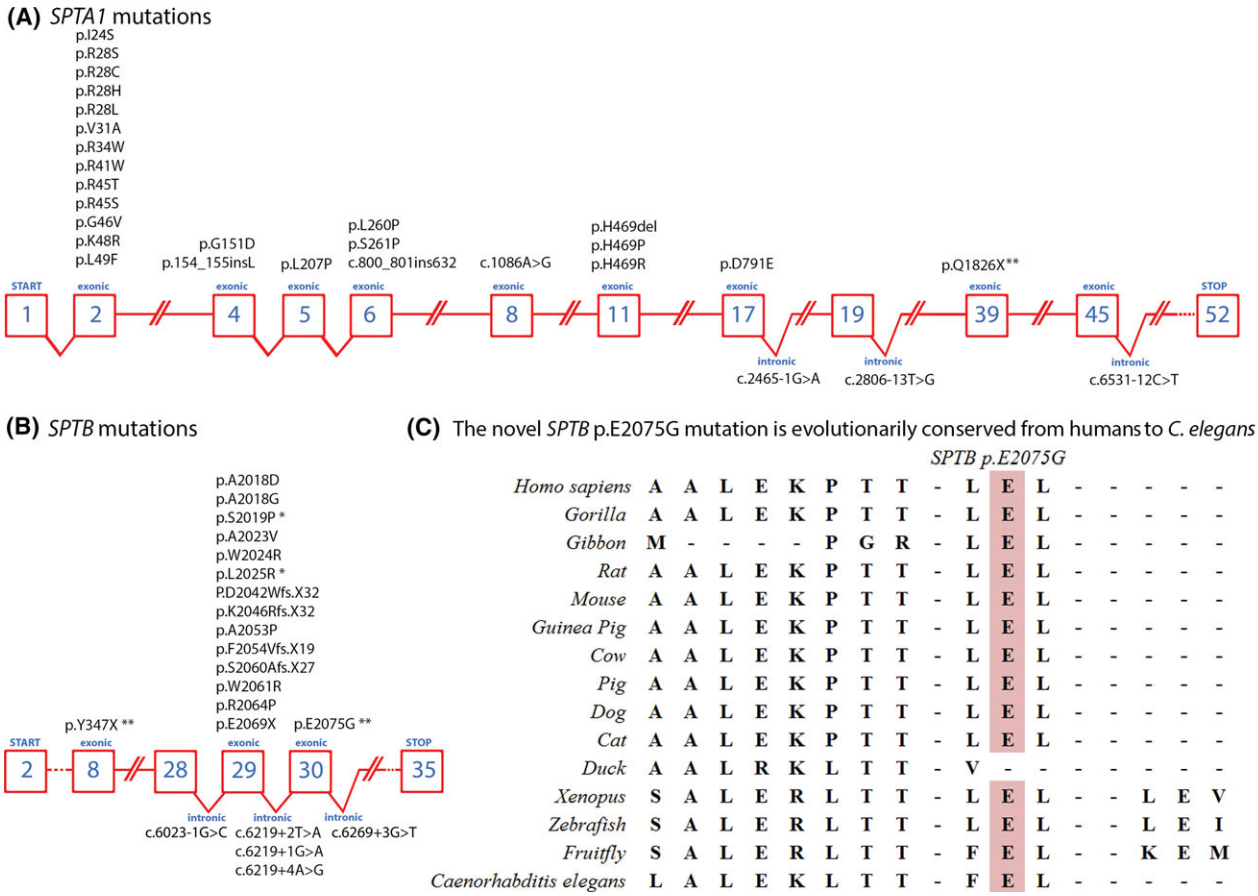

Fig 1. The *SPTB* (A) and *SPTA1* (B) mutations previously reported in the database for hereditary elliptocytosis and hereditary pyropoikilocytosis by the NHGRI Division of Intramural Research, National Human Genome Research Institute (https://research.nhgri.nih.gov/RBCmembrane/?mode=HE; accessed on 1 May 2018). Nomenclature is based on the reference sequences NM_003126.2 and NM_001024858.2 for *SPTA1* and *SPTB* respectively. Numbers shown within the boxes represent coding exons of each gene. Exons without reported mutations were skipped. The asterisk (*) and double asterisks (**) denote known and novel mutations identified in our patients. (C) Comparative analysis of conserved amino acid among species is shown for the novel spectrin Chiang Mai (*SPTB* p.E2075G mutation). [Colour figure can be viewed at wileyonlinelibrary.com]

haemolytic anaemia (Randon *et al*, 1994). The α-LELY is seen across ethnic groups, with a prevalence in Chinese of 22% (Marechal *et al*, 1995). In this study, the α-LELY was identified in three families. The co-inherited α-LELY did not seem to add to the clinical severity in the families with *SPTB* mutations. Similarly, co-inherited HE and β-thalassaemia has been reported to result in spectrin modification and enhanced haemolysis (Streichman *et al*, 1990). However, the effect of co-inherited α-LELY or thalassaemia in our patients could not be determined because the biallelic *STPA1* or *SPTB* mutations already result in severe anaemia.

In summary, the majority of Thai patients with HPP harbour mutations in *SPTB*. With a combined 2·34% prevalence of carriers of the three most common mutations, spectrins Providence (c.6055T>C), Chiang Mai (c.6224A>C) and Buffalo (c.6074T>G), the risk of HPP is as high as 1·4 in 10 000 pregnancies in this population. The information from this study expands the insights of molecular epidemiology of RBC membrane disorders in the Southeast Asian region.

Acknowledgements

The authors would like to thank the Thailand Research Fund (DPG6180001), the Chulalongkorn Academic Advancement Into Its 2nd Century Project, and The Newton Fund for the support for the study. The authors have no competing interests. PC and VS designed the study. RN, FT, LS, CC and PC enrolled the cases. CI, KS and VS performed the whole exome sequencing and data analysis. PC collected and analysed the data. PC, CI, KF and VS wrote the manuscript. All authors gave critical comments, revised and approved the final manuscript.

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Keywords: fetal anaemia, hereditary pyropoikilocytosis, hydrops fetalis, red blood cell membrane disorders, whole exome sequencing

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Whole exome sequencing method.

Table SI. Summary of the *SPTB* and *SPTA1* mutations detected in this study.

Table SII. Summary of the genotypes**,** zygozity status and WES read depths of the reference and alternative alleles.

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