Table 1. Comparison of LES PS21 and LES 9F in wild-type *Pseudomonas aeruginosa* isolated from CF patients and the reference strain LESB58 (GenBank Accession No: FM209186).

Target	Submitted GenBank Accession Number	Closest BLAST sequence match	Similarity	Position (in relation to FM209186)
LES PS21	FJ710791	*Pseudomonas aeruginosa* FM209186; PLES_26321	100%	np 2833095–2832811
LES 9F	FJ710792	*Pseudomonas aeruginosa* FM209186; PLES_23591	99%	np 2524896–2524524

demonstrated a single nucleotide deletion of an adenine base at position 2524879 (FM209186) of the LES 9F gene locus.

This small study demonstrates that LES PS21 and LES 9F are highly conserved in the wild-type CF *P. aeruginosa* isolates examined, even in the presence of several mutations in the pyoverdine gene locus. BLAST analysis of these sequences demonstrates the uniqueness of these sequences in nature, whereby only one match was obtained (GenBank accession number: FM209186). At this stage, the significance of this is unclear, but it is important to be able to note variation within these amplicons and possible variations in clinical disease states. Further analysis is now required of isolates from around the world, in order to examine potential geographical diversity. □

The authors wish to thank Dr. Craig Winstanley, University of Liverpool, for his advice and guidance in relation to studies into LES.

References

1 Rajan S, Saiman L. Pulmonary infections in patients with cystic fibrosis. *Sem Respir Infect* 2002; **17**: 47–56.

2 Winstanley C, Langille MG, Fothergill JL *et al*. Newly introduced genomic prophage islands are critical determinants of *in vivo* competitiveness in the Liverpool epidemic strain of *Pseudomonas aeruginosa. Genome Res* 2009; 1: 12–23.

3 Scott FW, Pitt TL. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* 2004; **53**: 609–15.

4 McCallum SJ, Gallagher MJ, Corkill JE *et al*. Spread of an epidemic *Pseudomonas aeruginosa* strain from a patient with cystic fibrosis (CF) to non-CF relatives. *Thora*x 2002; **57**: 559–60.

5 Al Aloul M, Crawley J, Winstanley C *et al*. Increased morbidity associated with chronic infection by an epidemic *Pseudomonas aeruginosa* strain in CF patients. *Thorax* 2004; **59**: 334–6.

6 Mohan K, Fothergill JL, Storrar J *et al*. Transmission of *Pseudomonas aeruginosa* epidemic strain from a patient with cystic fibrosis to a pet cat. *Thorax* 2008; **63**: 839–40.

7 Fothergill JL, Upton AL, Pitt TL *et al*. A multiplex PCR assay for the identification of the Liverpool, Midlands 1 and Manchester CF epidemic strains of *Pseudomonas aeruginosa. J Cyst Fibros*. 2008; **7**: 258–61.

8 de Chial M, Ghysels B, Beatson SA *et al*. Identification of type II and type III pyoverdine receptors from *Pseudomonas aeruginosa. Microbiology* 2003; **149**: 821–31.

9 Xu J, Smyth CL, Buchanan JA *et al.* Employment of 16 S rDNA gene sequencing techniques to identify culturable environmental eubacteria in a tertiary referral hospital. *J Hosp Infect* 2004; **57**: 52–8.

PRF1 gene mutation in a Saudi patient with haemophagocytic lymphohistiocytosis

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Haemophagocytic lymphohistiocytosis (HLH) is a rare autosomal inherited disease associated with activated macrophages that engulf erythrocytes, leucocytes, platelets and their precursor cells in bone marrow, lymph node, spleen and other tissues.[1,2] Patients with HLH manifest cellular immunological dysfunction of regulatory pathways that normally terminate in effector immune responses.[3]

Hereditary and sporadic cases of HLH have been reported mainly in children,[5] although the condition can affect other age groups.[6–9] The incidence of HLH is estimated to be 1.2 per 1,000,000.[4-6] Clinical manifestations include decreased fetal activity, neonatal hypotonia, neonatal feeding difficulties, hyperphagia with obesity, hypogonadism, short stature, small hands and feet, characteristic facial features, and mild to moderate mental retardation.

Haemophagocytic lymphohistiocytosis may be familial, or associated with a number of different infections, autoimmune disorders, or may occur together with malignancy.

The case present here is of an 18-month-old boy born at 34 weeks' gestation to a young couple (first cousins). Written informed consent was obtained from the parents of the patient for publication of this case report. The pregnancy suffered premature rupture of the membrane and neonatal polycythemia that required partial exchange of blood.

At the age of seven weeks the child was admitted to the paediatric intensive care unit with suspected septic shock syndrome. All microbiological tests were negative. Physical examination revealed hepatosplenomegaly and blood tests showed neutropenia (absolute neutrophil count: 400 cells/μL), thrombocytopenia (platelet count: 23,000/μL) and anaemia (haemoglobin [Hb]: 55 g/L). The patient recovered and his blood counts showed partial improvement.

At the age of three months he was admitted to the paediatric ward with fever and low blood counts that required frequent blood and platelet transfusion. Bone

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marrow aspirate showed no evidence of haemophagocytic cells, but showed erythropoietic hyperplasia with dysplastic changes in erythroblasts. No evidence of a metabolic storage disorder was seen. Cerebrospinal fluid (CSF) showed no sign of inflammation, malignancy or evidence of haemophagocytic cells.

