Thalassaemia and Haemoglobinopathy in the Waikato and Bay of Plenty:

A Review of Testing and Prevalence of Haemoglobinopathy at Pathlab Waikato.

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Abstract

Haemoglobinopathies are amongst the most common genetic disorders in the world but remain relatively overlooked. The World Health Organisation estimates around 5.2% of the global population may be carriers of a pathogenic haemoglobinopathy due to a mutation in one of the haemoglobin genes. For the past decade Pathlab Waikato has established a database of all diagnosed haemoglobinopathy and thalassaemia, with the intention that this data may be used to assess and improve the New Zealand national screening service. The purpose of this research was to analyse this database to investigate 1) What is being diagnosed in our region? 2) How common are these haemoglobinopathies? and 3) What is being missed? Answers to this will provide a better understanding of the pathology and prevalence of this disease in our region, and to provide recommendations and strategy for future testing.

The haemoglobinopathy database was analysed alongside data from incidental abnormal haemoglobin detection through testing for HbA1c. This information was run through RStudio for statistical analysis of ethnicity data to confirm that ethnicity was a significant predictor of the presence of abnormal haemoglobin (p value <0.001). Prevalence of different haemoglobinopathies was calculated and this was compared to previously reported prevalence both globally and within certain ethnic groups. Using population information from StatsNZ and the past three New Zealand Censuses (2023, 2018, 2013) the ethnic makeup of our testing population was also determined. With all of this information combined, differences and shortfalls in our database can be identified when compared to calculated estimates of prevalence of abnormal haemoglobin.

From the database, Pathlab performed 6,589 thalassaemia screens with a positive detection rate average of 53% with a steady increase in haemoglobinopathy testing every year. The most commonly diagnosed haemoglobinopathies (in order) were α thalassaemia, β thalassaemia, heterozygous HbE, and heterozygous HbS. Using known ethnic prevalence from the literature review and the ethnic makeup of our community from the census, it was found we are detecting less than what would be expected for our population. For example, 5.85% of our population has identified themselves as Indian, and based on previous reports at least 3% of them should be carriers for β thalassaemia. This would come to roughly 1,179 people. However, during our screening period, only 621 confirmed diagnoses of

β thalassaemia were made. Furthermore, using the Hardy-Weinberg equation, it is estimated there may be over 10,000 and 3,000 carriers of HbE and HbS, respectively. This is compared to only 358 and 171 detected in our database. Even if the actual allele frequency is half of what was calculated, the shortfall is stark.

With all this in mind, Pathlab are already introducing an extra step through the screening of abnormalities found incidentally in HbA1c. However, there are further steps we can take to tackle this deficit, including the establishment of a national database and national screening service. The process to allow accurate haemoglobinopathy screening in New Zealand was outlined. If haemoglobinopathy screening was introduced as part of an antenatal screen, it would help to bridge the gap we have uncovered here to prevent negative health outcomes and further strain on our health care system.

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1. Aim and Objectives

Haemoglobinopathy is a common disease and a public health issue. There is little data available for thalassaemia and haemoglobinopathy in New Zealand despite its high prevalence in many of our populations. For the last seven years, Pathlab Waikato has been collecting data on Thalassaemia and Haemoglobinopathy screens. The aim of this thesis is to bridge this gap of knowledge by analysing this data to better understand the pathology and prevalence of this disease in our region, and to provide recommendations and strategy for future testing.

The haemoglobin genes are large; therefore the number of possible mutations is vast, and new haemoglobinopathies are being discovered frequently. Because of this, the focus of this thesis will only be the haemoglobinopathies that are common, both in the global population and within New Zealand. While some of the less prevalent haemoglobinopathies may be mentioned briefly, they will not be the focus of this study as they are not found in any significant number in our data.

Our main objective is to achieve a broad overview of the haemoglobinopathy testing conducted at Pathlab Waikato, including how many of each type of thalassaemia or haemoglobinopathy we are detecting or what the most common is. This is accomplished through analysis of our haemoglobinopathy database, from 2017 to 2023. Our methods and approach to testing will be compared to what is done in other countries around the world with varying degrees of haemoglobinopathy prevalence. Our data will then be compared to national census data to see if we are detecting the expected levels of haemoglobinopathy for the ethnic makeup of our regions.

Secondary is the data collected from HbA1c testing. This test is an indicator of glucose levels for diabetes diagnosis, but it is also capable of detecting abnormal haemoglobins.[1] Using this data, we can investigate how many abnormal haemoglobins can be detected this way, and how many of these incidental findings are previously unknown. Also given these datapoints include information on ethnicity, we can compare the prevalence of haemoglobinopathy in specific ethnicities within our community to the estimated prevalence from research statistics.

Finally, and most importantly, is to reflect on these findings and determine in what areas we are falling short. Once this is done, we can begin to investigate ways to improve our service and reach, and hopefully through this research we may discover what steps to take to successfully accomplish this.

1.1 Abbreviations:

- CE: capillary electrophoresis, the machines used in Pathlab are referred to as ‘CAPY’
- CBC: complete blood count
- Hb: haemoglobin
- RBC: red blood cell
- MCH: mean cell haemoglobin
- MCV: mean cell volume
- HPFH: hereditary persistence of foetal haemoglobin
- HPLC: high- performance liquid chromatography
- MS: mass spectrometry
- NTDT: non- transfusion- dependent thalassaemia
- NHS: National Health Service
- SCD: sickle cell disease
- TDT: transfusion- dependent thalassaemia
- NHI: National health index – a unique identifying number given to every individual in New Zealand

Greek letters used in haemoglobin descriptions (all mentions are in the lower-case form):

- Alpha: α
- Beta: β
- Gamma: γ
- Delta: δ
- Epsilon: ε
- Zeta: ζ

1.2 Glossary:

- **Haemoglobin** – the molecule in the blood responsible for carrying oxygen.
- **Red cell indices** – refers to the parameters in a complete blood count (CBC) that are used to describe the red blood cells. These are: RBC, haemoglobin, MCV and MCH.
- **Wild type** – the most predominant form of an allele in nature, as opposed to one that is mutated.
- **HbA** – the majority of haemoglobin found in adults, made from two α chains and two β chains.
- **HbA1c** – the glycated form of HbA, HbA1c occurs due to glucose in the blood changing the haemoglobin. This is a commonly used measure of blood glucose in diabetes as it gives a measure of long term glucose control.
- **HbA2** – the second most prevalent haemoglobin found in adults, made from two α chains and two δ chains.
- **HbF** – otherwise known as foetal haemoglobin, this is the majority haemoglobin found late in the prenatal stage of life and persist usually only up to 12 months of age. Found in only very small amounts in adults and is barely detectable.
- **α Thalassaemia** – haemoglobinopathy caused by the loss of α genes through mutations such as large scale deletion. There are four α genes and the number of genes lost determines the specific name of that α thalassaemia:
    - α thalassaemia trait: loss of one or two α globin genes.
    - HbH Disease: loss of three α globin genes, HbH (bodies) is also the name given to the tetramer of β chains that forms in the absence of available α chains.
    - Hb Barts: loss of four α globin genes, it is also the name given to the tetramer of γ chains that forms in the absence of available α chains, this is normally found in slightly lower levels than HbH bodies.
- **β Thalassaemia** – haemoglobinopathy caused by the loss of α genes through mutations such as large-scale deletion.

- **β Haemoglobinopathy** – mutations in the β gene that cause haemoglobinopathy. Most commonly mentioned are:
    - HbS – sickle cell trait/anaemia, with a mutation that causes red blood cells to form an abnormal "sickle" shape.
    - HbD
    - HbE
    - HbC
    - HbO
- **i-lab α-thal** (or i-LAB αTHAL) is a commercial diagnostic kit that is used to confirm α thalassaemia through immunochromatographic test strips that detect γ globin chains.
- **Concomitant** – a thalassaemia or haemoglobinopathy (commonly α thalassaemia) that is diagnosed with one or more other kind of haemoglobinopathy. The patient carries mutations for both therefore the mutations (or diseases) are concomitant.
- **Haemolytic anaemia** – low haemoglobin caused by lysing of red blood cells. This can be due to many different factors such as genetic disease, severe infection, autoimmune disease or as a reaction to toxins or medication.
- **Splenomegaly** – an increase in the size of the spleen. It is a non-specific symptom that can be caused by a variety of factors.

2. Literature review

2.1 Haemoglobin

Haemoglobin (Hb) is a protein in erythrocytes (red blood cells (RBC)) that carries oxygen (Fig 2.1A). It is made of four globin chains (Fig 2.1B), each with a heme component that contains an oxygen binding iron molecule. Max Perutz was awarded a Nobel Prize in chemistry for being the first to describe the three-dimensional structure in 1962 using x-ray crystallography.[2] There are seven different variations of Hb comprised of six different globin chains: epsilon (ε) zeta (ζ), two gamma (γ) genes, delta (δ), and beta (β) (Fig 2.1B). The levels of these present in RBC change between infancy to adulthood (Fig 2.2). This was first observed by Ernie Huehns in 1964, who discovered there were two switches during human development, one from embryonic Hb to foetal Hb, then the second from foetal to adult Hb.[2]

The most common Hb in healthy adults is HbA, which constitutes around 95% of all haemoglobin. HbA is made of two alpha globin chains and two beta globin chains (Fig. 2.1). In a foetus or newborn baby, most of the haemoglobin is HbF, which is two alpha globin chains and two gamma globin chains (Fig 2.1). HbF begins to slowly decrease at 34 weeks gestation, then two weeks after birth production rapidly decreases and it is almost entirely replaced by HbA by 9 months (Fig 2.2).[3] HbF persists in adults at a very low, almost undetectable level, but high levels can be found in adults due to various medical conditions, medications, or in certain haemoglobinopathies. Not present in foetal blood, but the second highest concentration in adults is HbA2. This is typically 2-3% of all Hb in healthy adults. Embryonic Hb is also known as Hb epsilon due to the globin chains of the same name. It is only present for the first 5-10 weeks post conception, and is made up of Hb Gower (1 and 2) and Hb Portland.[3]

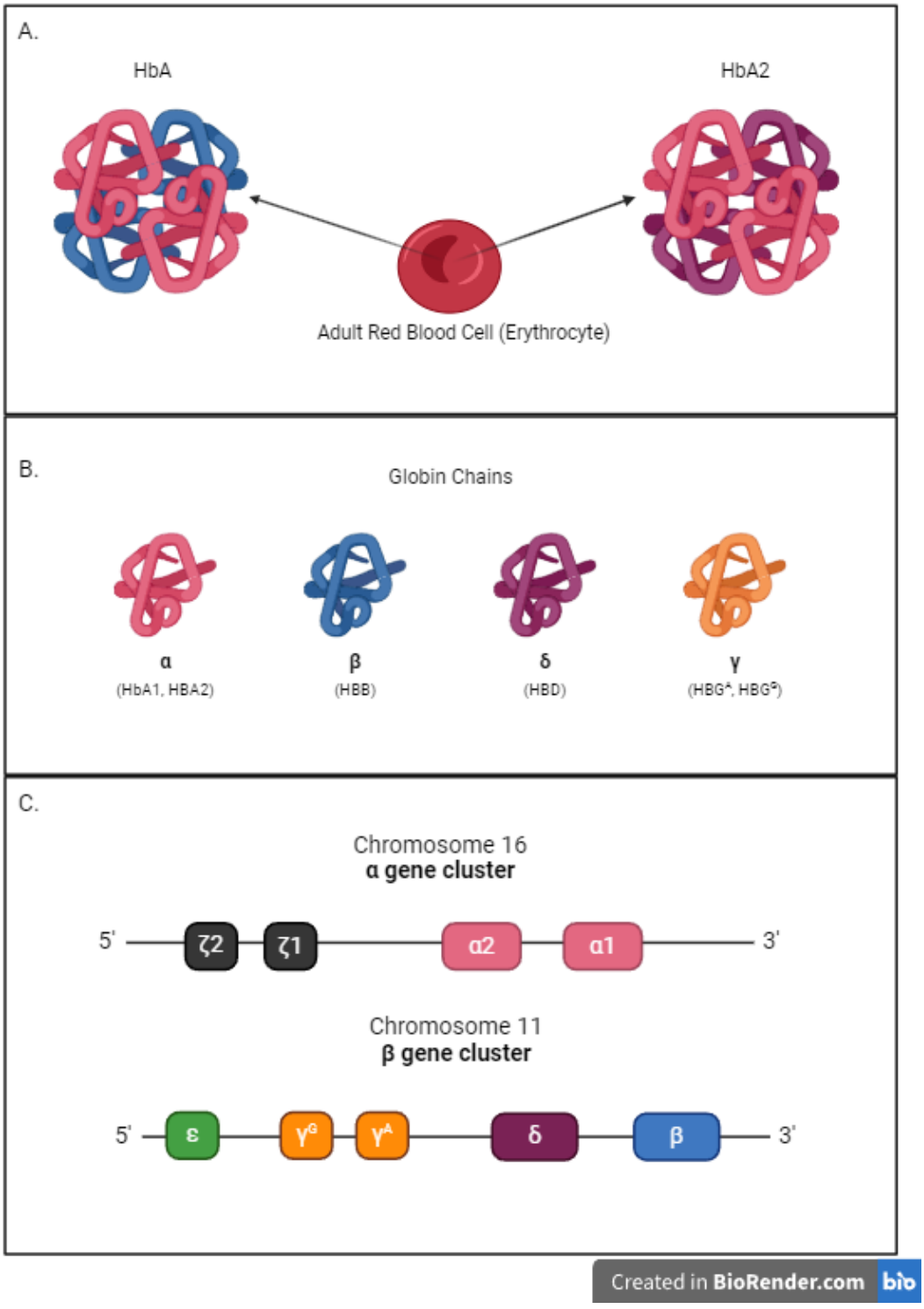


Figure 2.1: Globin chains and their genes

A) HbA and HbA2 are the two prominent fractions of haemoglobin found in the red blood cells of adults. B) The different colours represent the most common types of globin chain present in different forms of haemoglobin, they are listed here along with their corresponding genes. HbF mirrors HbA2, with two gamma (γ) chains instead of two beta (β) chains. C) Simplified diagram of the gene clusters for each of the chains.

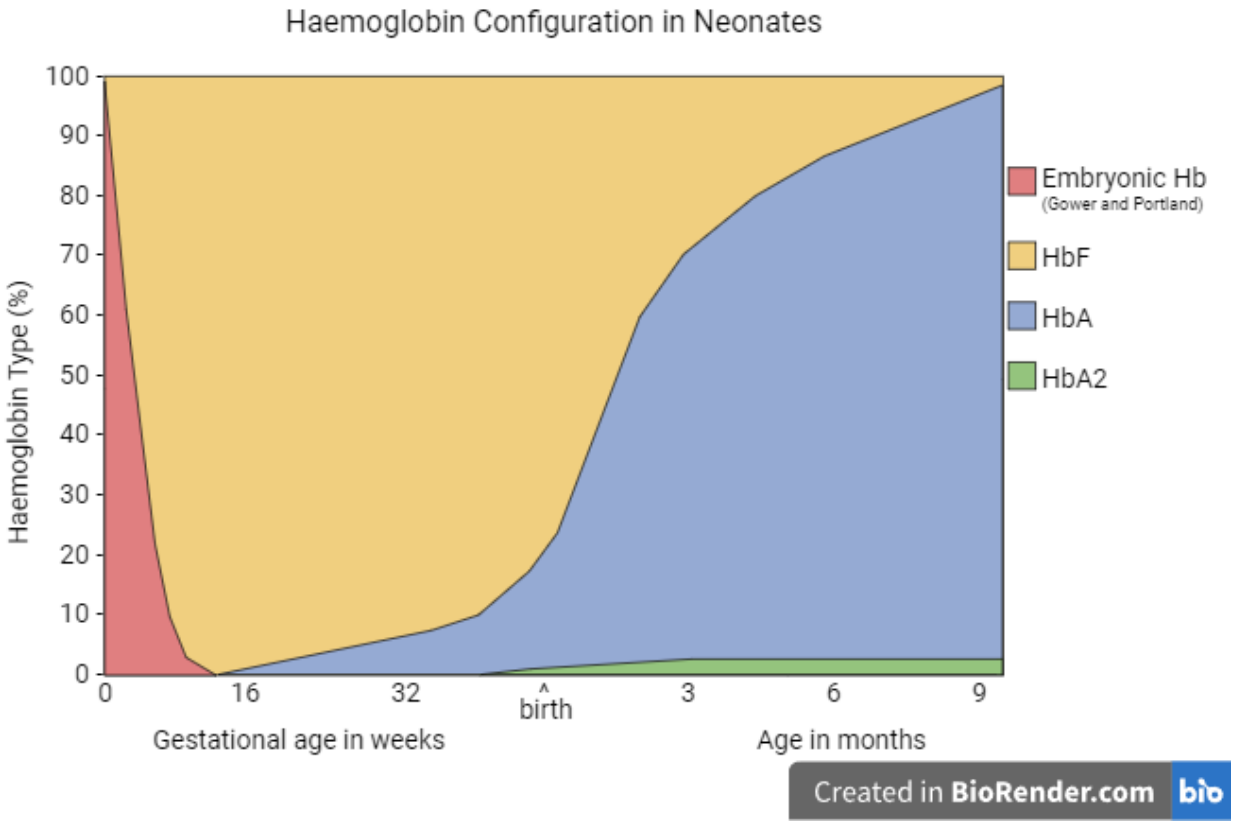


Figure 2.2: Haemoglobin changes in neonates, a basic representation of the change in haemoglobin during gestation to early infancy.

Haemoglobinopathy refers to mutations in the genes encoding these globin chains on human chromosomes 11 and 16 (Fig 2.1C). If these mutations cause a decrease in protein production of a certain globin chain, then this is called a thalassaemia. Any other mutations that cause a change in shape or function can be generally grouped and referred to as haemoglobinopathies, including the prominent sickle cell anaemia. A summary of all the most common forms of haemoglobinopathy can be found in Appendix 1, Table 7.1.

2.2 Origin of thalassaemia and haemoglobinopathy

One of the most common, and arguably the most widely known of the haemoglobinopathies is autosomal recessive inherited sickle cell anaemia (HbS). This was first described in 1910 by Dr James Herrick, who noted an anaemic patient had “peculiar, elongated sickle-shaped erythrocytes” described in Fig. 2.3[4; 5]. But it was not until 1949 that it was discovered by

Dr Linus Pauling, who found the origin of these anaemias was an abnormal haemoglobin structure, and he coined the term “haemoglobin S”.[4; 5] This disease was also seen in pioneering biochemistry in the 1950’s when Vernon Ingram proved that an amino acid change caused the RBC to become “sickled” in shape.[4; 6] Another breakthrough was made in the 1970’s with sickle cell anaemia becoming the first human disease proven to be caused by a single nucleotide substitution of A to T.[2] This became a significant ethical issue in the United States of America (USA) where this research was conducted, as at the time sickle cell anaemia was seen almost exclusively in patients of African descent. This was an important factor in the civil rights movement to highlight racial inequality in the American healthcare system. Because of this, the Sickle Cell Disease Association of America was established to increase funding for research, health care, and education. In 1972, the National Heart, Lung, and Blood Institute established the Cooperative Study of Sickle Cell Disease with funds from the USA government’s Sickle Cell Anaemia Control Act.[4]

The first description of thalassaemia was reported in 1925 when Dr Thomas Cooley studied Italian children in Detroit, USA with severe symptomatic anaemia. Thus, it was originally named “Cooley’s Anaemia”. In 1936, the name “thalassaemia” was first used by Whipple and Bradford, from the Greek “θαλασσα” (thálassa) meaning sea, due to the high prevalence in Greek and other Mediterranean ethnicities.[7] Similarly to HbS, it was in the 1940’s that the cause of the anaemia was discovered to be an abnormal haemoglobin.[4] It was also in the 1940’s that it was proposed that thalassaemia provided protection against malaria, a life-threatening mosquito-borne disease.[4] This was largely due to epidemiological data that showed high proportions of thalassaemia were present in populations with endemic malaria.[8] Research since has proven a more defined pathological resistance to malaria due to increased phagocytosis of infected RBC, specifically for *Plasmodium falciparum* in HbS, which again are both much more common in parts of Africa, the Middle East, and South America.[8; 9] Fresh blood transfusions were given as an effective treatment beginning in the 1960’s and are still used today, however due to iron overload from the excess heme, most of these early patients passed away.[4]

Figure 2.3: RBC shapes.
Normal red blood cells on the left showing a disk or doughnut shape, with a thicker outside and indent in the middle. The cells on the right are typical of sickle cell anaemia, showing the elongated or "sickled" shape caused by polymerisation of the abnormal globin chains.

