tracts of up to 50% of healthy adults, now has the ability to become pathogenic to the host.

Recently, we have shown that long-term use of azithromycin in adult CF patients has led to a macrolide-resistant population of viridans group streptococci (VGS) isolated from patients' sputum, in comparison to similar VGS populations originating from non-CF patients not treated with azithromycin for long periods of time.[7] In addition, complete gene homology in the macrolide resistance determinants, particularly *erm*(B) and *mef*(A) is also shared with other closely related genera, including *Gemella, Enterococcus* and *Granulicatella*. Presence of multiple macrolide-resistance determinants occurring at high frequency in VGS commensal organisms is of potential importance to the CF patient, other CF patients and the non-CF population. The presence of bacterial pathogens in CF sputum generally reflects those bacterial genera commonly associated with CF lung disease, including *P. aeruginosa* and *B. cenocepacia*. Normally, these pathogens do not constitute an infection risk to the healthy non-CF individual. However, the presence of such macrolide-resistance determinants in VGS organisms may be problematic for the healthy non-CF individual, as these may act as a reservoir of resistance determinants for other respiratory pathogens, particularly *Streptococcus pneumoniae*, where these commensal flora are transmitted from the CF patient to non-CF individuals (e.g., between CF and non-CF siblings within a household). Furthermore, the existence of macrolide-resistance determinants has been described in environmental waters (GenBank accession number: EU168331), farm animals (GenBank accession number: EU168331) and domestic animals, highlighting the important ecological evolution and transmission of these genes globally.

Finally, the presence of highly resistant commensal organisms and their genetic resistant determinants is of potential interest to infection control in the hospital setting, particularly in relation to CF and non-CF patients (i.e., are CF patients an important reservoir of resistance determinants?) For instance, given the amount of β-lactams taken by CF patients during their lifetime, we do not known at present whether or not their intestinal flora react by becoming extended-spectrum β-lactamase (ESBL) producers. Therefore, more research is required urgently to assess what genetic mutations and resistance determinants are being selected naturally by the commensal flora of the CF patient and whether or not these mutations are important in terms of their transmissibility to hitherto sensitive pathogens.

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Hb Owari associated with α-thalassaemia-1 in a Taiwanese subject

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An abnormal haemoglobin, Hb Owari (*HBA2*:c,364 G>A; or HBA1) was first described in Japan by Hrano *et al*. in 1986.[1] It is a non-pathological α-chain variant characterised by a mutation at the α^{121} position that changes valine to methionine (121[H4]Val>Met). It produces a neutral-to-neutral amino acid substitution in the α-chain. The site of amino acid substitution (α121) can be determined by the chymotryptic digest fingerprinting of the core fraction of the α-chain, with the oxidised counterpart of the abnormal peptide (α118–22) easily found as an extra spot. The clinical presentation of heterozygous Hb Owari is normal, and the proportion of abnormal HbX is 12.7–19% of total haemoglobin. However, the compound heterozygote with other haemoglobinopathies had previously not been reported. This study presents a case of a compound heterozygote of Hb Owari with α-thalassaemia-1.

A 25-year-old Taiwanese visited the haematology out-patient department as microcytic anaemia was noted at regular check-up in June 2009. Peripheral blood examination showed a microcytic hypochromic anaemia, and the red cells showed mild microcytosis and hypochromasia. The haemogram was as follows: Hb 13.4 g/dL, RBC 5.81×10^6/μL, MCV 72.5 fL, MCH 23.1 pg and MCHC 31.9 g/dL. White blood cells and platelets were within the normal range. Serum iron and ferritin levels (92 μg/dL and 95.3 ng/mL, respectively) were within the normal range. High-performance liquid chromatography (HPLC; Primus CLC 385, Primus, Kansas City, USA) showed an abnormal HbX peak (36.2%) at a retention time of 4.76 min (Fig. 1).

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Electrophoresis of freshly prepared haemolysates in cellulose acetate at pH 8.6 showed no abnormal Hb band (Fig. 2A).