Analysis of peripheral blood cells (BD FACSCalibur system)[1] revealed increased CD4 T-cell count (2156 cells/μL; normal range: 1000–1800), reduced CD8 count (280 cells/μL, normal range: 800–1500) and high CD4:CD8 ratio (7.7; normal range: 1.0–1.6). There was a decrease in B-cell and natural killer (NK) cell counts. Other laboratory findings included a high ferritin level (2730 μg/L; normal up to 330) and increased triglycerides (2.9 μg/L; normal up to 1.7).

The perforin (*PRF1*) gene was amplified from extracted genomic DNA using a previously described method,[2,3,5] and polymerase chain reaction (PCR) products were sequenced (ABI3130 DNA sequencer, PE-Applied Biosystems, Foster City, CA). *PRF1* sequence analysis revealed the presence of homozygous c.1349C>T (T450M). Analysis of the parents' *PRF1* gene showed the same heterozygous mutation, although there was no family history of HLH.

The patient was referred subsequently to the bone marrow transplant unit for stem cell transplantation and was successfully transplanted with unrelated donor umbilical cord blood stem cells.

The pathogenesis of HLH remains controversial; however, uncontrolled inflammation reflected by T-cell and macrophage activation remains the hallmark of HLH.[4] Genetic studies of familial HLH reveals a link between mutations in the perforin (*PRF1*; MIM170280), *MUNC13-4* and *STX11* genes. Mutations in all thee genes have been found in up to 50% of familial HLH families.

Lee *et al*. investigated *PRF1* gene mutations in a cohort of 50 HLH families using direct sequencing. Overall, *PRF1* gene mutations were found in at least 50% of the families, with the mutations occurring anywhere in the *PRF1* gene.[5] Stepp *et al*.[6] sequenced the *PRF1* gene in eight unrelated HLH patients and found four with homozygous non-sense mutations and four patients with missense mutations.

The present study reports a homozygous mutation of the *PRF1* gene (MIM 170280). This mutation occurs in the EGF-like domain of the *PRF1* gene (exon 2) and was reported previously in a one-month-old female with HLH. That patient showed reduced NK cell activity (5%), reduced cytotoxic lymphocyte activity (41%), and reduced PRF1 protein activity shown by Western blotting.[7]

To the authors' knowledge, this is the first report of a *PRF1* gene mutation in a Saudi HLH patient. Diagnosis will enlighten and increase the awareness of such rare genetic diseases, especially in cases of consanguineous marriage. □

The authors thank staff at King Abdullah International Medical Research Centre for their support.

References

1 Al Qouzi A, Al Salamah A, Al Rasheed R *et al*. Immunophenotyping of peripheral blood lymphocytes in Saudi men. *Clin Diagn Lab Immunol* 2002; **9** (2): 279–81.

2 Suga N, Takada H, Nomura A *et al*. Perforin defects of primary haemophagocytic lymphohistiocytosis in Japan. *Br J Haematol* 2002; **116** (2): 346–9.

3 Ueda I, Morimoto A, Inaba T *et al*. Characteristic perforin gene mutations of haemophagocytic lymphohistiocytosis patients in Japan. *Br J Haematol* 2003; **121** (3): 503–10.

4 Molleran Lee S, Villanueva J, Sumegi J *et al*. Characterisation of diverse PRF1 mutations leading to decreased natural killer cell activity in North American families with haemophagocytic lymphohistiocytosis. *J Med Genet* 2004; **41** (2): 137–44.

5 Stepp SE, Dufourcq-Lagelouse R, Le Deist F *et al*. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* 1999; **286** (5446): 1957–9.

6 Ishii E, Ueda I, Shirakawa R *et al*. Genetic subtypes of familial hemophagocytic lymphohistiocytosis: correlations with clinical features and cytotoxic T lymphocyte/natural killer cell functions. *Blood* 2005; **105** (9): 3442–8.

7 Imashuku S, Ueda I, Teramura T *et al*. Occurrence of haemophagocytic lymphohistiocytosis at less than 1 year of age: analysis of 96 patients. *Eur J Pediatr* 2005; **164** (5): 315–9.

8 Arico M, Danesino C, Pende D, Moretta L. Pathogenesis of haemophagocytic lymphohistiocytosis. *Br J Haematol* 2001; **114** (4): 761–9.

9 Zur Stadt U, Beutel K, Kolberg S *et al*. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11 and RAB27A. *Hum Mutat* 2006; **27**: 62–8.

Determination of optimum incubation time for release of bacteria from sputum of patients with cystic fibrosis using dithiothreitol (Sputasol)

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Cystic fibrosis (CF) is the most common inherited fatal disease in persons originating from a Caucasian and European background, and currently affects approximately 8000 individuals in the UK.[1] The defective gene carrying the mutation is carried by approximately one in every 25 people in the UK population. This means that more than two million people in the UK are symptomless carriers of the defective gene.[1]

Cystic fibrosis is an autosomal recessive condition whereby two alleles carrying a polymorphism in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene

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