INTRODUCTION
Thalassaemia disorders are the most common single gene disorders affecting 5% of the worlds population. The frequency of this group of hereditary anaemias differs in different populations and is as high as 40% to 90% in those populations where they are common. Migration makes this group of disorders an increasingly important health issue in Australia. Alpha thalassaemias are caused by mutations on chromosome 16 encoding the alpha-globin genes. Antenatal, prenatal and postnatal screening including molecular diagnosis essential for effective diagnosis, counselling, and management of the thalassaemias. In our laboratory, the increasing use of DNA sequencing of both the alpha-globin genes to identify mutations has led to the identification of novel mutations in both the alpha 1 and the alpha 2 globin genes.

Alpha thalassaemia resulting from non deletional mutations has been reported most frequently in the alpha 2 globin genes and have a more severe effect on the phenotype than alpha 1 globin gene mutations. We report two novel mutations which result in the same previously reported Hb variants in addition to confirming the recently reported novel mutation causing Hb Stanleyville II. Hb Watts, was originally reported to be caused by a tri-nucleotide deletion (codon 74 or 75, -GAC, Asp>0), in the alpha 2 globin gene. The loss of the tri-nucleotide yields a novel protein sequence having one less amino acid. Hb Watts has only been reported in the alpha 2 globin gene. We report here the identification and phenotype of Hb Watts resulting from the same tri-nucleotide deletion (codon 74 or 75, Asp>0) in the alpha 1 globin gene. As previously reported, Hb Agia Sophia, a hypermutable variant, was originally reported to be caused by a tri-nucleotide deletion leading to Valine (−GTT) at position 62 of the alpha 1 globin gene in the Greek population. A novel mutation causing this variant Hb (codon 62-63, -TGG) has been identified in our laboratory. In the original reports, the AAC>AAA mutation in codon 78 in the alpha 1 globin gene of Hb Stanleyville II, was linked to an alpha 3,7 deletional mutation. In this report our findings for individuals with Hb Stanleyville-II have been documented.

METHODS
Haematological data was obtained from laboratories referring blood samples collected in EDTA for DNA analysis for haemoglobinopathies. DNA was isolated from blood samples using the Magnapure automated DNA extractor from Roche Diagnostics Australia Pty. In this method, DNA was obtained using magnetic beads from proteolytic products of white blood cells in accordance with instruction by the vendor. The characterization of the alpha-globin genes were performed by direct sequencing of the amplicons specific to alpha 2 (primers C1, C3) and alpha 1 (primers H1, H2) globin genes. Sequencing for each gene was carried out using 3130xl Genetic Analyser from Applied Biosystems, Australia using common primer C1, C5, C7 and specific primers C3, C26c for alpha 2 and alpha 1 globin genes, respectively (please see Table 1 for primer sequences). This data was analysed using the ATF (version 1.0.2.4.1) software developed in Western Australia by Conexio Genomics Pty Ltd.

RESULTS AND DISCUSSION
Haemoglobin Watts
Codon 75 and 76 in both the alpha globin genes code for aspartic acids. These mutations have been described yielding non-synonymous substitution of either of these two aspartic acids in both the alpha globin genes. Hb Watts is the only described mutation resulting from the deletion of one of these aspartic acid residues from the alpha 2 globin chain causing instability of the Hb molecule. The reported case for Hb Watts was of Mexican-American ethnic origin and the abnormally produced globin gene was observed in 9.8% of the total Hb. Our laboratory identified two unrelated cases of Hb Watts in two individuals from the same family and country of origin with the same mutation in the alpha 1 globin gene. The amount of abnormal Hb observed is less, as listed in Table 2.

Haemoglobin Agia Sophia
Three unrelated individuals were identified by direct sequencing in our laboratory to have a novel deletion causing Hb Agia Sophia (Figure 1). The ATF (sequence data analysis software) reading for the sequence and conversion of sequence using nucleotide ambiguity nomenclature is tabulated in Table 3 to illustrate the frameshift mutation. The deduced base change was codon 62-63 (-TGG). The haematological findings are documented in Table 4. All three patients are of Greek ethnicity and the IVS-1-38 (C>T) base change was found in conjunction with the -TGG mutation. The IVS 1-38 (C>T) base change for Hb Agia Sophia has been documented in previous reports.

Hb Stanleyville II
Haemoglobin Stanleyville-II is a Hb variant of African origin and is reported in the globin gene server as a AAC>AAA mutation in codon 78 of the alpha 1 globin gene. Our laboratory has documented thirteen Hb Stanleyville-II cases caused by AAC>AAA mutation in the codon 78 in the alpha 2 globin gene (please see Table 5). Twelve of these cases where of African origin and one was of unknown ethnicity. Four of eight Hb Stanleyville-II cases of Sudanese origin were associated with an alpha 3,7 deletional mutation; three were not associated with an alpha 3>AAA deletion and one was not investigated. One of the cases of Somali origin was associated with an alpha 3>AAA deletion mutation and the other was not tested for the mutation. One of the cases of Hb Stanleyville-II was from the Democratic Republic of Congo and the ethnicity of one was unknown.

In summary, six were associated with alpha 3-7 deletional mutation, five not associated with this mutation and two were not tested. Haemoglobin Stanleyville-II was first described in the Democratic Republic of Congo in the alpha 1 globin gene (Codon 78, AAC>AAA). Recent reports have found this mutation to be mainly in the alpha 2 globin gene (codon 78, Hb AAC>AAA) with or without association with alpha 3-7 in European and South American origin and in the alpha 1 globin gene in a person of Asian (Chinese) origin which was found to be in association with Hb Constant Spring II. Our observation confirms the suggestion of Pimentel et al. (2011) that the AAC>AAA mutation in alpha 2 globin gene is more common than the AAC>AAG for codon 78 in the alpha 1 globin gene.

CONCLUSIONS
Molecular identification of the underlying mutation is critically important for the study of hemoglobinopathies if a better understanding of phenotypic complexity. Although the alpha 2 globin gene is more dominant than the alpha 1 globin gene at the mRNA transcription level, uncertainty remains about the translational efficiency of both of the genes. It is noteworthy that the amount of Hb Watts detected for the alpha 1 or the alpha 2 alpha globin gene in our series varied and is consistent with the hypothesis that the alpha 2 globin gene has a higher translation efficiency than the alpha 1 globin gene. Mutations and their location on the gene may also give valuable information about ethnicity. Hb Watts has been observed in Mexican Americans in the alpha 2 globin gene and in USA and the alpha 1 globin gene in individuals from the Indian subcontinent in Australia, reported here. Ethnicity should not just be assumed as default from the observed wide distribution of the AAC>AAA Hb Stanleyville-II mutation. Although this mutation has a high frequency in Africa, it has also been observed in Europeans (Caucasian), South Americans and Asians (Chinese). The South American observations point to African migration to Brazil. The Hb Stanleyville-II AAC>AAA mutation has so far been reported only in, Western Africa (around Congo, where it was first described). In our laboratory, the only sample having Hb Stanleyville-II from Congo was also found to have the AAC>AAA mutation. We also observed that two different frameshift mutations can result in the same amino acid deletion from the globin chain, resulting in the Hb Agia Sophia mutation.

REFERENCE
5. Yang, Q., Yang, D., H. Watts (347FS), Hb Stanleyville (66PGG) and Hb Stanleyville (78MFG) in Chinese patients, Hb Stanleyville (66PGG) and Hb Stanleyville (78MFG) in Chinese patients.