2.3 Genetics of haemoglobin and its genes

The genes involved in the synthesis of the human globin chains are found in two clusters, one on chromosome 16 and the other on chromosome 11 (Fig 2.1C). The alpha gene cluster is found on chromosome 16p13.3 and spans 28 kb with a number of pseudo genes that are transcribed but not translated, and two expressed alpha genes – Haemoglobin Subunit Alpha 1 (*HSA1*) and Haemoglobin Subunit Alpha 2 (*HSA2*).[5] Interestingly, the two alpha genes have 100% identity in their three coding exons but differ slightly over the 5' untranslated regions and the two introns, and they differ significantly over the 3' untranslated regions. *HSA1* spans 843 nucleotides and is transcribed into a 577 bp mRNA transcript whereas *HSA2* spans 835 nucleotides and is transcribed into a 576 bp mRNA transcript. But both result in the translation of a 142 amino acid protein with a molecular weight of 15,258 Da.[10]

Chromosome 11p15.4 contains the beta cluster, a region comprised of five genes: Haemoglobin Subunit Epsilon (ε) 1 (*HBE1*), Haemoglobin Subunit Gamma (γ) 1 (*HSG1),* Haemoglobin Subunit Gamma (γ) 2 (*HSG2*), Haemoglobin Subunit Delta (δ) (*HSD*), and Haemoglobin Subunit Beta (β) (*HBB*). The two types of gamma chains differ at amino acid residue 136 where glycine is found in the G-gamma product (HBG2) and alanine is found in the A-gamma product (HBG1).[10] All the expressed globin genes show a high level of conservation at the amino acid level, and all have the same structure of three exons.[5]

Like all genes, these globin genes are subject to germline (inherited) or de novo (spontaneous) mutation(s). These may be in the form of a point mutation or a larger deletion or insertion. Due to genetic redundancy, many of these point mutations do not alter the structure of the haemoglobin, and if a change of amino acid occurs, the same change may be caused by a number of different mutations.[5] When a mutation occurs that causes a change in structure, this is what is known as a haemoglobinopathy. If a mutation (usually a deletion) specifically causes a loss in the rate of synthesis, this haemoglobinopathy is then referred to as thalassaemia, though this can occur concurrently with a change in structure which will also be covered in this chapter. Thalassaemia can occur in alpha, beta and delta genes. Delta thalassaemia is of no clinical significance, with the only detectable change being a decrease in HbA2. This is only of note as it can complicate diagnosis by masking beta thalassaemia. For the purposes of this thesis, rather than using the official gene names, we are using the simplified Greek gene symbols that are recognised by the Standard Operating Procedures at Pathlab Waikato, NZ.

2.3.1 Alpha Thalassaemia

The α globin chain has two genes, α1 (*HBA1*) and α2 (*HBA2*), therefore inheritance is commonly expressed as αα/αα for individuals with four normal copies. These genes are not expressed equally, the ratio of α2 to α1 protein synthesis is usually around 3:1.[5] Therefore, the location of the mutation will affect the level of change or loss of function, and thus the phenotype or severity of the subsequential anaemia. Alpha thalassaemia occurs when one or more of the α genes contains a mutation that decreases protein synthesis. Most commonly this is a deletion of some, or all of the gene. This causes an unbalanced production of the normal globin chains, in this case leading to excess β chains which can be seen diagnostically and causes some of the pathological effects seen in α thalassaemia, such as increased haemolysis. Excess of the β chains forms a tetramer known as haemoglobin H, and less common is the excess γ chains which form a tetramer named Hb Barts. The severity of anaemia in α thalassaemia is incredibly varied, from asymptomatic to a condition called Bart's hydrops fetalis, which causes neonatal death.[5] The severe condition comes from a loss of all four α globin genes, whereas a loss of only one may go unnoticed for life.
Due to the nature of the α genes, a two gene deletion thalassaemia can be balanced or unbalanced. This refers to the loss of either both genes on the same chromosome, or the loss of one from each chromosome. These genotypes are known as α^0 (alpha zero, expressed as

--/αα when homozygous) and α^+ (alpha plus, expressed as -α/-α when homozygous), respectively.

The most common deletions for an α^+ thalassaemia are $\alpha^{3.7}$ and $\alpha^{4.2}$, with the name of each of these referring to the number (in kb) of nucleotide information lost. The mutation $\alpha^{3.7}$ encompasses a group of three 3.7kb deletions that differ slightly in the exact location of deletion, ranging from the 3' end of the α2 gene to the 5' end of the α1 gene, creating a fusion (α2α1) gene.[5] The $\alpha^{4.2}$ deletion occurs entirely in the α2 gene. Both of these deletions result in a reduction in protein synthesis of the α chain by around 50%, due to upregulation of the α1 gene compensating for the loss of α2, which usually makes up 70% of synthesis.[5] The $\alpha^{3.7}$ deletion is common in a range of ethnicities including New Zealand Māori, Polynesian, African, and Greek.[5] In the heterozygous state, an α^+ thalassaemia can be completely undetectable, with a normal complete blood count (CBC) or only slightly decreased mean cell volume (MCV) and mean cell haemoglobin (MCH). Patients with homozygous α^+ thalassaemia will usually have a more evident microcytic, hypochromic anaemia.
The most common α^0 mutations are named after the geographical regions they were first found, and span almost the entire α gene region, these are: $--^{SEA}$, $--^{FIL}$, $--^{THAI}$, and $--^{MED}$ (for South East Asia, Philippines, Thailand, and Mediterranean, respectively).[5] These are all large-scale deletions that encompass both α1 and α2 genes. Unlike α^+ thalassaemia, it is less common in individuals with Mediterranean or African heritage, and more common in Asian ethnicities, particularly Chinese and Southeast Asian (SEA). Even in the heterozygous state, patients with α^0 thalassaemia will show a microcytic hypochromic anaemia with a raised RBC count, and infants will show detectable levels (5-10%) of Hb Barts.[5] This anaemia would be more severe than in individuals with homozygous α^+ thalassaemia.

There is also a third kind of α thalassaemia, which is non-deletional and therefore sometimes referred to as a 'thalassaemic haemoglobinopathy'. By far the most common non-deletional α thalassaemia is named haemoglobin Constant Spring (HbCS, shown as α^{CS}), due to the first diagnosis occurring in the Constant Spring region of Jamaica.[5] HbCS is the result of a point mutation (TAA→CAA) in the α2 gene (*HBA2* c.427T>C; rs41464951) that has the downstream effect of an elongated α1 gene, through the change of a stop codon adding 31 more amino acids. This mutation creates unstable mRNA, which causes a significant decrease in protein synthesis leading to the thalassaemic property of this haemoglobinopathy. HbCS follows a similar geographical incidence as α^0 thalassaemia and is most common in Southeast

Asia and China. Clinically, heterozygosity for this disease produces symptoms similar to an α^+ thalassaemia and may be completely asymptomatic. However, homozygous Hb CS produces a more severe anaemia than is seen in homozygous α^+ thalassaemia.

Haemoglobin H (HbH) disease occurs when a patient is heterozygous for both α^+ and α^0 thalassaemia, meaning loss of three out of four α genes (- -/-α), for example an individual of South East Asian descent may be at risk of the genotype $--^{SEA}/-\alpha^{3.7}$.[5] HbH disease may also be due to co-inheritance of a non-deletional α thalassaemia such as Hb CS. Using the same example this may be $--^{SEA}/\alpha^{CS}\alpha$. HbH disease that is caused by a non-deletional thalassaemia appear to be more severe than the alternative, however, these all cause a severe hypochromic, microcytic anaemia, with detectable levels of HbH bodies (β chain tetramers).[5] The HbH bodies have a very high oxygen affinity, causing poor oxygenation of tissues. It is also prone to oxidation, which causes instability leading to a higher turnover of RBC or haemolytic anaemia. This is responsible for the clinical symptoms of anaemia and splenomegaly due to phagocytosis of RBC. These symptoms are more evident when compounded by other causes of anaemia such as during pregnancy, infection or certain deficiencies. Iron overload may also be seen in HbH disease, and in other severe thalassaemia.[5; 11]

When all four α genes are lost, there is no production of the α chains, therefore no HbA, HbA2 or HbF. This results in Hb Barts hydrops fetalis, which is typically incompatible with life causing foetal death. Due to previously described epidemiology, this occurs most commonly in parents of South East Asian or Chinese descent, though it is also seen in other ethnic groups and significantly in Mediterranean regions.[5] Hb Barts (γ chain tetramer) is similar to HbH in that it has a higher affinity for oxygen therefore unable to effectively oxygenate tissue in the body. The effect of this disease begins during the embryonic switch from Hb Gower and Portland to Hb F, where the embryonic haemoglobins can deliver oxygen, but the lack of α chains produces only Hb Barts, not the desired Hb F.[5] Without transfusion, this causes organ failure and other abnormalities leading to death.

Haemoglobinopathies in the α gene result in a mutated form of both HbA and HbA2 (or HbF, dependent on age). These are less common than α thalassaemia and usually of no clinical significance due to being entirely asymptomatic. Examples of these are Hb G-Philadelphia and Hb J, which are both asymptomatic and rare, therefore not discussed further here.

2.3.2 Beta Thalassaemia

The β gene cluster contains the genes for the rest of the globin chains found in adult haemoglobin. This includes the β, δ, and γ chains found in *HbA*, *HbA2*, and *HbF*, respectively (Fig. 2.1). Reduction of synthesis due to homozygous mutation in the β gene results in β thalassaemia (major) or in the heterozygous form β thalassaemia trait (or β thalassaemia minor). A large variety of mutations are known to cause β thalassaemia across a wide range of ethnicities. Greater than 400 mutations have been reported to cause β thalassaemia, and these may be point mutations or large deletions.[5; 12] The genotype – phenotype is typically more straightforward in β thalassaemia when compared to α thalassaemia, as there are only two alleles of the one gene. However, due to the large number of possible mutations, the disease can be divided into two groups similarly to α thalassaemia: β^+ or β^0 thalassaemia.[5] As is the case in α thalassaemia, β^+ thalassaemia mutations cause a decrease in synthesis but not total absence of the β chain even in homozygous state. Different mutations cause anaemia in varying degrees from mild to severe. The mutations classed as β^0 thalassaemia are either large scale deletions or an abnormality that inhibits expression. Therefore, in the homozygous state no β chains are synthesised leading to absence of HbA entirely.[5] It is possible for compound heterozygosity to occur when a patient has two different mutations to each of their β genes, therefore they have no normal or wild type copy.

Heterozygosity of β thalassaemia (or β thalassaemia minor) is reportedly asymptomatic, but with a reduced MCV and MCH seen in a CBC. However, some patients do have mild symptoms such as fatigue or increased susceptibility to infection.[5] These patients may even require blood transfusions under certain conditions such as pregnancy to relieve the anaemia. This may contribute to iron overload, as discussed earlier. β thalassaemia minor causes a longstanding excess of iron due to ineffective haematopoiesis, and this may be exacerbated by clinicians administering iron due to an incorrect diagnosis of iron deficiency anaemia, which has nearly identical clinical characteristics.

β thalassaemia major, caused by homozygous or compound heterozygous β thalassaemia results in a severe, transfusion dependent anaemia. The anaemia is typically evident at early in infancy, becoming significant and requiring treatment when the switch from HbF to HbA occurs, as β globin is not required for HbF production. First symptoms include failure to thrive and bone deformity, particularly in the skull.[5] Transfusions need to begin early in life

for the patient to survive into adulthood. The excess of α globin chains compounds the effect of the anaemia by decreasing RBC life span.[5]

A variety of homozygous β^+ and heterozygous β^0 mutations (or a compound heterozygosity for β^+ and β^0) may result in a disease termed 'β thalassaemia intermedia' which is a symptomatic anaemia, but does not require blood transfusions.[5] For example, homozygosity of a very mild β^+ thalassaemia such as the IVS1 6 T>C, or heterozygosity of β^0 thalassaemia such as IVS1 1-5 G>C that is ameliorated by a co-inherited α thalassaemia trait (-α/-α or --/ αα). Due to the wide variety of causes, β thalassaemia intermedia is only classified by the clinical symptoms of the disease.[5] As with β thalassaemia minor, some patients may require transfusion during times of haematopoietic stress. However, these patients may also have symptoms of β thalassaemia such as anaemia, bone deformity, or splenomegaly. As the mutations causing this condition are varied, so too are the severity of the symptoms seen.

2.3.3 Beta Haemoglobinopathy

There are many reported β haemoglobinopathies, the most common being HbS (sickle cell anaemia), HbC, HbD, HbE, and HbO. New haemoglobinopathies are constantly being discovered and classified, with a majority of these being asymptomatic. For the sake of simplicity, we will only discuss the most common.

HbS is always caused by a change from the amino acid glutamic acid to valine at position 6 of the β chain.[5] This change causes the haemoglobin to become unstable when deoxygenated, causing polymerisation that leads to the characteristic sickle shape of the cells. This shape makes the cells more rigid and more likely to adhere to the endothelium of blood vessels, causing restricted blood flow. The disease is most common in individuals of African, Middle Eastern, Indian, and South American descent.[5] As discussed earlier, the geographical spread of HbS appears to have occurred where malaria is endemic due to the purported protective benefits. HbS can be homozygous as sickle cell anaemia (β^S/β^S), or heterozygous as sickle cell trait (β/β^S). Sickle cell trait is known to be asymptomatic, however new studies are being conducted to see if chronic conditions can be caused by the heterozygous state, and oxidative stress or hypoxia can cause symptoms similar to those seen in the homozygous disease.[5; 13] In sickle cell anaemia no normal β chains are produced,

therefore no normal HbA is present. Even with treatment patient life expectancy is greatly decreased, and symptoms such as bone malformation, splenomegaly, haemorrhage, or infarction can be seen from 6 months of age. Other thalassaemia or haemoglobinopathies may cause a more severe disease when co-inherited with HbS. A patient with heterozygous HbS will have the same features as sickle cell anaemia if they also carry the β mutations HbC or HbD, or if they have co-inheritance with β thalassaemia.

HbC is caused by a mutation in the same site as that found in HbS, but the change is from glutamic acid at position six to lysine.[5] HbC can also form crystals in RBC, but unlike HbS, these crystals occur in the oxygenated state then dissolve once deoxygenated. So, they are unlikely to cause significant damage to cells or blood flow. HbC is thought to have originated from a particular area in West Africa spanning Burkina Faso, Ghana, and Côte d'Ivoire.[5] It is therefore more commonly found in individuals of African descent, and like HbS, its spread can be partially attributed to its ability to offer some protection from Malaria. HbC can also be present in heterozygous and homozygous states. Homozygous HbC or 'HbC disease' can cause a mild haemolytic anaemia. Heterozygous HbC is again of no clinical significance alone and is usually detected as an incidental finding. However, this is an important finding due to the possible implications of co-inheritance with another thalassaemia or haemoglobinopathy. As HbC, HbS, and β thalassaemia are all common in the same regions of the world, co-inheritance is also somewhat common.[5] For example, if inherited with a heterozygous HbC, a β^+ thalassaemia produces a clinically similar disease to β thalassaemia intermedia.

Haemoglobin D covers a range of mutations, the most significant of these is HbD-Punjab, also known as Hb D-Los Angeles (*HBB*: c.364G>C; rs33946267). This point mutation causes a change from glutamic acid to glutamine in the one hundred and twenty first position in the expressed protein.[5] Heterozygous HbD is entirely asymptomatic, and patients will have a completely normal CBC. Homozygous HbD or HbD disease has an almost undetectable phenotype, with a possible slight anaemia or haemolysis. Therefore, the only significance of HbD is if it may be inherited with another β haemoglobinopathy.

After HbS, HbE is the second most common haemoglobinopathy in the world.[5] Due to its high prevalence in South East Asia, we see many cases of this in New Zealand. HbE is caused by a point mutation (GAG → AAG; c.79G>A) resulting in an amino acid change

from a glutamic acid to a lysine, which is similar to HbC, but in the twenty sixth residue position.[5; 14] This substitution mutation is so common that some reports have the incidence being as high at 50-70% in some parts of Thailand.[14] While HbE causes an abnormal haemoglobin, it also results in a decrease in synthesis, meaning it is a thalassaemic haemoglobinopathy, similar to HbCS as discussed earlier. These are the only two hemoglobinopathies in this category that appear in our database, therefore they are the only ones to be discussed in this paper. The decrease in haemoglobin is due to abnormalities in the mRNA produced by the mutated β globin gene, resulting in decreased post transcriptional processing.[5] This means that anaemia is usually present in even heterozygous HbE, and thalassaemic red cell indices can be seen. Compounding this, the haemoglobin produced with HbE is less stable during oxidative stress leading to increased red cell degradation.[5] However, in the heterozygous state the anaemia is usually mild and most often asymptomatic. Homozygous HbE, or 'HbE disease' may also be largely asymptomatic, though there are reports of possible splenomegaly or jaundice as a result of this disease.[5] On a CBC, the red cell indices appear similar to that of a β thalassaemia trait with reduced MCV and MCH. Again, due to the overlap in geographical incidence of HbE and β thalassaemia, co-inheritance is common. HbE β thalassaemia is the most common form of clinically severe β thalassaemia in Asia, and accounts for around 50% of all cases worldwide.[14] This usually presents as at least a β thalassaemia intermedia, with a range of symptoms from a moderate microcytic hypochromic anaemia to transfusion dependence and multiple organ involvement.[5; 14]

Hb O-Arab is caused by a change in the same glutamic acid as HbD, but in this case the amino acid is changed to a lysine. It is not frequently found in New Zealand, and though it seems to be found in a wide range of other countries, it is not common in any of those either. Despite the name given to this haemoglobinopathy, it is though that it too originated in Africa then spread to the Ottoman empire and to the Mediterranean and Middle East.[5] Much like HbD, even in the homozygous state HbO is of no clinical significance and entirely asymptomatic. Again, the diagnosis of HbO is only important when related to co-inheritance of other haemoglobinopathy or thalassaemia such as HbS or β thalassaemia.