Direct DNA sequencing of the α- and β-globin gene was performed and the results demonstrated a homozygous mutation at *HBA1*:c.364G>A (Hb Owari) of the α1-globin gene (Fig. 2B). The *HBA2* and the *HBB* genes were normal. However, due to the low MCV, MCH and MCHC levels, the high proportion of HbX was not consistent with an Hb Owari heterozygote. The differential diagnosis was between homozygous Hb Owari and compound heterozygous Hb Owari with the deletion form of α-thalassaemia. Owing to the high prevalence of thalassaemia in Taiwan, a compound heterozygote of the α-chain variant with the deletion form of α-thalassaemia was considered. Subsequent analysis of the deletion form of α-thalassaemia using the gap-polymerase chain reaction (PCR) method[2] showed that the patient was a compound heterozygous Hb Owari with α-thalassaemia-1 south-east Asian (SEA) type (Fig. 2C).

Analysis of the α1- and α2-globin genes was performed, and DNA was isolated from white blood cells using standard methods. The α1-globin gene was amplified with primer P1 (forward primer, 5′ non-coding area): 5′-CTC TTC TGG TCC CCA CAG AC-3′ and P2 (reverse primer, 3′ non-coding area): 5′-CCA AGG GGC AAG AAG CAT GGC CA-3′, to amplify the entire coding region and two introns. The α2-globin gene was amplified with primers P1 and P3 (reverse primer, 3′ non-coding area): 5′-CAG GAA GGG CCG GTG CAA GGA G-3′, to amplify the entire coding region and two introns.

Briefly, the PCR conditions were as follows: amplification was performed in a 50 µL volume consisting of 200 ng genomic DNA, 10 µmol each primer (P1 + P2 or P1 + P3), 5% DMSO, 10 mmol each dNTP, 1×PCR buffer and one unit of *Thermus aquaticus* (*Taq*) polymerase (Perkin Elmer, Norwalk, USA). Cycling conditions were 5 min at 96°C (hot start), 15 cycles of 30 sec at 96°C (denaturation), 30 sec at 62°C (annealing), then 20 cycles of 30 sec at 96°C (denaturation), 30 sec at 58°C (annealing), 40 sec at 72°C (extension), and a

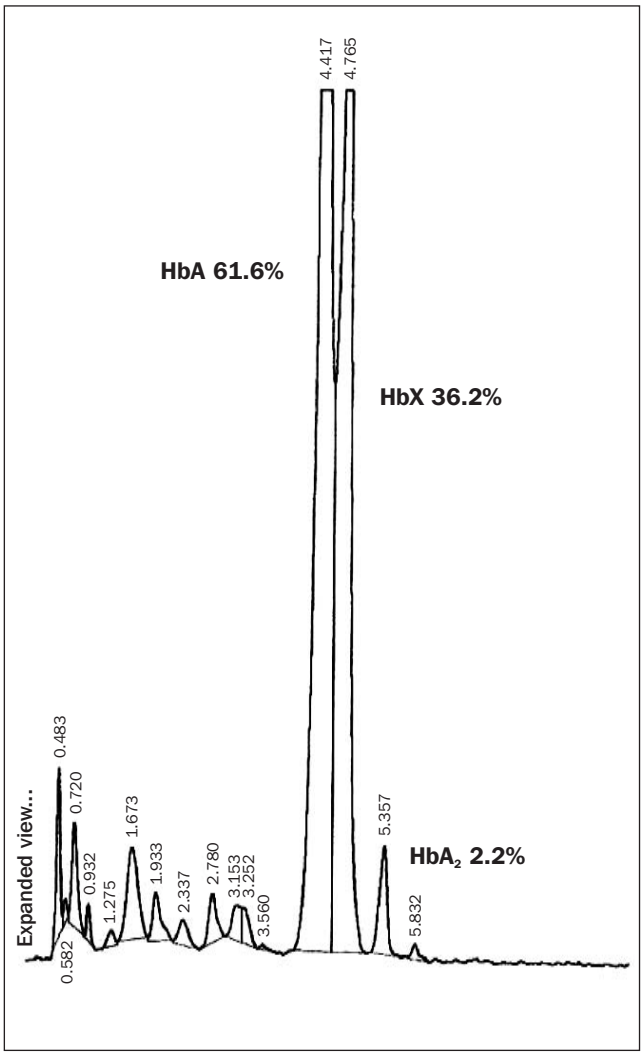

Fig. 1. An abnormal peak of HbX (36.2%) at a retention time of 4.76 min was found next to the HbA peak in the HPLC chromatogram.