2.3.4 Concomitant thalassaemia and haemoglobinopathies

As previously mentioned, due to overlapping high incidence regions, it is not uncommon for co-inheritance of different thalassaemia's and haemoglobinopathies. Different mutations interact in different ways, some have compounding effects and result in a more severe anaemia, but others balance each other out and produce milder symptoms.
Co-inherited α thalassaemia and β thalassaemia is an example of balanced mutations, as there is a decrease in production of both chains. This results in a slightly milder anaemia than what would normally be seen in β thalassaemia, and as there are no excess of either chain, the haemolysis and ineffective erythropoiesis is lessened. A similar response to α thalassaemia is seen across most β haemoglobinopathies, as fewer α chains are available to create abnormal haemoglobin with the mutated β chains. In the case of HbH disease, a co-inherited β thalassaemia trait will make the symptoms milder with fewer HbH bodies present, but some patients may still be transfusion dependent. In most cases, when co-inheritance occurs with a β thalassaemia and a β haemoglobinopathy, the anaemia or disease is more severe. Examples of this are HbS and β thalassaemia or HbE and β thalassaemia.[5; 9]

2.4 Symptoms and treatment

The symptoms of haemoglobinopathy vary greatly depending on the mutation present, as discussed above. As these are disorders of haemoglobin, these symptoms are largely related to anaemia or the effects of damaged RBC. Treatment for severe thalassaemia is blood transfusion, either regularly or as required depending on the type of thalassaemia present. Iron overload must be considered when giving regular transfusions, therefore iron chelation therapy is usually given concurrently.[15; 16] In New Zealand, iron chelation may be in a pill form (deferasirox) taken daily, which is funded but costs between $9.86 - $39.46 NZD per pill depending on dose (according to Pharmac volume 13, February 2025). An Australian study found that therapy costs for an adult haemoglobinopathy patient taking daily deferasirox costs more than $48,000 AUD annually.[17]

With sickle cell disease being the most common haemoglobinopathy, there has been more research into its treatment. Unlike thalassaemia, blood transfusion may be ineffective or cause further sickling of red cells and therefore other therapies must be considered. Hydroxyurea is a common treatment for sickle cell anaemia as this increases the level of HbF

present to compensate for the lack of HbA. This is still considered the ‘gold standard’ for treatment. In the USA the cost is around $2.20 USD per day, and in New Zealand it is funded but likely to cost less than $1 NZD per day (depending on dose).[18; 19] In fact, a study in 2000 by Moore et al.[19] found the cost of hydroxyurea for a group of sickle cell disease patients for one year to be substantially less than the cost to treat the control group receiving a placebo.[19]

Other pharmaceutical therapies have been developed specifically for sickle cell anaemia such as Voxelotor. This drug inhibits polymerisation of the abnormal haemoglobin chains, which increases the flexibility of the RBC therefore prolonging their half-life.[18] This was approved by the FDA in 2019 but a report in 2023 by Archarya et al.[18] found a clinical trial had 4 fatal adverse reactions to this drug in a group of 81. Also noted by these authors is the high cost of Voxelotor (sold under the brand name Oxbryta by Pfizer), estimated at $416.67 USD a day or a lifetime cost of around $1.2 million USD annually.[18]

Bone marrow or stem cell transplantation may be considered for treatment of any of the haemoglobinopathies, however this is an extremely expensive option and finding suitable donors can be difficult. If no family members can donate, then the risk of graft-versus-host disease increases and requires sufficient immunosuppression of the recipient patient. The benefit of treatment would have to outnumber the risks involved, and the likelihood of long-term medication. Therefore this is only recommended for SCD patients who have very severe symptoms.[18] The cost is also very great, in the USA this ranges between $350,000 - $800,000 USD without insurance.[19]

Gene therapy is also being developed, similar to a stem cell transplant, but with genetically altered cells of the SCD patient returned to them to produce normal RBC.[18] Recent advances in genetic therapy have made a cure possible, and this was first approved in 2018 after successful clinical trials with multiple versions being engineered since.[20] However, this groundbreaking technology also broke records for its price, approval for the β thalassaemia genetic therapy drug named Zynteglo was granted in 2022, and the cost per treatment was around $2.8 million USD.[21; 22] The drug is produced by Bluebird Bio, who claim this is less than the total cost to treat a β thalassaemia over their lifetime, which they estimated at up to $6.4 million USD.[21] This drug was not approved for use in Europe partly due to the high price tag, but it is still available in the USA.[23]

2.5 Diagnosis of a haemoglobinopathy

Currently in Pathlab Waikato, the tests performed to diagnose a haemoglobinopathy are the capillary electrophoresis, sickle solubility test, and 'i-LAB α thal' immunochromatographic α thalassaemia test. Any abnormalities not identifiable by these methods are sent to Canterbury Health Laboratories (CHL) for either mass spectrometry or genetic sequencing. Other methods are available such as high-performance liquid chromatography, which can be used in parallel with capillary electrophoresis or for confirmation testing. However, to increase efficiency and reduce cost, the decision was made by Pathlab that capillary electrophoresis alone is sufficient. The secondary testing applied by Pathlab is the use of the Sebia capillary electrophoresis machine with the HbA1c settings and reagents.

2.5.1 Capillary electrophoresis

Pathlab Waikato currently performs Capillary Electrophoresis (CE) on the Sebia Capillarys 3 Tera (CAPY) (Fig 2.4). Capillary electrophoresis is the next step in technology from gel electrophoresis where it has the added benefits of being faster, more precise and requires a smaller sample volume, at around 300µL. This volume was ascertained by laboratory staff as the minimum volume, however, the manual states at least 1ml of sample is required for dead volume, with only 20µL required for analysis.[24] The earliest records of the technology used in capillary electrophoresis come from the 19th century, when electrophoresis was carried out in a tube in 1860, though this was not used for separation until 1910.[25] Arne Tiselius was the first to use a version of electrophoresis to separate serum globulins in 1937, and he was later awarded a Nobel Prize in 1948 for his work.[25] Electrophoresis with a free solution in a capillary was established in the 1960's largely due to work by Frans Everaerts and Stellan Hjertén.[25] Modern CE was developed in 1981 by Jorgenson and Lukacs with the first commercial CE instrument coming in 1989[26]. This technology was applied to haemoglobin in 1994 by Hempe and Craver, who suggested the technique could be used to identify and quantify haemoglobin variants rapidly with high sensitivity.[27]

The CAPY analysers are used to measure glycated haemoglobin or HbA1c as a measure of blood glucose levels. High levels of glucose cause a non-enzymatic reaction to the N-terminal amino acids in haemoglobin over the roughly 120-day lifespan of a RBC. This is used as an index for diabetes control and insulin resistance. The CAPY is able to quantify the level of HbA1c present by separating the different haemoglobin molecules out according to size

through electrophoresis in silica capillaries. The charged molecules are separated out by their electrophoretic mobility in an alkaline buffer with a specific pH in a free solution. Whole blood (preferably EDTA) has a haemolysing solution added then is aspirated through the anodic end of the capillary, where a high voltage protein separation is performed. Haemoglobin variant detection is performed at the cathodic end of the capillary at 415nm (this wavelength is specific to haemoglobin). Detection of haemoglobin fraction occurs in the following order: A2/Hb C, HbE, HbS, HbD, HbF, HbA, HbA1c.

Figure 2.4: The Sebia Capillary electrophoresis (CAPY) 3 machines at Pathlab Waikato.

Thalassaemia and haemoglobinopathy detection on these machines is performed using a different reagent kit and alkaline buffer (with a pH of 9.4). Mutations in the globin genes that cause the haemoglobinopathies also cause a change in the charge and electrophoretic mobility of the haemoglobin, creating a separation from the wild type HbA and HbA2. CE has high resolution and is therefore able to distinguish between similar haemoglobinopathies or haemoglobin fractions that other methods may not be capable of, such as HbD and HbS, or HbE from HbA2 or HbC. An example of this can be seen in Figure 2.5, showing some common haemoglobinopathies such as beta Thalassaemia.

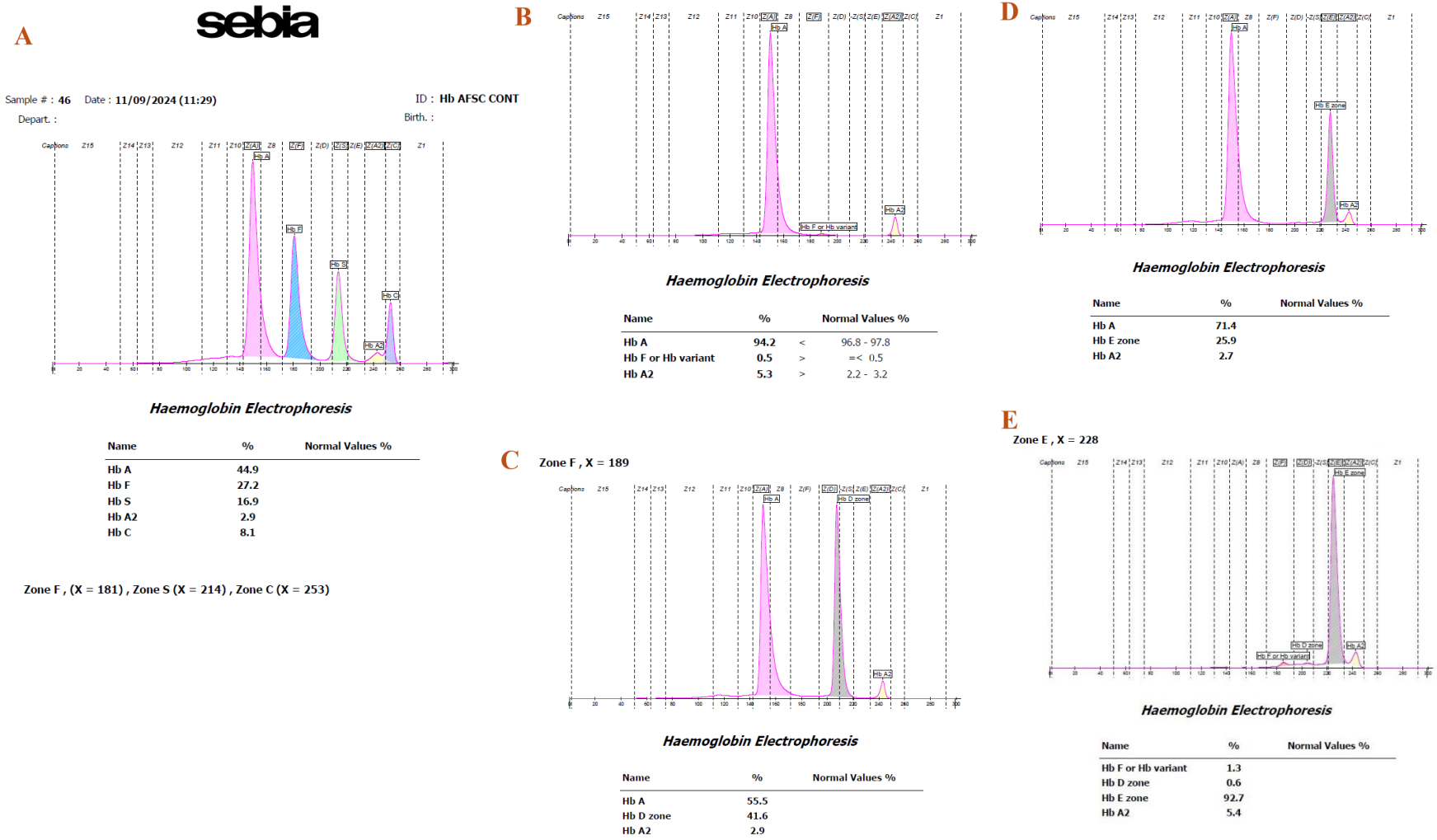


Figure 2.5: Capillary electrophoresis results as electropherograms.

A) An artificial control mix produced by Sebia showing the correct positions of haemoglobins A, F, S, A2 and C. This is used for calibrating the CAPY. B) a classic example of a beta thalassaemia, with a raised HbA2 and a slightly increased HbF. C) an example of HbD, showing the slight but perceptible change in position from HbS. D) a heterozygous HbE showing defined separation of the HbE and A2. E) homozygous HbE, showing the total lack of normal HbA, a slight increase in HbF and the A2 is less easily defined. Note the degraded HbE showing as a small peak in zone D.

2.5.2 High Performance Liquid Chromatography

Cation-exchange high performance liquid chromatography (HPLC) replaced gas chromatography (GC) from the late sixties. GC was only capable of separating volatile compounds by passing them through a gas-filled column. A chemist by the name of Albert Zlatkis from the University of Houston, USA organised meetings for "Advances in Chromatography" where the new technology was introduced in 1969.[28] HPLC had the benefit of being able to analyse many different kinds of molecule, including those that were polar or too large for GC. Though these meetings are regarded as the turning point for the popularity and widespread use of HPLC, the first mention of the technology is in a 1967 paper by Horvath et al. titled "Fast liquid chromatography: an investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers".[28]

HPLC is similar to CE in that it separates molecules based on charge. It can be broken up into two phases: stationary and mobile. The stationary phase measures adsorption of the molecules to a negatively charged chromatographic column. The mobile phase is the elution of these molecules by a liquid containing cations flowing through the column. The adsorbed haemoglobin molecules elute from the surface at different rates based on their affinity, and how easily the cations replace them. For example, the more positively charged haemoglobin molecules HbS and HbC have a longer retention time and therefore elute slowly and are detected last. The detection occurs optically via sensors. This method does have some overlap between certain haemoglobinopathies, therefore does not give a result as precise as CE. In both methods the location of the abnormal haemoglobins is determined by relation to a known or normal haemoglobin (such as HbA2)

2.5.3 Confirmation testing

A number of confirmatory tests can be performed before a diagnosis is made. These are used to distinguish between multiple haemoglobinopathies that move close together, or if a diagnosis cannot be confidently made based on the CE or HPLC. The process of haemoglobinopathy diagnosis in Pathlab can be seen in Fig 2.6, which shows the decisions made at each step of analysis.

The first of these is the sickle solubility test, used to confirm HbS. Haemoglobin variants such as HbD-Punjab travel in close proximity to HbS due to similar electrophoretic mobility,

so it is important to distinguish between them, as HbS is more clinically relevant. The principle of the sickle solubility test is to create a low oxygen environment to force HbS cells to form into the sickle shape. The test can be affected by a low haematocrit, severe anaemia, or if the HbS is present in reduced amounts due to a co-inherited thalassaemia.

There are two supplementary tests currently in use for the confirmation of alpha thalassaemia. The first of these is the HbH stain, this is done on any patient that has microcytic hypochromic indices. The reticulocyte stain new methylene blue is used (brilliant cresyl blue may also be used), and this is incubated with whole blood at 37˚C for 90 minutes, then two films of the solution are set up and viewed by two scientists. This is a sensitive and specific method for detecting HbH bodies that occur due to excess beta chains in alpha thalassaemia. Secondly is the i-LAB α thal test strip, which is a chromatographic immunoassay for excess γ chains. Packed red cells are used to avoid heterophile antibodies or autoantibodies that may cause false positive results, these are lysed and a test strip added. Once washed, a band is visible to indicate the presence of γ chains, confirming alpha thalassaemia. This test cannot be used in patients with a raised HbF (>5%) due to the increase in γ chains in these patients, which may cause a false positive.

2.5.4 Mass Spectrometry

This technique can be used when screening cannot give a definitive result, as a next step to avoid full gene testing. The technology required for Mass spectrometry (MS) originated from the “hunt for the electron” in the first three decades of the 20th century by physicist JJ Thomson.[29] This technology was commercialised and improved through 1940 to the 1980’s to reduce the footprint and increase sensitivity. In the late 1980’s ionisation techniques such as electrospray ionisation (ESI) and matrix-assisted laser desorption/ionization (MALDI) were developed, which are both used today to study proteins and peptides.[29] MS is a biochemical technique that separates out molecules based on their charge and size and gives a profile of the substance present. This profile can be compared to known profiles from a database to determine what the genetic composition or what amino acids are present. This can be used to confirm or rule out certain haemoglobinopathies to save the time and cost of sending a sample to a genetic testing facility. As an example of this, we currently send all samples that require further testing to Canterbury Health Laboratories, NZ, who perform ESI-

TOF (time-of-flight) mass spectrometry. This allows assessment of the α, β, δ, and γ globin chains.

2.5.5 Genetic testing

Genetic analysis to detect mutations in the globin genes can be carried out if the abnormality is unknown or unable to be diagnosed, or if the exact type of thalassaemia needs to be determined. For example, to determine the risk to a child, it might be necessary for genetic studies of the parents to determine what β thalassaemia mutation they carry. This is also useful for determining the α thalassaemia mutations present, as this can only be achieved using genetic techniques, and it may be important to determine if a patient is heterozygous or homozygous, and what the size of the deletions are. Polymerase Chain Reaction (PCR) of extracted RBC DNA and *Hb* gene specific primers followed by Sanger Sequencing can be performed to analyse or diagnose haemoglobinopathy that is abnormal, novel or otherwise unable to be confirmed by other methods.[5] Copy number variations that occur in thalassaemia can be detected via methods such as multiplex ligation-dependent probe amplification (MLPA) for large scale deletions that may be otherwise difficult to detect.[30]

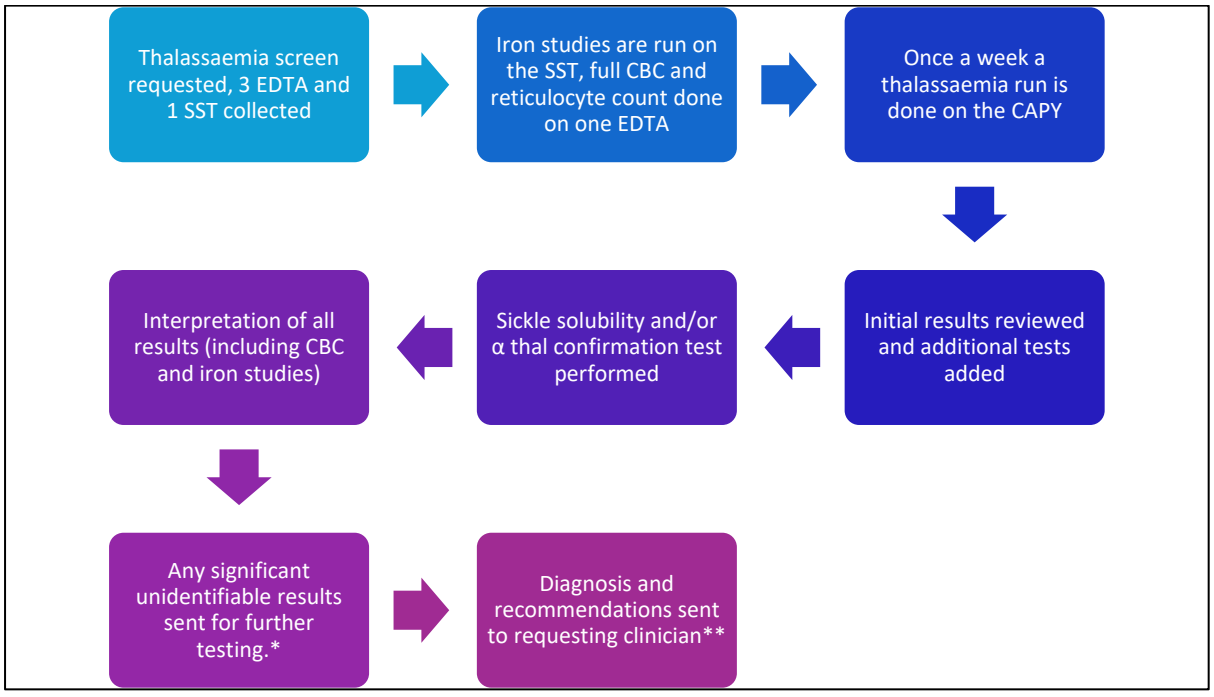


Figure 2.6: Flow chart of haemoglobinopathy screening in Pathlab Waikato.

**The testing to be done is determined by the Haematologist. If there is a variant to confirm then mass spectrometry may be enough. If a significant thalassaemia is suspected, then a genetic screen may be required to confirm.*

*** The format for the results includes a link to an information leaflet for the relevant haemoglobinopathy and a recommendation for family testing if the result is significant.*

2.6 Epidemiology

Haemoglobinopathy is the most common inherited mutation. A report by the WHO in 2006 suggested around 5.2% of the total world population may be carriers of a pathogenic haemoglobinopathy, and over 7% of pregnant women. Therefore, each year over 300,000 infants are born with a haemoglobinopathy or thalassaemia.[31; 32] Though sickle cell anaemia makes up 70% of these births, the carrier rate of thalassaemia is higher than haemoglobinopathy.[32] However, the prevalence of carriers of thalassaemia and haemoglobinopathy is difficult to predict, as these individuals are asymptomatic, this largely requires diagnosis by incidental finding or through family studies of effected individuals.

The increase in global travel and immigration will be changing the prevalence and type of haemoglobinopathy and thalassaemia seen in different geographical areas. Historically, global events such as European colonisation, the African slave trade, globalisation and humanitarian crises have significantly affected the ethnicities of certain regions, and therefore also the epidemiology of haemoglobinopathy.[33] An example of a humanitarian crises affecting the prevalence of haemoglobinopathy in a population is in 2011-2012 Myanmar had the highest number of net migration arrivals into Australia, an ethnically diverse but relatively low risk country for haemoglobinopathy.[17] In the Myanmar population, carrier prevalence for α, β, and HbE are 36.8%, 27.2% and 24.9%, respectively.[17]

This is emphasised in a 2008 report on global epidemiology by Modell et al. who state that haemoglobin disorders were originally only endemic in 60% of countries, potentially affecting 75% of births, but they are now common in 71% of countries affecting 89% of births.[31] In 2021, the reported world-wide number of thalassaemia cases was 1,310,407, with a prevalence rate of 18.28 per 100,000 people.[15] Haemoglobinopathy and thalassaemia are responsible for around 3.4% of mortality in children under five, but in Africa this increases to around 6.4%.[15] Table 2.1 contains prevalence data from the 2003 research by Modell et al. broken up into the WHO defined regions. This contains information on: α thalassaemia, significant haemoglobinopathy including β thalassaemia and HbS, and any variant including haemoglobinopathies that are not clinically significant.[31]

Table 2.1: A summary of the percentage of the population in the specified WHO regions that carry variants.[31]

WHO Region*	Significant variant**	α^+ thalassaemia (Het. or hom.)	Any variant***
Africa	18.2%	41.2%	44.4%
America	3.0%	4.8%	7.5%
Eastern Mediterranean	4.4%	19.0%	21.7%
Europe	1.1%	2.3%	3.3%
South-East Asia	6.6%	44.6%	45.5%
Western Pacific	3.2%	10.3%	13.2%
World	**5.2%**	**20.7%**	**24.0%**

* WHO regions (for full list – see appendix 1)

**Significant variants include HbS, HbC, HbE, HbD, β thalassaemia, α^0 thalassaemia

***Clinically non-significant haemoglobinopathies including some that are co-inherited with α or β thalassaemia that are not disease causing.

Alpha thalassaemia is the most common monogenic disease, effecting up to 20% of the world's population.[9; 31] The 2012 paper by TN Williams and DJ Weatherall states the annual number of births with HbH disease is 9,568 and the number with Hb Bart's Hydrops is 5,183.[34] It is common in many countries in Asia, with some regions of China reporting a carrier prevalence of up to 12%, and HbH disease incidence of 0.2%.[9] In India, α thalassaemia prevalence varies from 10-25%, with some regions reporting even higher. In Southeast Asia, prevalence of α thalassaemia ranges from 17.3% in Malaysia to over 50% in Vietnam.[9; 31] In Papua New Guinea, α thalassaemia is nearing fixation in the population with prevalence reportedly around 80-90%. Incidence rates are lower in Europe, but around the Mediterranean prevalence ranges from 3-4% in Turkey and Italy, to up to 60% in Eastern Saudi Arabia.[9; 35] In Africa, some countries such as Kenya and Nigeria have reported up to half the population to be carriers to some extent.[9; 35] Closer to home, α^+ thalassaemia is common in individuals of Māori and Pacific Island descent. However, only one paper was sourced from the current literature that focused on the prevalence of the $\alpha^{3.7}$ mutation in the Māori population, and this was written in 1989 by LS Parker et al.[36] This paper in the New Zealand Medical Journal suggested the incidence of this mutation in the combined Māori and Pacific Island population was 16.3%.[36]

According to a 2008 report by the WHO, over 40,000 babies are born each year with β thalassaemia, and over half of these are transfusion dependent.[37] The expected proportion of these births per region are 51% in Southeast Asia, 25% in the Eastern Mediterranean region, 2.5% in Europe and 1% in the Americas.[37] The overall carrier rate is expected to be 1.5% worldwide, though again this can be hard to estimate.[37] Even within regions, allele frequencies vary greatly. β thalassaemia follows a somewhat similar pattern of incidence to α thalassaemia, with reportedly between 1-16% in Southern China, 3-4% in India 4-11% in Middle East countries, and 1.5-3% in North Africa.[9] The countries surrounding the Mediterranean appear to have a high prevalence of β thalassaemia, with Cyprus being as high as 17% incidence before implementation of prenatal screening, which has caused this to drop to around 12%.[9] Incidence rates of β thalassaemia have risen and fallen in different parts of the world for a number of reasons, largely migration or prevention and screening programmes.

Sickle cell disease is known to be more common in regions where there is also a high level of malaria. The majority of cases of HbS come from Africa or from ethnic groups that are of African descent. The higher prevalence of sickle cell anaemia in many parts of the Americas and other parts of Europe can be largely attributed to large migrations from Africa due to historic events and humanitarian crises such as the slave trade. The study in 2008 by Modell et al. stated the worldwide total annual number of conceptions with a sickle cell disorder was 276,168.[31] And around 84% of these were from the WHO African region, with the next two largest contributions being the Southeast Asia region (9.4%) and the American region (3.3%).[31]

HbC is also most common in Africa where it is thought to have originated from, particularly the area including Ghana, Togo, Benin, Burkina Faso, and Côte d'Ivoire. In Ghana and Côte d'Ivoire the incidence reaches 40-50%.[5] Because of this origin it is also found in individuals of African descent from the Caribbean (3.5%), United States of America (2%), United Kingdom and Canada.[5] This mutation is also found in lesser amounts in Southern Europe, the Middle East and Southeast Asia.[5]

The highest incidence of HbD is found in the Punjab population, which is why it was named as such. However, this is only reported as being 2-3%.[5] Again, this mutation is also found in individuals of African descent and also in low frequencies in Caucasian populations across

Europe.[5] The relatively high incidence in some areas of the United Kingdom are purportedly due to the British stationing of large regiments in India in the 19th-20th century.[5]

HbE is the second most common haemoglobinopathy after HbS and is overwhelmingly seen in Southeast Asia compared to other regions. For example, in some regions of Thailand the prevalence is estimated to be 50-70%.[14; 38] The most common HbE co-inheritance is with β thalassaemia, which reportedly accounts for around 50% of all severe β thalassaemia disease globally.[38] This is likely due to the fact that there is significant overlap in the countries with high incidence of both mutations.

2.7 Screening Programmes

Many countries offer screening programs as part of an antenatal screen, newborn screen, or in some high prevalence countries there is a premarital screen. For this review, the screening services of many countries were assessed. These all follow a similar pattern, for high prevalence countries such as India and China, there are many systems already in place to monitor and diagnose haemoglobinopathy and Thalassaemia. However, both countries face the same issues, having such a large and diverse population over many different geographical and political areas makes consistency for screening difficult, and it is likely still underdiagnosed in many populations.

In India the average estimated prevalence of β thalassaemia is around 3-4% (though ranges between 1-17%), and HbE has a prevalence as high as 40-50% in some regions.[39] In China there are an estimated 30 million carriers with 300,000 individuals with either thalassaemia major or intermedia.[33] A number of European countries are also beginning to screen for haemoglobinopathy, and many of these cite increasing immigration as one of the main causes for an increase in incidence. Spain, Great Britain, the Netherlands, and Denmark all offer some form of screening, and Germany now offers sickle cell screening as part of the newborn screen introduced in 2021. A study from Australia in 2016 suggested the need for a centralised screening and reporting centre or registry. Reports from all of these countries made similar conclusions, that the first step was a robust registry or database where all diagnoses of haemoglobinopathy can be stored. Once this is established a decision is to be made on who to screen and who is at risk. Conflicting reports are given on screening only

"high-risk" ethnicities versus screening all of a population group (such as antenatal or newborn screening). However, this is largely down to the perceived risk in the country and projected strain to be placed on the healthcare system, either by the increase in screening or the increase in patients with severe haemoglobinopathy requiring lifelong treatment. The Danish study calculated that to offset the cost of the screening programme, they would only need to reduce the number of transfusion dependent patients by one every two years.[40] Prevalence is still low in Denmark, and even though most of their cases of haemoglobinopathy are in individuals who have immigrated to Denmark, targeting screening of at risk ethnicities may need to transition to a universal screening service due to the anticipated increase in incidence.[40]

The guidelines for thalassaemia and haemoglobinopathy screening used by Pathlab are based on the United Kingdom's National Health Service (NHS) Sickle Cell and Thalassaemia Screening Programme.[41] As the population of high risk ethnicities in the United Kingdom is increasing, they now offer both antenatal and newborn screening. It is estimated that thalassaemia major affects 1 in every 27,000 pregnancies, therefore around 20-30 babies are born with the disease each year.[41] The NHS is separated into different "Trusts" that cover different areas, similar to the New Zealand health system where Te Whatu Ora involves the entire country but former District Health Board's or hospitals have their own regions. These trusts are deemed high or low risk for haemoglobinopathy, with a threshold of ≥2% of screens being positive for high-risk. In these high-risk regions, all women are screened regardless of ethnicity. In low risk only those identified as potential carriers are tested, for example with CBC results suggestive of thalassaemia or family history. However, all women should be offered screening for thalassaemia. These guidelines also stress the importance of screening the father when a mother is high risk or diagnosed with a haemoglobinopathy. Pathlab use these designations for "high risk" vs "low risk" carrier states for certain haemoglobinopathy or thalassaemia. This changes the comments made for diagnosis and whether or not partner or family testing is recommended. For higher risk patients this also determines immediate referral to a haematologist. The classification used by Pathlab can be summed up in the following table based on that used in the NHS sickle cell and thalassaemia screening handbook (Table 2.2)

The NHS screening programme handbook was also referenced when establishing the processes for screening at Pathlab. CE technology is used through the CAPY on a thalassaemia programme for the screen, and on a HbA1c programme for the confirmation. Additional testing is carried out to confirm HbS through sickle solubility testing, and confirmation of α thalassaemia is through HbH staining and chromatographic immunoassay (i-LAB α thal kit).

Table 2.2: Combinations of parental carrier states and their risks to the unborn child.

		Father Carrier of:								
		HbS	β thalassaemia	δβ thalassaemia	HbE	HbO^{Arab}	HbC	HbD^{Punjab}	HPFH	Not a carrier
Mother carrier of:	HbS									
	β thalassaemia									
	δβ thalassaemia									
	HbE									
	HbO^{Arab}									
	HbC									
	HbD^{Punjab}									
	HPFH									
	Not a carrier									

	Serious risk – referral for genetic and haematological counselling, prenatal diagnosis done
	Less serious risk – referral for counselling, may require further investigation.
	No risk – No further action required.

3. Methods

3.1 Statement on Human Ethics

Ethics approval was granted by the University of Waikato Human Research Ethics Committee for the research conducted for this thesis (HREC(Health)2023#45). Approval and permission were also granted by Pathlab from the former CEO Dianne McQueen and Haematology lead of speciality Alan Neal. The ethics submission and approval, along with a letter of support from Pathlab can be found in the Appendix 1 (Documents 7.1 and 7.2, respectively). In accordance with the human ethics approval, all data used for this research had any identifying patient information such as name, date of birth, or doctor information removed before analysis began. This data is held and only accessible on password protected computers on site at Pathlab. Databases with no identifying patient information can be made available upon reasonable request at the discretion of Pathlab and Alan Neal.

3.2 Screening

At Pathlab Waikato a thalassaemia screen is performed in this precise order:

1. CBC, reticulocytes and iron studies carried out.
2. Once a week whole blood collected in EDTA are electrophoresed using the thalassaemia programme on the CAPY machine.
3. The samples are then run again using the HbA1c programme.
4. Initial interpretation is conducted, and additional tests are added as required.
5. Sickle solubility and α thalassaemia confirmation test strips are completed.
6. Any required send-away tests such as mass spectrometry or DNA sequencing are discussed with the haematologist and sent to corresponding laboratories.
7. Final interpretation is completed, and the result is confirmed by a second person before a result is released to the requesting clinician.

Initial interpretation is based on the presence or absence of any abnormal haemoglobin, the amount of HbA and HbA2 reported, and the red cell indices in the CBC. Any microcytic hypochromic red cell indices are checked for other possible causes such as iron deficiency or anaemia of chronic disease. These can be distinguished by following the rules outlined in

Table 3.1. For patients with no obvious cause of low MCV and MCH, and no HbH bodies detected, an i-LAB α thal test strip is done. For these patients, if the strip is negative, the result given is "Unable to exclude alpha thalassaemia, recommend family testing." This is due to the fact that single α gene deletions are very difficult to detect as they are very mild. If an abnormal haemoglobin is detected and runs in proximity to HbS (214nm), then a sickle solubility test is done to confirm or rule out HbS. This is done for all suspected HbD-Punjab as this abnormal haemoglobin runs at around 208nm. The other common haemoglobinopathies can be diagnosed based on the location of the peak, and the results go out with a comment that recommends partner testing for individuals of reproductive age, or for testing of family members for those that are not of reproductive age (the age range of 16-50 is used by Pathlab Waikato). The result includes a link to an information leaflet for the patient giving a brief outline of the disease and the risks to the patient (see Appendix 1, Document 7.3 for an example).

Table 3.1: Common causes of low MCV and MCH and diagnosis using full iron studies.

Serum Iron Test	Thalassaemia	Iron deficiency	Anaemia of Chronic Disease
Ferritin	Normal to High	Low	Normal to High
Iron	Normal to High	Low	Low
Transferrin	Low to Normal	High	Low to Normal
Iron Saturation	Normal to High	Low	Low

3.3 HbA1c

As previously mentioned, the HbA1c method used at Pathlab Waikato is capable of detecting abnormal haemoglobins. Every time an abnormality is detected, a comment is added to the HbA1c result by a Biochemistry Scientist. These comments vary depending on the abnormality detected, the effect this has on the reading of HbA1c, and the status of any pending haemoglobinopathy diagnosis, and will be specified as one of the following:

- "Abnormal haemoglobin peak detected which may have clinical significance (and interference with HbA1c result). Thalassaemia /haemoglobinopathy screen recommended - unless known case."

- “Abnormal haemoglobin peak detected during HbA1c analysis. This variant haemoglobin may cause haemolysis (see CBC) and therefore HbA1c cannot be measured accurately. When screening for diabetes, a fasting glucose and/or glucose tolerance testing should be used. Suggest thalassemia screen if result is unexpected-unless known case.”
- “Abnormal haemoglobin peak detected which may have clinical significance. This sample is awaiting a thalassemia screen for confirmation. The HbA1c result is correct by this method.”

If the patient has previously been diagnosed or a diagnosis is given on the request form, the HbA1c comment will be “This patient has a known haemoglobinopathy/Thalassemia.” If any of the previous comments are added to a woman of childbearing age (16-50), an email is reflexed to a haematology scientist to follow-up. This involves checking to see if the patient is pregnant or planning pregnancy, if they have been previously diagnosed by Pathlab or Waikato hospital, and whether or not the abnormality is significant. For unknown significant abnormalities in pregnant (or suspected pregnant) women, a phone call is made to the requesting clinician to strongly suggest adding a thalassaemia screen for this patient, and to recommend their partner also be tested for haemoglobinopathy. As this is a genetic disease and has implications beyond the patient, we require permission from both the clinician and the patient before testing is performed. If a significant abnormality is confirmed, a comment is added to the result to recommend this result be discussed with the high-risk pregnancy team at Waikato Hospital, who are notified of the patient’s status. If partner testing is performed and the partner is also found to have a haemoglobinopathy, this is urgently passed on to the Haematology department (and/or the on-call Pathlab Haematologist) to follow-up with the patient and their primary care clinician (midwife or general practitioner (GP)).

All other patients who have one of the comments in their HbA1c result but do not fit the above criteria, are compiled into a weekly list. This is analysed by a haematology scientist and a presumptive diagnosis is given based on the CBC and HbA1c result. If this diagnosis is determined to be significant then a formal letter is sent to the requesting clinician, asking that a thalassaemia screen be done on this patient due to a high suspicion of the relevant haemoglobinopathy. The uptake or effectiveness of these letters is difficult to determine, as only the occasional thalassaemia request form will disclose this, and it is not recorded anywhere.

3.3.1 Significance Criteria

As previously stated, many decisions are made based on the proposed significance of a haemoglobinopathy. This is largely determined by the guideline set out by the NHS antenatal thalassaemia screening guidebook, and this can be seen in Table 3.2. For the abnormal HbA1c screening as described above, the exact criteria are shown in this table.

Table 3.2: The criteria used to determine if the abnormal haemoglobin detected during a HbA1c test is significant and therefore requires follow-up. Conditional significance applies to patients that are female and between the ages of 16-50. All non-significant results will still have the relevant comment attached to the HbA1c that recommends thalassaemia.

Presumptive Diagnosis	Always Significant	Conditional Significance	Not Significant
Alpha thalassaemia	✓		
Alpha Variant			✓
Beta thalassaemia	✓		
Raised A2 with normal CBC		✓	
Beta Variant			✓
Hb Constant Spring	✓		
Delta thalassaemia			✓
Delta Variant			✓
Delta Beta Thalassaemia	✓		
Het or Hom HbD-Iran	✓		
Het or Hom HbC	✓		
HbD can't excl. D Punjab		✓	
Het HbD + α thalassaemia	✓		
HbH	✓		
Het HbE		✓	
Het HbE + α or β thalassaemia		✓	
Het HbE + other		✓	
Hom HbE	✓		
Het HbS	✓		
Het HbS + α or β thalassaemia	✓		
Het HbS + Other	✓		
Hom HbS	✓		
HPFH/Raised HbF		✓	
Unknown – normal CBC			✓
Unknown – abnormal CBC	✓		

3.4 Entering Medical Information and Results into the Pathlab Internal Haemoglobinopathy Database

After every run of thalassaemia screens completed at Pathlab, any patient with a positive result for thalassaemia or haemoglobinopathy has their metadata entered into a confidential online internal database (Microsoft SharePoint list). Therefore, it is updated in real-time and accessible to all authorised Pathlab staff. Figure 3.1 shows a representative image of the information that is collected from the patient such as their name, national heath index (NHI) number and lab number. It also contains detailed information on the haemoglobinopathy or thalassaemia detected. The columns for this are as follows: Thal type, HbA2%, HbF%, HbH result, Alpha RDT kit result, sickle solubility result, and variant zone position and percentage. Red cell indices and ferritin are also included, along with an attached image of the capillary result, and boxes to indicate if genetic testing has been done or a partner NHI if provided.

3.5 Census population data

Data from the previous three census (2013, 2018, 2023) can be found online at stats.govt.nz. Through the Aotearoa Data Explorer tool on this website, under “Society”, select “Ethnicity, culture, and identity”. From here select the “Birthplace, ethnicity (detailed total responses level 3), and age for the census usually resident population count, (RC, TALB, SA2, Health), 2013, 2018, and 2023 Censuses”. Under these dimensions, for the most relevant data for our research, filter only for the areas as defined by “health region/health district”. With this filter we can further define only the Waikato and Bay of Plenty, under Te Manawa Taki. Ethnicities can be filtered for, but for a broad overview we can select the subcategories: European, Māori, Pacific Peoples, Asian, Middle Eastern/Latin American/African, other ethnicity, total stated – ethnicity, and not elsewhere included. Following this, for the graphs and data used in this research, the layout is changed so that the rows contain only ethnicity, and the columns are both area and census year.