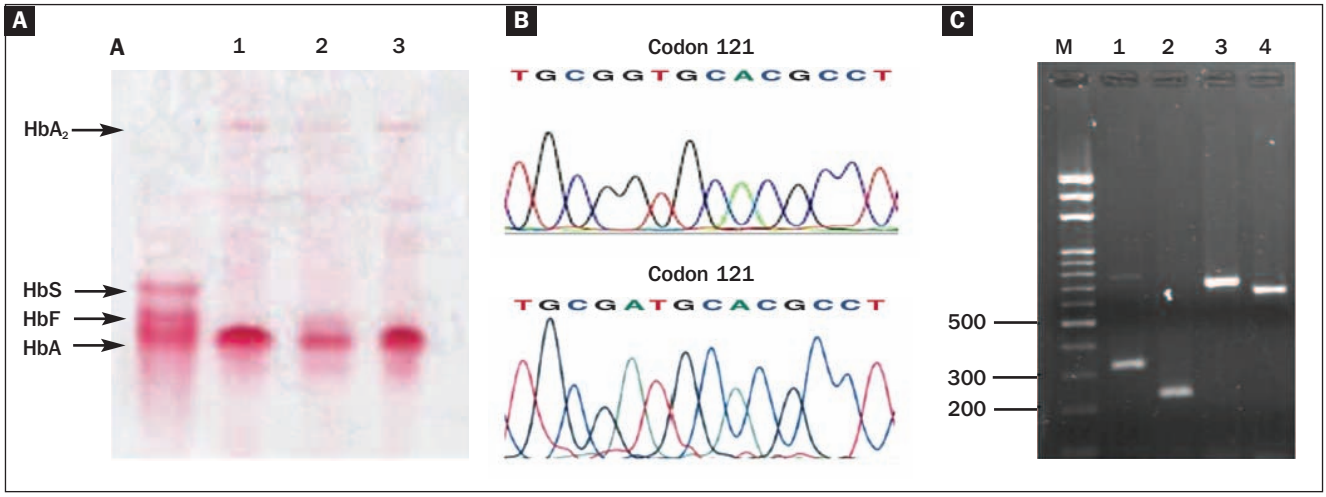

Fig. 2. A) The results of cellulose acetate Hb electrophoresis. Lanes 1–3 represent normal individual, α-thalassaemia-1 and the patient, respectively. B) The results of direct sequencing of the α1-globin gene show a homozygous G>A substitution at nucleotide 364; the upper panel is the normal control sequence, the lower panel is the Hb Owari sequence. C) The gap-PCR result of the α-thalassaemia-1 SEA type. The amplified product of the normal portion of the α-globin gene cluster (Lane 1) is 314 bp. Lanes 2–4 are the PCR products of the α-thalassaemia-1 SEA amplified with different specific primers; the sizes are 195 bp, 710 bp, and 620 bp, respectively. M represents the 100 bp ladder marker. *See these images in colour at www.bjbs-online.org*

final extension of 7 min at 72°C (Perkin Elmer Cetus PCR thermocycler 9700). Products were purified and sequenced as described previously.[3]

Some 411 Hb variants occur at the *HBA1* and *HBA2* genes and show considerable variation in severity.[4] Although the majority do not manifest clinically, the interaction with other thalassaemias may have the potential to cause haematological abnormalities. Clinical and laboratory presentation of these rare haemoglobin variants could be helpful in the evaluation of new cases and in genetic counselling, especially in high-prevalence areas such as Taiwan, south-east Asia and southern China.[5] If the direct sequencing of an Hb variant shows a homozygous mutation, the differential diagnosis should include a compound Hb heterozygote with the deletion form of thalassaemia. Owing to the presence of the non-pathological Hb Owari variant, this patient's clinical phenotype is the same as α-thalassaemia-1.

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