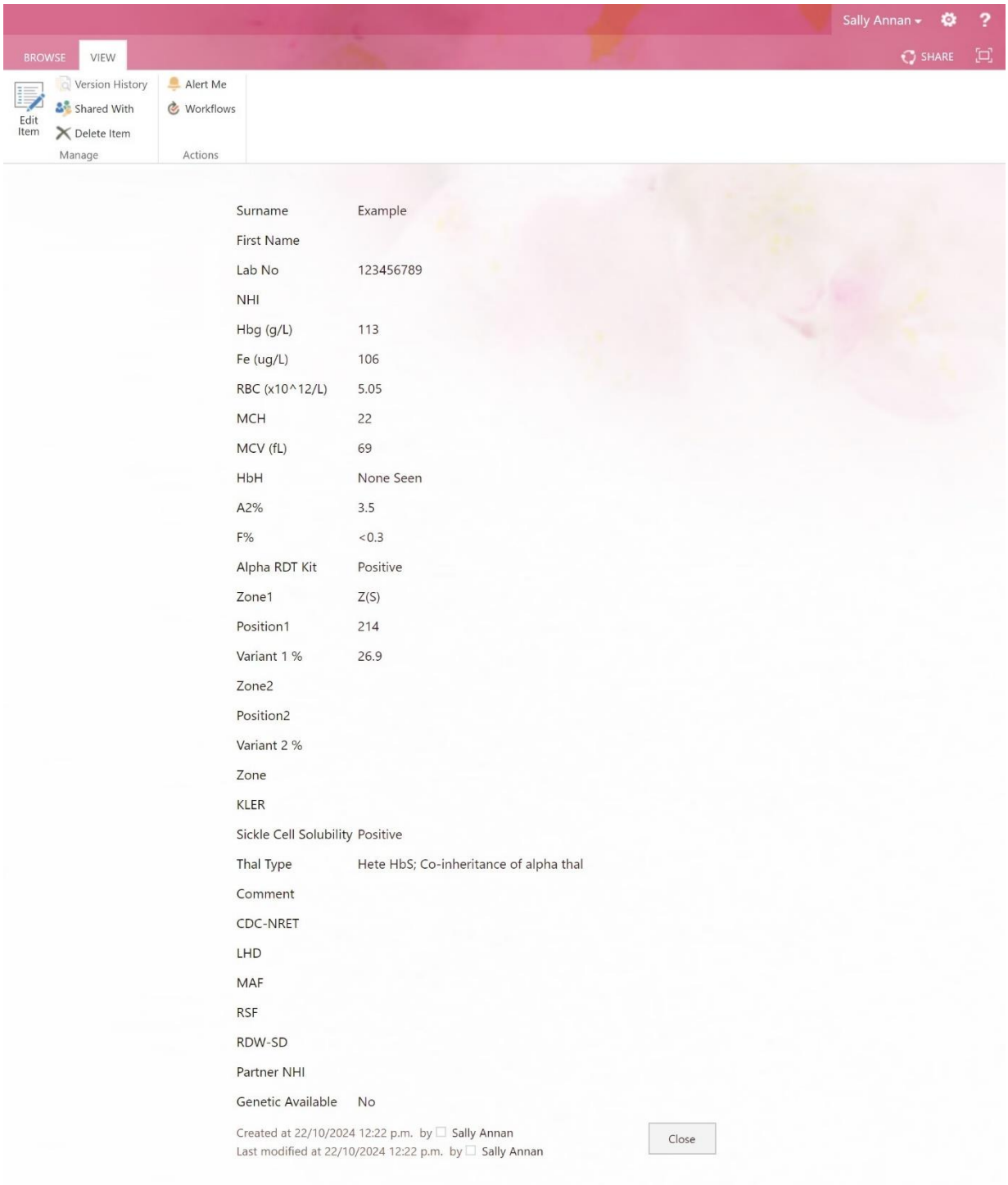


Figure 3.1: An example of an entry into the database.
In this example the HbH stain was negative, a sickle solubility test was positive, and α thalassaemia test strip ("Alpha RDT Kit") was positive. It shows an abnormal haemoglobin was detected in the HbS zone (travelling at 214nm) and made up 26.9% of the haemoglobin present. All this information supports the diagnosis given at the bottom which is heterozygous HbS with a co-inheritance of α thalassaemia. These results are copied from an actual patient entry into the database, with all identifying information removed.

3.6 Literature Review

A thorough review of relevant literature was conducted, using a variety of online search engines. Foremost was the University of Waikato Library search, as this has access to many different publications. For the past 3 years an ExLibris search query has been ongoing for any new papers published containing the search terms ‘thalassaemia’ or ‘haemoglobinopathy’. Subscriptions to both the British Journal of Haematology and the American Society of Haematology Journals were used to stay up to date on any new research or published articles. Pathlab Waikato maintains a collection of books and papers relevant to practices, including the 3rd Edition of Haemoglobinopathy diagnosis by Barbara J. Bain, and both a physical copy and links to the NHS Sickle cell and thalassaemia screening programme. Key words used for online database searches were: haemoglobinopathy, thalassaemia, sickle cell anaemia, beta thalassaemia, and haemoglobinopathy prevalence and incidence.

3.7 Hardy-Weinberg Equation

The Hardy-Weinberg law was discovered in 1908 by mathematician G.H. Hardy and geneticist W. Weinberg.[42] It is used to calculate gene and allele frequencies in a population. The equations works under a number of assumptions, firstly and most importantly is that there is no selective breeding (i.e. only random mating), and other assumptions that can be bent slightly are that the population is not finite, stable with no migration, and that there is no mutation.[42; 43] Mitton (2002)[42] explained that frequencies are accurate for over 100 breeding individuals and it is not biased by mutations that occur at the rates expected for most nuclear genes.[42] The equation is depicted by Equation 3.1:

$$p^2 + 2pq + q^2 = 1$$

Equation 3.1: Hardy-Weinberg Equation. With p being the allele frequency of the dominant allele, and q being the allele frequency of the recessive allele. p^2 is the frequency of homozygous dominant genotype, q^2 is the frequency of the homozygous recessive genotype, and 2pq is the frequency of the heterozygous genotype.

3.8 RStudio

The data was analysed using Rstudio (version 2024.09.0+375 (Cranberry Hibiscus)). An annotated copy of the code and all the produced tables of data can be found in Appendix 1. This was used to determine the relationship between abnormal haemoglobinopathy and ethnicity. Both analysis of variance (ANOVA) and an estimated marginal means (emmeans) analysis was performed. The level of statistical significance was set at a p value of less than 0.001.

4. Results

Between a seven-year period, 2017 to 2023, Pathlab Waikato performed 6,589 thalassaemia screens. Of this, 3,525 cases were positive. The number of tests done increased steadily for each of these years with the exception of 2022. This may be due to the effect of the COVID-19 pandemic decreasing immigration or lockdowns decreasing regular testing. However, in 2023 there were the highest number of tests on record with 1229 performed and 671 found to have a haemoglobinopathy. This can be seen in Figure 4.1 with the upward trend clearly visible.

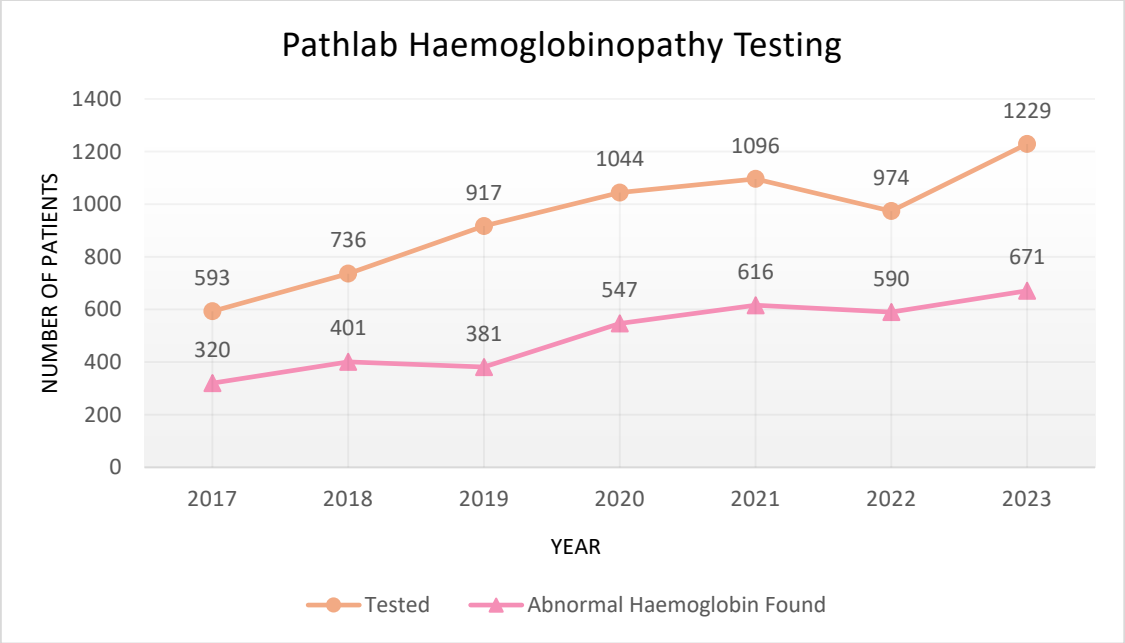


Figure 4.1: Total number of thalassaemia screens performed at Pathlab from 2017-2023 (orange), compared to the number of positive screens for this period (pink).

4.1 Haemoglobinopathy Database

Our database contained 3,525 entries of various haemoglobinopathy diagnosed by Pathlab Waikato between 2017-2023. The largest group is the "unable to exclude α thalassemia", which is a designation used when α thalassemia is suspected due to a MCH of less than 26, and no evidence of iron deficiency, but is unable to be confirmed as α thalassemia due to lack of HbH bodies and a negative i-LAB α thal test strip. These alone make up around 27%

(or 1,022 individuals). The other two most common singularly inherited categories are α and β thalassaemia, being 19% (732) and 18% (711), respectively. This information can be better visualised by Tables 4.1, 4.2, and Figure 4.2 below.

Table 4.1: Summary of all the haemoglobin abnormalities detected between 2017-2023 at Pathlab.

	HbE	HbS	HbD	HbC	HPFH/ raised HbF	Delta Beta
Het	358	171	135	49	67	11
Hom	38	4	0	0		
Het + co-inherited	105	15	7	1	1	

	Alpha	Beta
Unable to exclude	1,076	109
confirmed	820	723

	Other/ unable to be identified	Delta variants
Not clinically significant	257	11
Clinically significant	21	

Table 4.2: A table showing all the different haemoglobinopathy diagnoses between 2017-2023, and their prevalence based on the 2023 population.

Diagnosis	Confirmed diagnosis given	Unable to exclude**	Prevalence (%)
α Thalassaemia*	820	1076	0.0984
β Thalassaemia	723	109	0.0868
Heterozygous HbE	430		0.0516
Homozygous HbE	39		0.0047
Heterozygous HbS	186		0.0223
Homozygous HbS	4		0.0005
Heterozygous Hb D	144		0.0173
Heterozygous HbC	51		0.0061
Raised HbF/HPFH	71		0.0085
HbCS	14		0.0017
δβ thalassaemia	11		0.0013
Unable to be identified, Suggest Gene Studies (UHPA)***	44		0.0053
Unable to be identified, Normal RBC indices (UHPN)***	268		0.0322

Prevalence given is based on the population of Waikato and Bay of plenty regions from the 2023 census data. Note, if a patient had concomitant thalassaemia or multiple haemoglobinopathy they were each counted into their respective categories, therefore one patient may be counted more than once.

*Alpha thal includes HbH disease and any patient that was diagnosed with concomitant alpha thal. The other haemoglobinopathy present in that patient is also counted in the number of diagnoses for that type.

**Including those concomitant with another haemoglobinopathy.

***UHPA and UHPN include all α, β, and δ variants that are not one of the other common haemoglobinopathies named here

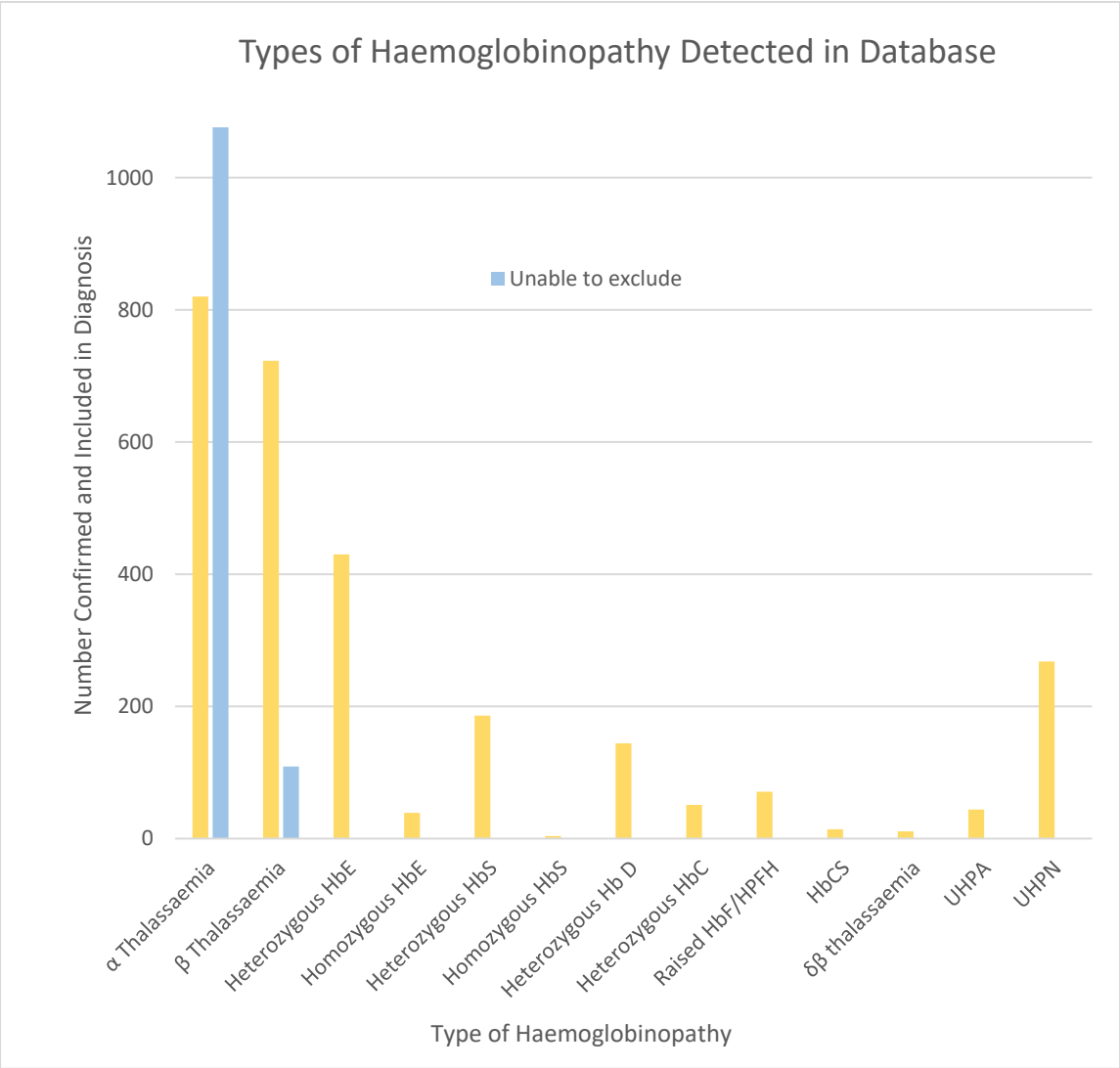


Figure 4.2: Types of haemoglobinopathy detected in the database.

4.2 Haemoglobin A1c data

In the year 2023, Pathlab Waikato performed 291,012 HbA1c tests. Of these, 859 had abnormal haemoglobin detected with only 219 of these being previously diagnosed haemoglobinopathy. Table 4.3 shows the breakdown of the ethnicity of all the patients who had the HbA1c test in this year. There were 16 different ethnic groups identified, the largest two being New Zealand European and Māori making up over 70%, and the smallest being Middle Eastern and Pacific Peoples not further defined with less than 700 individuals in each. We can also see how many of these individuals were found to have an abnormal haemoglobin, and how many of these were previously diagnosed (Fig 4.3 and 4.4).

Ethnicities	Proportion of Total Population	No Haemoglobin abnormality	Haemoglobin Abnormality	Proportion with abnormality	TOTALS	Proportion of abnormalities previously diagnosed
New Zealand European/ Pakeha	55.9	162455	260	0.16%	162715	10.38%
Māori	17.1%	49767	37	0.07%	49804	16.22%
Unknown	8.7%	25214	104	0.41%	25318	25.00%
European NFD	7.1%	20669	55	0.27%	20724	9.09%
Indian	4.1%	11837	149	**1.24%**	11986	21.48%
Asian NFD	1.9%	5452	104	**1.87%**	5556	28.85%
Chinese	1.2%	3563	22	0.61%	3585	13.64%
Southeast Asian	1.0%	2856	68	**2.33%**	2924	20.59%
Samoan	0.5%	1361	2	0.15%	1363	0%
African	0.4%	1203	20	**1.64%**	1223	25.00%
Latin American/ Hispanic	0.4%	1175	15	**1.26%**	1190	20.00%
Fijian	0.4%	1170	11	0.93%	1181	27.27%
Cook Island Māori	0.3%	996	1	0.10%	997	0%
Tongan	0.3%	783	3	0.38%	786	0%
Pacific Peoples NFD	0.2%	683	2	0.29%	685	0%
Middle Eastern	0.2%	619	6	0.96%	625	66.67%

Table 4.3: Ethnicity data from the testing of HbA1c for the year 2023.

Unsurprisingly, the populations with the highest percentage of previously diagnosed haemoglobinopathies are also those with the highest levels of haemoglobinopathy. The only group that does not fit this trend is those identified as Southeast Asian. This group has the highest percentage with haemoglobinopathy, yet less than 21% of these were found to be previously diagnosed.

Table 4.4 below shows the number of individuals tested for HbA1c that had an abnormal haemoglobin detected, against the number of individuals who were and were not previously diagnosed. We can see from this that only just over 20% of those with an abnormal haemoglobin were previously diagnosed. This can also be visualised for each ethnicity in Figure 4.4. It is important to note that to be deemed "previously diagnosed" this test either had to be done at Pathlab or Waikato Hospital or have been made evident on the request form by the clinician requesting HbA1c. Pathlab staff do not have access to patient data from tests done elsewhere (including overseas or within New Zealand), through portals such as clinical work station (CWS).

Table 4.4: Previous diagnosis status for all abnormal haemoglobins detected via HbA1c in 2023.

	No Previous Diagnosis	Previously Diagnosed Haemoglobinopathy*
No abnormal Hb detected in HbA1c	306812	0
Abnormal Hb detected in HbA1c	932	191

*excluding α thalassaemia

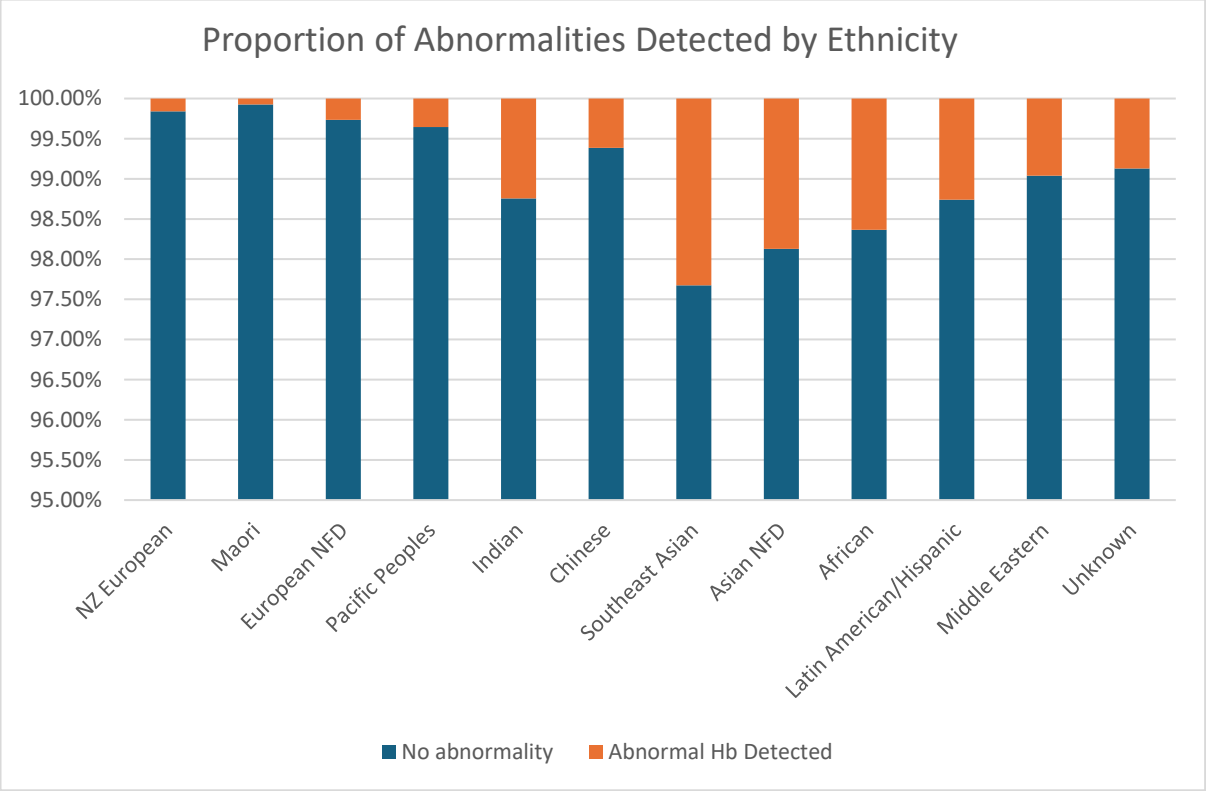


Figure 4.3: Percent of HbA1c tests that found an abnormal haemoglobin by ethnicity.

NOTE: y axis does not start at 0, no ethnicity had less than 95% of HbA1c test with no abnormality detected.

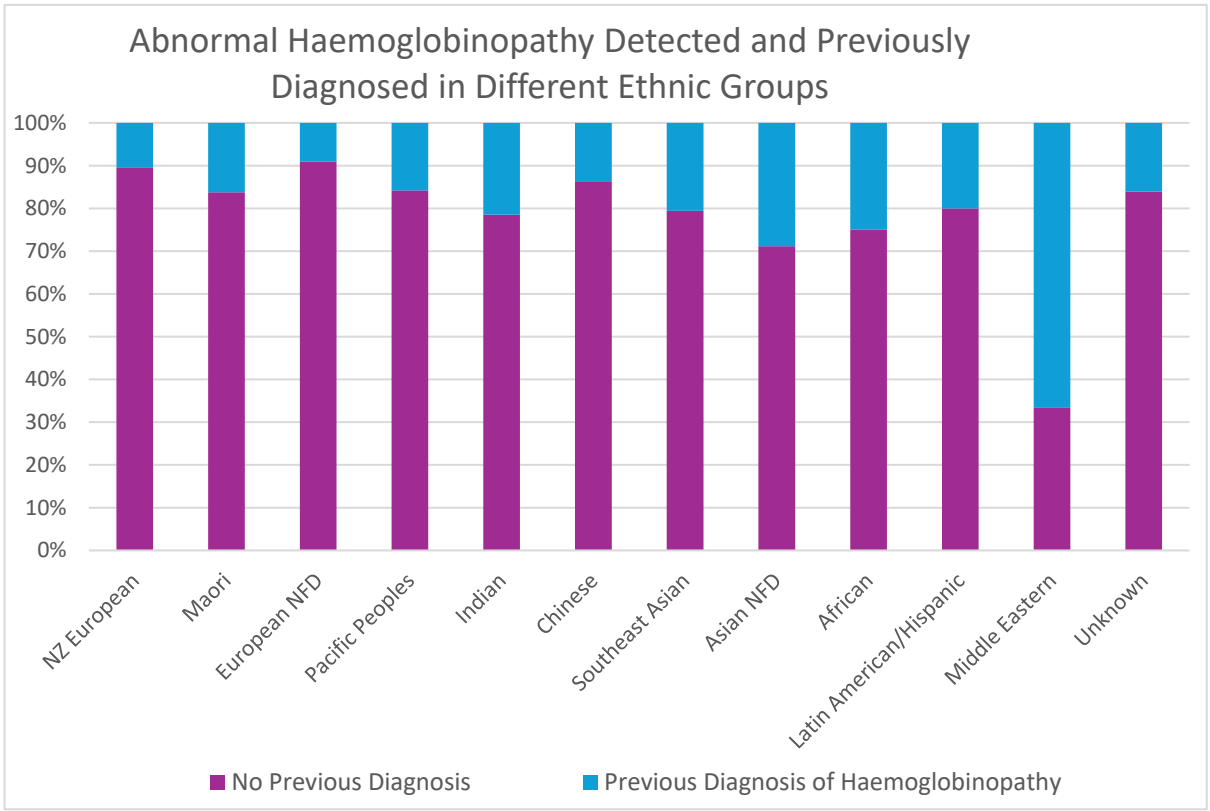


Figure 4.4: Proportion of abnormal haemoglobins detected in HbA1c for 2023 that were previously diagnosed, grouped by ethnicity.

Statistical analysis in RStudio was performed to determine if there were significant differences in the presence of abnormal haemoglobin between the ethnicities. A binomial general linear model was created using the HbA1c data, with ethnicity as the predictor and the presence of abnormal haemoglobin as the response. This determined that ethnicity was a statistically significant predictor of abnormal haemoglobin (p value <0.001). ANOVA and emmeans calculations were done to compare the ethnicities, and significant differences were found. Table 8.1 in Appendix 2 shows this comparison, and from this we can see that when compared to NZ European, most of the other minority ethnicities have a stronger correlation between ethnicity and the presence of abnormal haemoglobin, with the exception of Māori and Pacific Peoples. This may be partially due to the fact that alpha thalassaemia is common for individuals with Māori heritage, and this is not detectable on HbA1c.

Analysis was performed for every thalassaemia screen that was added by Pathlab Waikato due to monitoring for abnormal haemoglobin in the antenatal screen from 2019 to 2023. Interestingly, 153 new diagnoses were made in the four years since screening began. Of these, only two were not the expected result as determined by the HbA1c alone, and these were both cases of a raised HbA2 that was only confirmed to not be a beta thalassaemia by genetic testing. The majority of the diagnoses made were either heterozygous HbE or β thalassaemia trait with just under 35% each (see Fig.4.5).

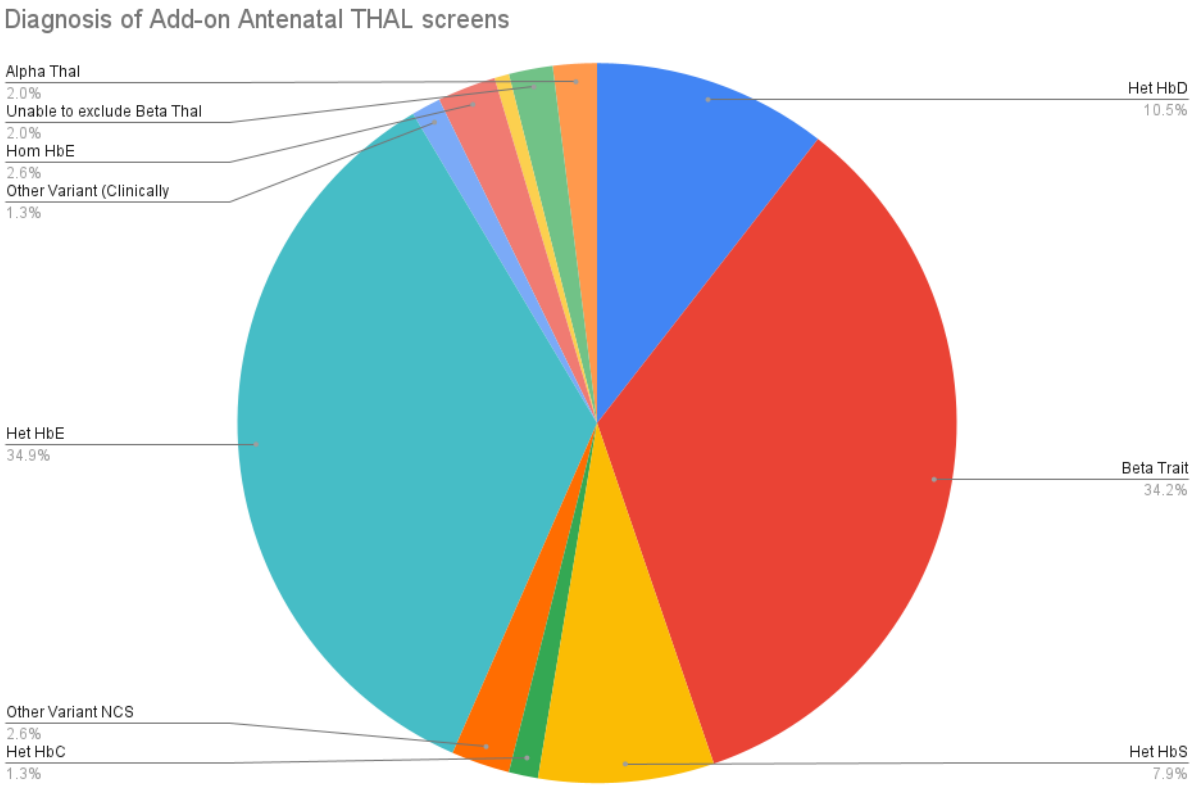


Figure 4.5: Summary of all diagnosed haemoglobinopathies that were added on by haematology staff at Pathlab Waikato as part of the screening that began in 2019.
Note: NCS= Not clinically significant, due to normal CBC

4.3 Population Statistics

During the 2013, 2018, and 2023 New Zealand Census, data was collected on self-reported ethnicity. In 2023, a total of 4,696,935 people responded, and from that 2,814,552 people (or around 60%) answered that they were New Zealand European only. The remaining 40% (around 1.9 million) are split into either one or a combination of the other following six ethnic groups: Māori, Asian, Pacific Peoples, European, Middle Eastern/Latin American/African, or other/not stated.

These are further broken down into individual nations shown in Table 4.5 and can be visualised in Appendix 1 Figure 7.1, which shows the relative proportions of each of these ethnicities. The Figure 7.2 in Appendix 1 shows the comparison and changes between 2013, 2018 and 2023.This data counts mixed ethnicities as a value for each ethnicity listed. One of the biggest changes is the increase in the Indian population, increasing by around 2% from 2013, to be 5.6% of the total population in 2023. Also of note is that between 2013 and 2023,

the percentage of people identifying as New Zealand European has dropped around 6% to 62%. This is also relevant when analysing the 2023 population breakdown, as there are now more ethnicities that account for >0.5% of the population. This threshold is used in Table 4.5 for ease of viewing.

This data also includes specific information for the regional council areas covered by Pathlab, the Waikato and Bay of Plenty. This has been divided into two graphs: Figure 4.6 and 4.7 to give slightly different perspectives. The first, Figure 4.6, only includes ethnicities that make up at least 0.5% of the total population of New Zealand. Whereas Figure 4.7 shows all the ethnicities recorded but excludes the two highest – NZ European and Māori, so that the ethnicities present in only very low amounts can still be visualised. The first graph shows that the Pathlab regions have a higher New Zealand European and Māori percentage than the New Zealand average, but lower Indian, Chinese, and Samoan populations. In the second graph it is possible to discern that Waikato, in particular, has a higher proportion of people of Dutch, African, and Cambodian ethnicities. Also that the Bay of Plenty has a higher Latin American population.

Combining population data and the HbA1c data from 2023 generates Table 4.6. This table shows the totals for each of these populations and how many of them are being tested for HbA1c. We can see there is potentially a large number of people in the Pacific Island regions who are not being tested for HbA1c. This is of note as there is a large amount of research surrounding the high prevalence of Diabetes in this region, and HbA1c is an important indicator of disease progression.[44] The data in Table 4.6 also gives a summary of the proportion of abnormal haemoglobins found in each ethnicity. While this is not a complete overview as it only shows data from one year, we can see immediately that there are five ethnic groups that have a much higher proportion of abnormalities found. These are Indian, Asian not further defined, Southeast Asian not further defined, Latin American, and African. This is concordant with what was found in the literature review about higher risk ethnicities.

Census year	2013				2018				2023			
Area	Total - New Zealand by regional council	2013 Total %	Waikato Region	Bay of Plenty Region	Total - New Zealand by regional council	2018 Total %	Waikato Region	Bay of Plenty Region	Total - New Zealand by regional council	2023 Total %	Waikato Region	Bay of Plenty Region
Ethnicity												
New Zealand European	2,727,009	67.98%	275,562	175,893	3,013,440	64.12%	316,251	209,817	3,099,858	62.07%	331,875	220,881
Māori	598,602	14.92%	83,742	68,943	775,836	16.51%	109,488	89,778	887,493	17.77%	125,574	102,387
Chinese	171,411	4.27%	7,710	2,406	247,770	5.27%	12,084	4,041	279,039	5.59%	13,266	4,788
Indian	155,178	3.87%	9,915	6,264	239,193	5.09%	17,295	10,335	292,092	5.85%	26,019	13,284
Samoan	144,138	3.59%	4,890	2,217	182,721	3.89%	6,972	3,354	213,069	4.27%	8,739	4,449
British and Irish	105,765	2.64%	9,147	6,642	121,986	2.60%	11,022	7,890	122,571	2.45%	11,046	8,217
Other European	81,228	2.02%	6,492	3,993	104,064	2.21%	8,883	5,790	129,189	2.59%	12,033	8,649
Cook Islands Maori	61,839	1.54%	4,842	2,640	80,532	1.71%	6,702	3,552	94,176	1.89%	8,424	4,515
Tongan	60,333	1.50%	2,370	1,458	82,389	1.75%	3,606	1,965	97,824	1.96%	4,848	2,511
Filipino	40,350	1.01%	2,883	1,428	72,612	1.55%	5,427	2,880	108,297	2.17%	9,126	4,488
Korean	30,171	0.75%	1,104	924	35,664	0.76%	1,548	1,557	38,934	0.78%	1,662	1,569
Dutch	28,503	0.71%	3,588	2,082	29,820	0.63%	3,843	2,262	30,948	0.62%	3,996	2,433
European nfd	26,469	0.66%	2,181	1,332	34,632	0.74%	2,823	2,268	21,834	0.44%	1,974	1,638
Niuean	23,880	0.60%	1,038	438	30,867	0.66%	1,590	687	34,944	0.70%	2,028	870
Australian	22,470	0.56%	2,082	1,308	29,349	0.62%	2,643	1,890	30,591	0.61%	2,946	2,130
Other Southeast Asian	21,090	0.53%	1,116	555	26,847	0.57%	1,449	813	33,708	0.67%	1,938	1,089
Middle Eastern	20,406	0.51%	1,053	351	27,990	0.60%	1,521	414	33,309	0.67%	1,953	567
Fijian	14,445	0.36%	1,089	489	19,722	0.42%	1,560	729	25,038	0.50%	2,076	975
Japanese	14,118	0.35%	651	480	18,141	0.39%	897	714	19,488	0.39%	942	831
African	13,464	0.34%	1,563	258	16,890	0.36%	1,872	381	21,795	0.44%	2,661	594
Latin American	13,182	0.33%	954	663	25,731	0.55%	1,749	1,392	38,154	0.76%	2,562	2,799
German	12,810	0.32%	894	663	16,818	0.36%	1,236	912	17,565	0.35%	1,356	1,083
Other Asian	12,513	0.31%	618	426	20,184	0.43%	1,371	606	30,462	0.61%	2,538	1,269
Sri Lankan	11,274	0.28%	582	243	16,830	0.36%	975	330	23,661	0.47%	1,821	594
Other Pacific Peoples	10,890	0.27%	1,017	507	15,039	0.32%	1,422	756	20,154	0.40%	1,950	1,302
Cambodian	8,601	0.21%	1,410	96	9,672	0.21%	1,593	198	11,514	0.23%	1,878	300
Tokelauan	7,173	0.18%	363	456	8,676	0.18%	444	546	9,822	0.20%	513	663
Vietnamese	6,660	0.17%	198	66	10,086	0.21%	321	195	14,157	0.28%	483	333
South Slav	5,370	0.13%	264	108	5,886	0.13%	369	168	6,702	0.13%	468	294
Asian nfd	4,623	0.12%	360	171	11,811	0.25%	849	492	12,645	0.25%	1,125	678
Italian	3,795	0.09%	339	192	5,352	0.11%	387	330	6,444	0.13%	453	450
Greek	2,478	0.06%	108	57	2,475	0.05%	141	75	2,823	0.06%	168	111
Polish	2,166	0.05%	111	72	2,871	0.06%	192	123	3,360	0.07%	216	156
Southeast Asian nfd	1,275	0.03%	96	15	6,219	0.13%	345	159	8,235	0.16%	606	297
Pacific Peoples nfd	1,029	0.03%	96	48	2,724	0.06%	246	162	2,271	0.05%	210	192
Total stated - ethnicity	4,011,399		382,536	250,584	4,699,755		458,202	308,499	4,993,923		498,771	334,140

Table 4.3: Data from Stats NZ from the 2013, 2018 and 2023 Census.

The filters applied highlight the breakdown by ethnicity of the total NZ population and the Waikato and Bay of Plenty district council regions, as these cover all areas where Pathlab is the primary laboratory service.

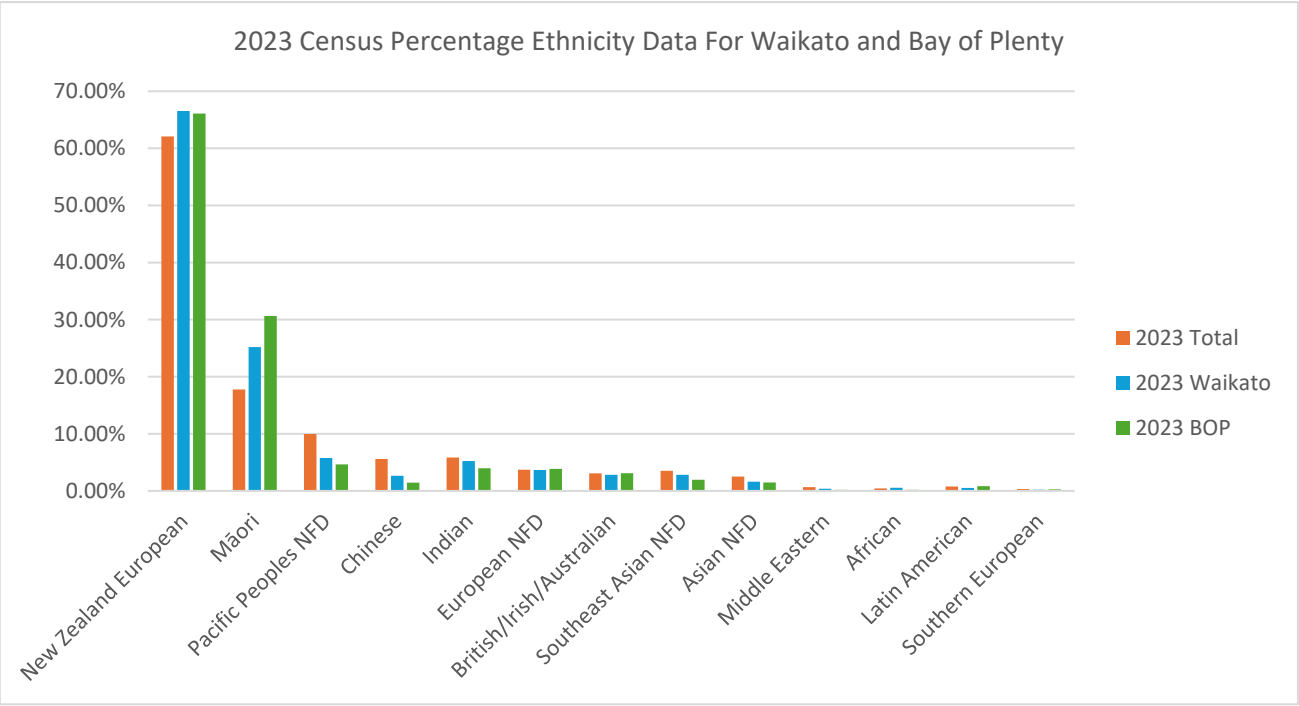


Figure 4.6: New Zealand 2023 census data graphed to show the difference in the proportions of ethnicities for our regions of interest compared to the general New Zealand population.

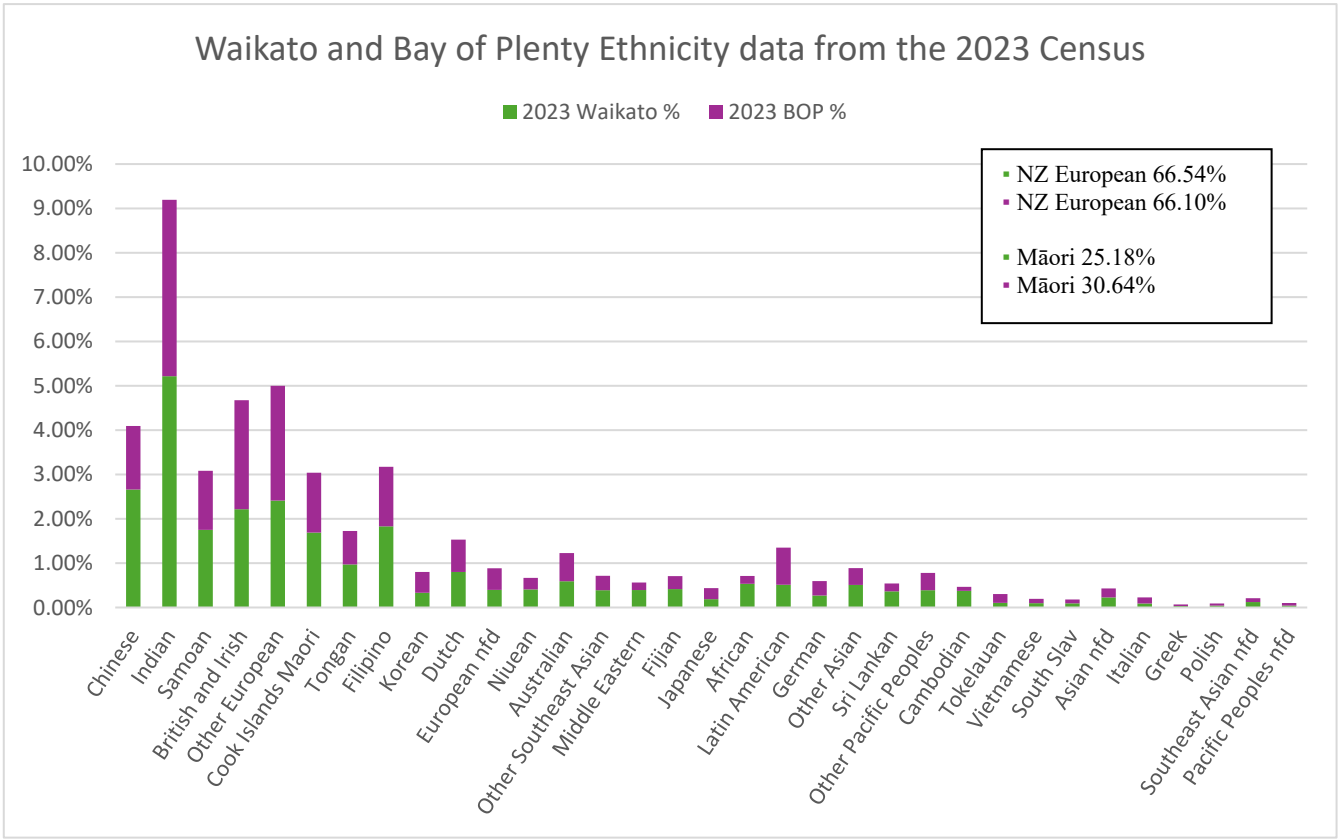


Figure 4.3: Ethnicity census data specially looking at the Waikato and Bay of Plenty in 2023 to compare the proportions of ethnic groups between the two.

Table 4.4: Data from Stats NZ on ethnicity for the Waikato and Bay of Plenty regions covered by Pathlab. This is combined and compared with previous 2023 HbA1c data to give the percentage of each ethnic group being tested. In the "Proportion tested for HbA1c" column, any ethnicities that are less than 15% are highlighted green. In the "% HbA1c tests with abnormality" column, any ethnicities that have a value higher than 1% are highlighted orange.

Ethnicity	Total - New Zealand	2023 Total	Waikato Region	Bay of Plenty Region	Pathlab regions (total)	Pathlab regions	HbA1c tests	Proportion tested for HbA1c	Proportion of HbA1c tests with abnormality
New Zealand European	3,099,858	62.07%	331,875	220,881	552,756	66.36%	162,715	29.44%	0.16%
Māori	887,493	17.77%	125,574	102,387	227,961	27.37%	49,804	21.85%	0.07%
Indian	292,092	5.85%	26,019	13,284	39,303	4.72%	11,986	30.50%	**1.26%**
Chinese	279,039	5.59%	13,266	4,788	18,054	2.17%	3,585	19.86%	0.62%
Samoan	213,069	4.27%	8,739	4,449	13,188	1.58%	1,363	**10.34%**	0.15%
European NFD	151,023	3.02%	14,007	10,287	24,294	2.92%	20,724	85.31%	0.27%
British and Irish	122,571	2.45%	11,046	8,217	19,263	2.31%			
Filipino	108,297	2.17%	9,126	4,488	13,614	1.63%			
Tongan	97,824	1.96%	4,848	2,511	7,359	0.88%	786	**10.68%**	0.38%
Cook Islands Māori	94,176	1.89%	8,424	4,515	12,939	1.55%	997	**7.71%**	0.10%
Asian NFD	43,107	0.86%	3,663	1,947	5,610	0.67%	5,556	99.04%	**1.91%**
Southeast Asian NFD	41,943	0.84%	2,544	1,386	3,930	0.47%	2,924	74.40%	**2.38%**
Korean	38,934	0.78%	1,662	1,569	3,231	0.39%			
Latin American	38,154	0.76%	2,562	2,799	5,361	0.64%	1,190	22.20%	**1.28%**
Niuean	34,944	0.70%	2,028	870	2,898	0.35%	174	**6.00%**	0.00%
Middle Eastern	33,309	0.67%	1,953	567	2,520	0.30%	625	24.80%	0.97%
Dutch	30,948	0.62%	3,996	2,433	6,429	0.77%			
Australian	30,591	0.61%	2,946	2,130	5,076	0.61%			
Fijian	25,038	0.50%	2,076	975	3,051	0.37%	1,181	38.71%	0.94%
Sri Lankan	23,661	0.47%	1,821	594	2,415	0.29%			
Pacific Peoples NFD	22,425	0.45%	2,160	1,494	3,654	0.44%	685	18.75%	0.29%
African	21,795	0.44%	2,661	594	3,255	0.39%	1,223	37.57%	**1.66%**
Japanese	19,488	0.39%	942	831	1,773	0.21%			
German	17,565	0.35%	1,356	1,083	2,439	0.29%			
Vietnamese	14,157	0.28%	483	333	816	0.10%			
Cambodian	11,514	0.23%	1,878	300	2,178	0.26%			
Tokelauan	9,822	0.20%	513	663	1,176	0.14%	176	**14.97%**	0.00%
South Slav	6,702	0.13%	468	294	762	0.09%			
Italian	6,444	0.13%	453	450	903	0.11%			
Polish	3,360	0.07%	216	156	372	0.04%			
Greek	2,823	0.06%	168	111	279	0.03%			
Unknown							25,318		0.41%
Total stated - ethnicity	4,993,923		498,771	334,140	832,911	66.36%			

4.4 Immigration Statistics

To further investigate how our population may be changing, immigration statistics from New Zealand's official data agency - Stats NZ were collected. The patterns from 2008-2023 from 13 countries with the highest numbers of immigrants entering New Zealand are shown below in Figure 4.8. From this data we can immediately see that migrant arrivals are increasing, with the 208,400 migrant arrivals in the year ending July 2023 being the highest on record for an annual period. Even with higher-than-average migrant departures (112,200), the annual net gain of 96,200 is still the highest on record. Fig 4.8 below shows similar patterns to what is seen in the census ethnicity data, with an increase in the Indian, Filipino, and Chinese populations. The immigration numbers follow a cyclic pattern, with December always having the lowest number of immigrant arrivals.

4.5 Predictions and Estimations

Comparing our HbA1c data collected from the year 2023, and the prevalence estimated for each ethnic group we can see areas where haemoglobinopathies are under-represented or under-tested. This is shown in Table 4.7 below. The estimates vary greatly between some countries, and this is only a rough estimate. However, even taking the most conservative estimates, we can see that many high-risk groups are being under diagnosed. For example, in the Indian population in New Zealand, only 1.24% of individuals who were tested for HbA1c in 2023 were found to have any abnormality, and only 0.68% were identified as β thalassaemia carriers. If we are to go from the estimated prevalence of 3% that would mean theoretically over 275 people with β thalassaemia trait are undiagnosed in the Indian population alone. The Southeast Asian population is another high prevalence and high-risk group that appears to be under-diagnosed.

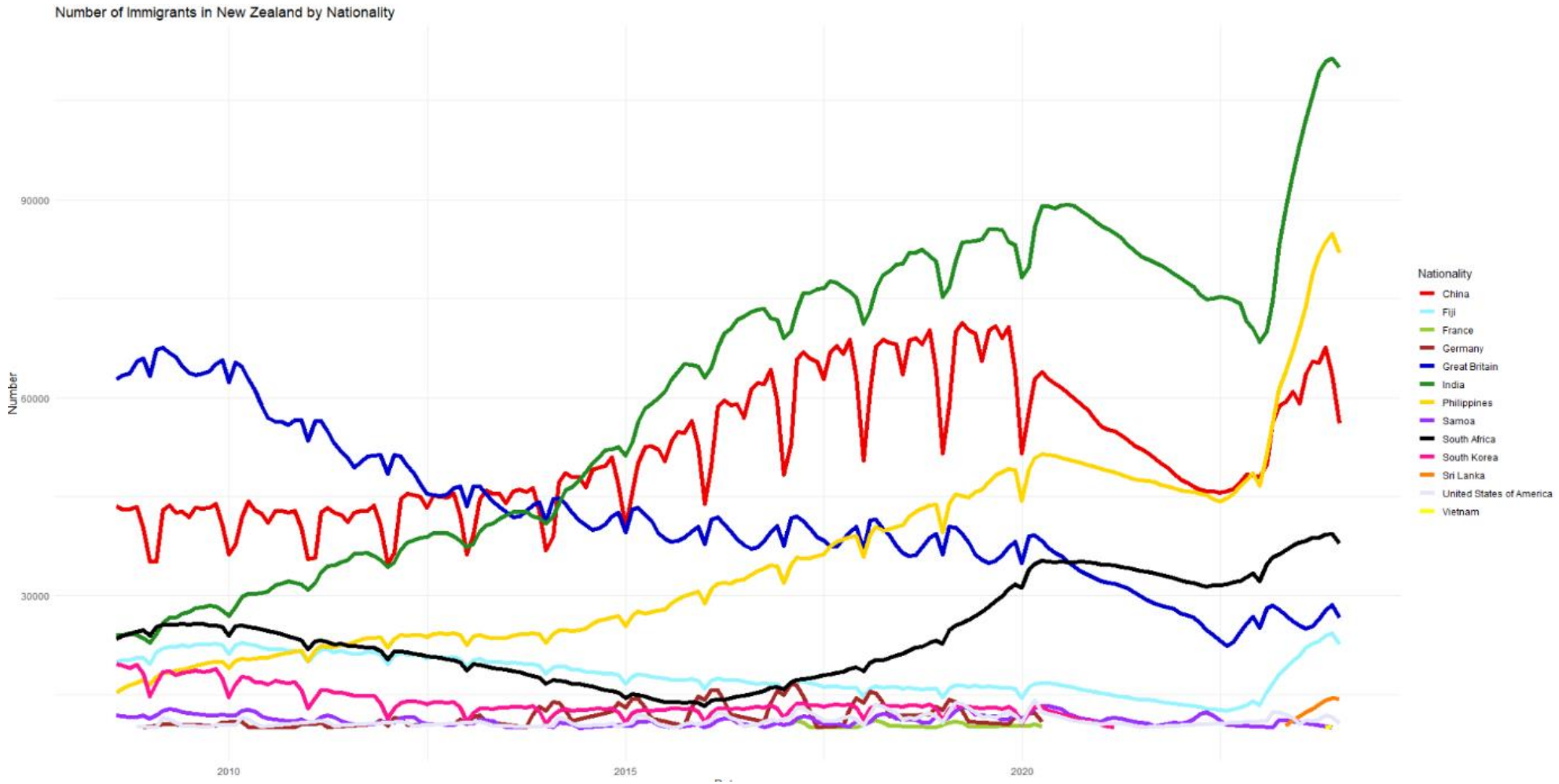


Figure 4.4: Graph showing the number of immigrants to New Zealand by country, for the years ending July 2008 – 2023. This shows only the top 13 countries of origin.

Table 4.5: Comparison of the variants found, and the prevalence expected for different ethnicities. Using data from the literature review table.[5]

Ethnicities	2023 NZ Census population data	HbA1c tests in 2023	Proportion with abnormality	Literature estimates
NZ European	3,099,858 (62.07%)	162,715 (52.84%)	0.16%	
Māori	887,493 (17.77%)	49,804 (16.17%)	0.07%	α: 5-10%
Pacific Peoples	497,298 (9.96%)	5,362 (1.74%)	0.27%	α: 0-45%
European NFD	354,462 (7.1%)	20,724 (6.73%)	0.41%	α: 0-10% β: 0-8% HbS: 0-34% HbC: 0.5-1%
Indian	292,092 (5.85%)	11,986 (3.89%)	**1.24%**	α: 5-33% β: 3% HbS: 0-35%
Chinese	279,039 (5.59%)	3,585 (1.16%)	**1.87%**	α: 0-9% β: 0.5-6%
Southeast Asian	175,911 (3.52%)	2,924 (0.95%)	0.61%	α: 5-29% β:1-25%
Asian NFD	125,190 (2.51%)	5,556 (1.80%)	**2.33%**	α: 3-20% β: 0-13% HbS: 0-35%
Latin American/ Hispanic	38,154 (0.76%)	1,190 (0.39%)	**1.64%**	HbS: 0-22% HbC: 0-7%
Middle Eastern	33,309 (0.67%)	625 (0.20%)	**1.26%**	α: 9% β: 2-20% HbS: 0-38%
African	21,795 (0.44%)	1,223 (0.40%)	0.15%	α: 0-14% β: 0-46% HbS: 0-46% HbC: 0-50%
Unknown		42,241 (13.72%)	0.93%	

4.6 Hardy-Weinberg Equation

Using Equation 3.1, we calculated the expected number of carriers of a haemoglobinopathy given the number of individuals who are homozygous for that haemoglobinopathy, with the assumption that the population are in Hardy-Weinberg equilibrium. Table 4.8 shows the calculations for HbE and HbS, as we have a defined number of homozygous patients in our region for these haemoglobinopathies (Table 4.8). These are done using the total population of the Waikato and Bay of Plenty regions in 2023 (832,911), and assumes that individuals without the selected for haemoglobinopathy have normal haemoglobin (i.e. p + q = 1).

Table 4.6A and 4.8B: Hardy-Weinberg Equation to estimate the carrier frequency of HbE and HbS in our population.

(A)

HbE: 38 homozygous patients in a population of 832,911 p (HbA) = 0. 99325 q (HbE) = 0. 00675	
Normal haemoglobin (HbAA)	p^2 = 0.98655
Homozygous HbE (HbEE)	q^2 = 0.00005
Heterozygous HbE (HbAE)	$2pq$ = 0.01341
Carrier frequency = 1 in 74.53 or 1.34%	

(B)

HbS: 4 homozygous patients in a population of 832,911 p (HbA) = 0.99781 q (HbS) = 0.00219	
Normal haemoglobin (HbAA)	p^2 = 0.99562
Homozygous HbS (HbSS)	q^2 = 0.00000361
Heterozygous HbS (HbAS)	$2pq$ = 0.00437
Carrier frequency = 1 in 228.66 or 0.44%	

If we follow the example of van Vliet et al.[45] and apply this to the number of children born in the region every year, we predicted around 110 children born annually that are HbE carriers, and just over 35 children that are HbS carriers. Below is this data in a table form, using information from stats NZ maternity web tool (Table 4.9).

This is possibly the most compelling calculation to show just how many people are being missed with our current methods. If these numbers are accurate then we should be seeing far more than 358 heterozygous HbE patients, and more than 171 heterozygous HbS patients if around 112 and 36 are being born each year, respectively.

DHB (birthplace)	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Averages
Waikato	5565	5471	5537	5325	5434	5171	5187	5206	5228	5261	5328	5399	5463	5911	5279	5384.33
Bay of Plenty	2950	2962	3000	2837	2938	2738	2767	2755	2875	3069	2991	3089	3118	3373	3097	2970.60
TOTAL	**8515**	**8433**	**8537**	**8162**	**8372**	**7909**	**7954**	**7961**	**8103**	**8330**	**8319**	**8488**	**8581**	**9284**	**8376**	**8354.93**
HbE Carrier frequency 1.34%	114.1	113.0	114.4	109.4	112.2	106.0	106.6	106.7	108.6	111.6	111.5	113.7	115.0	124.4	112.2	112.0
HbS Carrier frequency 0.44%	37.5	37.1	37.6	35.9	36.8	34.8	35.0	35.0	35.7	36.7	36.6	37.3	37.8	40.8	36.9	36.8

Table 4.7: DHB birth-rate data by birthplace, with estimated frequency of HbS and HbE carriers.

4.7 International Antenatal and Population Screening Programmes

A review of screening programmes available in other countries was performed in the literature review. These can be grouped into high-risk and low-risk countries. High risk or high prevalence countries such as Greece, Cyprus, and India have implemented more aggressive strategies for decades and therefore provide a large amount of useful information on efficacy. Lower risk countries are those who are seeing a rise in thalassaemia and haemoglobinopathy due to an increase in immigration, such as the United Kingdom, Australia, and Denmark. We can use the information from these reports along with our ethnicity statistics to determine an estimate of the high-risk population in New Zealand.

In India, a study aimed to introduce prenatal screening in a high risk area, as the overall prevalence of β thalassaemia ranges from 1-17%, with an average of 3-4%, and HbE has a prevalence as high as 40-50% in some regions.[39] This study found that while there were still many at-risk couples being screened, that number was decreasing with every year the screening was offered, and the time of testing had shifted to more couples being tested earlier in the pregnancy or pre-conception. This showed the power of awareness and education of the disease meaning more couples were now aware of their risk. However, the authors acknowledge that the reach is still limited, and reaching an "overwhelming majority" of people who live rurally would be a major challenge for successful implementation.[39] The paper ends on the note that only through "extensive education and awareness" can their efforts be successful.[39]

A review of thalassaemia in China was carried out in 2023 by Wang et al. due to China having the highest number of existing and new thalassaemia cases than any other country at around 30 million carriers and 300,000 thalassaemia major or intermedia.[33] The prevalence of α thalassaemia is around 5-15% and the annual birth-rate of HbH is around 2,500 – 4,300, and Hb Barts around 1,000 – 3,700.[33] The incidence of β thalassaemia is slightly lower at 3-4% but co-inheritance of α and β thalassaemia is common. The study estimated around 72 billion RMB (just under $17 billion NZD in 2025) is needed to treat the 15,000 people with thalassaemia major. Areas in China with high incidence have implemented screening programmes over the past few decades to reduce the number of thalassaemia major births. These used a multitude of tools such as free premarital medical exams, social media and educational material targeted at childbearing age groups, premarital, pre-pregnancy and prenatal exams and consultations with free interventions if required. These

saw a 10-fold decrease in the births of children with thalassaemia major and hydrops fetalis in targeted regions.[33] The authors created a flowchart of the policy in one such region for the control and prevention of thalassaemia that begins with education, availability of services, centres and networks, then finishes with a thalassaemia care system and research capacity. They finish by saying the change needs to begin with authorities accelerating policy development and increasing funding, followed by social awareness and increased education and specialisation of medical professionals and centres. Finally, they push for more funding into treatment and research into new therapies for the large existing population with thalassaemia, along with global initiatives and partnerships.

A 2022 review by Lee et al. analysed 13 years of thalassaemia epidemiological data in Korea, from 2006-2018.[46] They found the prevalence of thalassaemia increased during this time, from 0.74 per 100,000 (exactly 354 people) to 2.76 per 100,000 people (exactly 1577 people).[46] The authors suggested this increase is likely due to an increase in immigration, with number of foreign residents increasing from 1.8% to 4.6% during this period, with the majority being from China, Thailand, and Vietnam.[46] The focus of the study was the treatment and comorbidity of thalassaemia, and they acknowledge that early detection and timely intervention are required for the management of thalassaemia.

The Malaysian study by Ngim et al.[47] in 2015 found incidence rates of 4.1% for α thalassaemia, 4.5% for β thalassaemia, and 3-40% for HbE. The authors suggest poor knowledge about thalassaemia and low uptake for screening are part of the cause for the high incidence.[47] A screening programme was launched in 2004 that initially screened all 16 year old school children, but this was found to put too much of a strain on the healthcare system. A retrospective study of thirty children with TDT born between 2005-2012 found that in the majority of births of a TDT child, neither parent was aware of their haemoglobinopathy. They found that for the parents that were aware, most were provided with inadequate health care, support, and education or the opportunity to make an informed decision about the pregnancy. This is significant as in the study over 50% of parents retrospectively indicated that they would terminate an affected pregnancy. This includes a majority of parents that were not given adequate information, care, or informed reproductive choices, and who said they would have chosen to terminate had these been available.[47] The study mentioned other countries where screening is mandatory such as Cyprus, Iran, Saudi Arabia, and Palestine. In these high-risk countries, couples wishing to marry must first have

premarital screening for haemoglobinopathy. These couples maintain the right to marry regardless of the outcome (which a vast majority still do) but they will be informed of the risk to potential future children. The authors also mention the family screening performed in India, Pakistan, and Sardinia, which is carried out once one member of a family is diagnosed. They suggest both of these techniques could be applied in Malaysia, though to do so poses many challenges to the healthcare system and public uptake would likely be low due to lack of awareness or religious choices.[47] Though prenatal diagnosis is available in Malaysia, there still remains the issue of insufficient health care and both legal and religious perceptions surrounding termination of affected children. This paper came to a similar conclusion as many others, that the first steps to reducing incidence are increasing awareness, education, and healthcare support.

The United Kingdom National Health Service (NHS) established a sickle cell and thalassaemia (SCT) screening programme in 2001, and an antenatal screening service was implemented in 2008.[48] A retrospective study of this service was completed in 2020 by Weil et al. to assess its efficacy. The aim of the screening programme was to "*offer timely antenatal SCT screening to all women (and couples), to facilitate informed decision making*" and to do so required screening and diagnosis to be done before 12 weeks gestation.[48] The guidelines for this service were discussed in the literature review, as they have shaped the service currently offered by Pathlab Waikato. β thalassaemia is the most common haemoglobinopathy in the UK, with 20 to 30 babies born annually with β thalassaemia major, affecting around 1 in every 27,000 pregnancies.[48] They also estimate that there are around 12,500-15,000 people with sickle cell disease currently living in the UK. The study found that during the study, 4,935,902 screens were done on antenatal bloods, and 102,477 women were found to have a significant haemoglobinopathy, with around 38% and 24% being HbS and β thalassaemia, respectively.[48] The authors agreed that antenatal screening remains the best pathway to reach the most people for thalassaemia and haemoglobinopathy, but did highlight that this must be done as soon as possible after conception. They found that only around 50% of women were being tested by 10 weeks gestation, and that the timing of the diagnosis had significant impact on the reproductive choices made by parents. The authors suggested that while positive changes had come about in recent years to improve this – such as including a question on family history of thalassaemia or SCD on self-referral forms – that to reach their early screening targets, policies around preconception care, patient and

healthcare genetic literacy and education, and more information around the importance of timely screening should be made available.[48]

In Spain, a nationwide registry was created in 2013 to monitor all rare anaemias. This data was studied, and a review published in 2024 by Sánchez et al.[49] The study found that the most common disease in the database was SCD (homozygous HbS), with a prevalence in Madrid of 1 per 6,250 births, and neonatal haemoglobinopathy prevalence was 5.57 per 1,000 births.[49] The authors stated that worldwide the annual number of newborns with SCD is estimated at 305,800 in 2010, and that this was likely to increase by around a third by 2050. The study found that while most of the patients in the database were born in Spain, the majority of the parents of these patients were not. They state that the number of immigrants living in Spain from Africa, Central and South America, and Asia had almost doubled in the last 20 years.[49] These are all areas with a high prevalence of haemoglobinopathy, therefore this could partially explain the increase in the number of diagnoses seen. However, the authors state this is likely to also be due to an increase in screening and awareness.[49]

Germany introduced newborn screening only for SCD in 2021, with the estimated prevalence being between 1:5,000 to 1:7,500.[50] In a retrospective study between 2018-2021, 339 patients were diagnosed with a haemoglobinopathy, including 13 paediatric patients with SCD.[50] The authors of this study suggested that the increase in immigration and asylum seekers in Germany is a major contributing factor to the increase in haemoglobinopathy diagnosis. This review found many of the patients in the database were not diagnosed until adulthood.[50] Also with neonatal testing, many parents were unaware they were carriers of haemoglobinopathy. Aramayo-Singelmann et al. (2022) suggested that while the testing was a step in the right direction, and awareness seemed to be improving, further steps need to be taken to improve education and testing, such as referral for genetic testing.[50]

For the low risk or low incidence countries, papers from Australia and Denmark have enough similarities that these can be easily applicable to the situation in New Zealand. Both studies reference that the WHO has recommended all its member countries have a screening programme in place for haemoglobinopathy. In Australia it is estimated that 28% of their residents are born outside Australia, and in Denmark the number of immigrants or descendants of immigrants was estimated to be 14.4% in 2022 and is expected to increase.[17; 40] A 2008 report from the WHO estimated that 4.6% of Australians are carriers

of α or β globin gene variants.[17] The 2016 Australian review by Crighton et al. suggested the first step in prevention is establishing the number of Australians currently affected by creating an updated registry and national surveillance system.[17] They aim to target screening and prenatal diagnosis by creating national guidelines and policies, which would include education and informed consent for patients. The authors did not have any estimated cost available but gave the lifetime cost to treat β thalassaemia major in the UK in 1998 was over £800,000, and in the USA in the early 2000s was $460,000 USD. They did estimate that one patient on daily iron chelation therapy (deferasirox) would cost $48,000 AUD annually.[17] The study had mixed recommendations on whether to only test woman of high-risk ethnicity or to screen all pregnant women, but agreed with other studies that more education and policy is required with increased testing. The study in Denmark in 2024 by Gravholt et al. suggested screening may be premarital, antenatal or postnatal, but again mentioned both targeted screening and universal screening depending on the prevalence.[40] However, in Denmark since 2007, screening for haemoglobinopathy has been carried out as part of an antenatal screen for women with high-risk ethnicities. The high-risk ethnicities as defined by the Danish Health Authorities listed in the study were: Africa and Afro-American, Northern Mediterranean, Middle Eastern, most of Western and Central Asia, all of Southeast Asia, and Oceania.[40] Therefore, this would include New Zealand Māori, New Zealand European, Australian, and Pacific Peoples as high-risk. The authors did suggest that they may need to change to a universal screening service due to the predicted increase in prevalence and immigration. The cost to treat TDT is estimated at €28,000 per year, therefore estimated €1,200,000 lifelong cost per TDT patient, not including costs such as loss of income or social benefits.[40] They counter this by saying the cost for a screening programme in 2022 is estimated to be €555,000, therefore this would be cost neutral if they decreased the number of TDT births by just one every two years.[40] Again, the authors finish by stating a good strategy is to increase education and awareness.

In 2022 van Vliet et al. published two papers on thalassaemia and haemoglobinopathy screening in the Netherlands.[45; 51] While there is no antenatal screening currently performed, in 2007 sickle cell anaemia was added to the heel prick test done on infants, leading to around 800 heterozygous HbS and 35-40 homozygous HbS diagnoses being made every year since then.[45] In 1998 the decision was made in the Netherlands that a screening programme was not required. The authors suggest that much has changed since then, and preconception or antenatal screening is now appropriate and should be implemented with

standardised testing and reporting. They mention the 2006 WHO recommendation that encourages a national screening programme be put in place when there are more than 20 affected births per year.[45] The authors propose that carrier screening for haemoglobinopathy should occur in the community with GPs in collaboration with midwives and specialised childcare or genetic counsellors as necessary. They conducted interviews of GPs or primary care professionals and found all of them believed they were lacking in knowledge about haemoglobinopathies and expressed a desire for further education.[51] The requirements were broken down into three steps: 1) more information should be made available on haemoglobinopathies, 2) improved guidelines on referral or further testing for patients, and 3) clearer policies and understanding of the roles of different organisations in the care and diagnosis of haemoglobinopathy.[51] One of the papers published goes further by providing a list of strategies to meet these requirements, and these can be applicable to any screening service world-wide. These include providing information about the disease when giving a diagnosis and offering it in a variety of different languages so that patient may understand it better. Also suggested is providing an online training course or documents for healthcare professionals, or for those who would prefer in-person training, setting up workshops and conferences, while having a designated “expert” in each region that other clinical staff can go to.[45]

5. Discussion

The purpose of this research was to analyse the haemoglobinopathy data from the Waikato and Bay of Plenty regions of New Zealand, and compare this to global data available to evaluate our methods, forecast the risk for our population, and consider our testing reach. Unexpectedly, from our data analysis we have proven there are a significant number of haemoglobinopathies not being detected in our community. There are growing populations of individuals with at-risk ethnicities in our regions therefore the number of haemoglobinopathies is also likely to be increasing. The implications of these results are that there is a substantial gap in haemoglobinopathy testing in New Zealand, and this requires action and attention to prevent negative health outcomes and further strain on our health care system.

5.1 Haemoglobinopathy Database

The results from the Pathlab database show a steady increase in haemoglobinopathy testing every year. The overwhelming majority of reported cases were thalassaemia, and while there were similar numbers of confirmed α and β thalassaemia, the number of diagnoses of "unable to exclude α thalassaemia" was almost double the number of confirmed β thalassaemia. This may reflect the population data we have seen, with α thalassaemia being high prevalence in many different ethnicities. Most notable is the high prevalence of the $\alpha^{3.7}$ thalassaemia in the New Zealand Māori population. This mutation can be difficult to detect when heterozygous which may account for some of the "unable to exclude" diagnoses, as this is the result given when there is suspicion of α thalassaemia due to microcytic hypochromic RBC indices, but there are no HbH bodies detected, and an 'i-LAB α thal' test-strip is negative.

The testing methods and processes developed at Pathlab are largely based on the NHS thalassaemia screening handbook.[41] As previously mentioned in the literature review, within the NHS, a region or 'trust' is deemed high-risk for haemoglobinopathy if greater than 2% of haemoglobinopathy screens are positive. On average around 53% of the haemoglobinopathy screens conducted at Pathlab are positive (Figure 4.1). While this is significantly higher than the high-risk threshold, it is likely biased due to the reasons for testing being very different. As there is no general screening in New Zealand, testing is only

done if thalassaemia is already suspected by the clinician due to CBC results, family history, or if an abnormal haemoglobin has been detected on HbA1c with a comment sent to the requesting clinician.

One measurement of our detection rates compared to global rates is using the WHO report from 2006, which claimed the worldwide the carrier rate of β thalassaemia was 1.5%.[37] The prevalence calculated from our database (Fig 4.2) was only 0.0868%. While this estimated global prevalence is affected by areas with very high levels of β thalassaemia, it is still over 17 times higher than the prevalence we detected, which suggests we are not detecting all the β thalassaemia patients that may be in our community.

The Hardy-Weinberg equation (Equation 3.1) and its implications are mentioned by van Vliet et al. 2022[45]. They calculated the number of babies born that were carriers for either β thalassaemia or HbS. They found the calculated number of carriers was far greater than the number detected in some areas. From Equation 3.1 it is determined that there are a significant number of potential carriers of HbE and HbS in our community that are undiagnosed (Table 4.8). The equation estimated 1.21% of the population may be carriers of HbE, or over 10,000 people across the Waikato and Bay of plenty, where there has only been 372 detected. Similarly, for HbS the calculated carrier rate was 0.38% or 3,165 across the Waikato and Bay of Plenty, where there has only been 167 detected. It is important to note however, that these predictions make certain assumptions about the population that may not be accurate, but even if we are conservative with our calculations there is still a substantial shortfall in our testing reach. In 2006 the WHO recommended that a screening programme be implemented if there are more than 20 affected births per year.[32; 45] From Equation 3.1 and Table 4.9, we estimate there to be an annual average in the Waikato and Bay of Plenty of 112 and 36.8 children born as carriers of HbE and HbS, respectively. The implications of this many carriers in these two regions suggest that it is likely New Zealand would meet this criterion.

From the above predictions it is clear that there are patients in our community who may be unaware that they are carriers of significant haemoglobinopathies. If a national screening service is introduced, the aim would be to reduce this number so that people can receive genetic and reproductive counselling to make informed decisions or seek out pre-pregnancy options such as preimplantation genetic diagnosis or in vitro fertilisation. Antenatal screening

would be an ideal way to target those planning on having children, even if it is too late for the current pregnancy, it means they have information and options available for any subsequent pregnancies.

5.2 Haemoglobin A1c data

The HbA1c data gives us an indication of the ethnicities in our population, and the proportions of haemoglobinopathy found in these. This information is useful to identify where we are not detecting the predicted levels of haemoglobinopathy for these groups. The statistical analysis performed proves that ethnicity is a significant predictor of haemoglobinopathy, however, it is my opinion that testing should not be decided based on ethnicity. Screening should be available to the entire population, because not only are there potential concerns with self-reporting of ethnicity, the risks for different haemoglobinopathies vary greatly between different groups or even within the same ethnicity. Also, in a country with increasing immigration, screening everyone is another way to ensure no one with a significant haemoglobinopathy is missed.

Data from the testing of HbA1c and detection of abnormal haemoglobin from just one year (2023) was analysed due to the high volume of testing done annually. Patient demographics for this year generally matched what is predicted for the different ethnicities, with patients who have identified as Southeast Asian having the highest proportion of abnormal haemoglobin. This is followed by Asian not further defined, African, Latin American/Hispanic, then Indian. It is important to consider that there were very few patients in the Latin American/Hispanic and African groups (1,175 and 1,203, respectively) when compared to the larger minority populations such as Indian or European not further defined (11,837 and 20,669, respectively). This could explain why these have a smaller than expected number of abnormalities detected. However, this data contained prevalence statistics that could be compared to the expected prevalence found during the literature review. As a broad example, in 2006 the WHO estimated around 5.2% of the global population may be carriers of a pathogenic haemoglobinopathy,[31] and in 2023 only 0.33% of all patients tested for HbA1c had an abnormal haemoglobin detected. While a non-specific statistic such as this cannot be applied to most populations, it gives an idea of what applications this data has.

Specifically analysing different ethnicities gives a good indication of where our testing may be falling short. There is significant difference between predicted and calculated prevalence in the high-risk ethnicities such as Indian, Southeast Asian, and African (Table 4.7), which shows we are not even reaching half of what is expected by these estimations. Taking the example again of those patients who have identified as being of Indian ethnicity. In many different sources the estimated prevalence of haemoglobinopathy in India is 3-4%, whereas of all the individuals who identified as Indian in our database, abnormal haemoglobins were detected in only 1.24%. Again this is less than half of what we should be theoretically expecting.[15; 31] As another example, reports on prevalence of haemoglobinopathy in Southeast Asia are more varied, but all are significantly higher than the calculated 2.33% from the HbA1c data, with some estimates being as high as 40%.[5; 31] These results are all excellent indicators that we are not reaching everyone in our population with our current haemoglobinopathy screening service. This is also reflected in the number of haemoglobinopathies detected via this method that were not already diagnosed (Table 4.3), which is reasonably high for some ethnicities (Table 4.3). With the exception of those of Middle Eastern ethnicity, at least 70% of the abnormalities in other ethnicities were not previously diagnosed.

As mentioned, ANOVA analysis was used on this data to determine that there was a statistically significant relationship between ethnicity and presence of haemoglobinopathy in our population (p value <0.001). A second analysis was conducted using emmeans to compare the ethnicities (Appendix 2, Table 8.1). From this it is evident that when compared to NZ European the ethnicities African, Asian NFD, Indian, Latin American/Hispanic, and Southeast Asian were significantly more likely to have an abnormal haemoglobin (p value <0.001). While screening for haemoglobinopathy should not only be offered to certain ethnic groups, it may be beneficial to know who should be targeted for increased information regarding testing or screening awareness.

While the HbA1c data gives perspective on the calculated shortfalls already discussed, if Pathlab is the only laboratory in the country actively looking for haemoglobinopathies in the HbA1c screening, then the coverage here will likely be much better than other areas. If every laboratory that offered a haemoglobinopathy testing service in New Zealand were to also screen using the HbA1c, I believe the number of undiagnosed haemoglobinopathies for the nation would decrease dramatically. If staff members from Pathlab Waikato or even Sebia

representatives were able to travel to the other centres to teach them how the HbA1c is utilized, I believe it would be an efficient and effective way to expand the testing reach nationwide. The validity of using the HbA1c was proven by former Pathlab scientist Kate Mclaughlin, and the importance of this research should be shared with other laboratories.[1]

5.3 Limitations

While sources of error and bias have been carefully considered, there are certain limitations to this data and available analyses. Firstly, the database relies on manual entry, which increases the chances of human error. This was minimised by combing through the diagnoses made and confirming they fit the given information such as location of variant haemoglobin. Mentioned previously in the discussion is the potential challenge of self-reporting ethnicity. From the Stats NZ data, there are a growing number of individuals in New Zealand who are mixed ethnicity. An example of this could be a patient who is of Fijian Indian descent. This individual could choose to self-report their ethnicity as Indian or Fijian, and these have vastly different risks of certain haemoglobinopathies. To mitigate situations such as this, I believe we should not rely on ethnicity when screening. This is further motivation for a universal national testing service through antenatal screening. Another limitation for our database is that Pathlab only covers the Waikato and Bay of Plenty, therefore it is difficult to extrapolate this data to the rest of New Zealand. For this analysis to have wider applications, a national database of all regions would be required. Finally, due to the large amount of data available, time constraints and the nature of the work, a more thorough and complete statistical analysis could not be performed. A dedicated and experienced health statistician or bioinformatician would be able to provide a more in-depth analysis for more accurate predictions and models.

5.4 Screening service in New Zealand

From the data we have collected it is strongly suggested that New Zealand needs a coordinated national screening service. This is also recommended by the WHO for all its member countries.[32] It does not have to be as encompassing as other countries have implemented, but should offer an effective way to screen the general population of New Zealand for high risk haemoglobinopathy. I believe, based on the services provided in other

countries, that the best way to do this is through antenatal screening. This is for two reasons, firstly to detect individuals that are at risk of having children with thalassaemia major, so that they can make informed reproductive decisions. Secondly, it is a group that are already routinely tested, and the tests that are presently performed can be utilised in this screening. Below is a flow chart (Fig 5.1) that shows the suggested workflow of screening using the parameters already tested for in an initial antenatal screen.

It is suggested that if this were implemented across the country in a coordinated effort from the public and private health services, we could greatly improve the reach of our screening services. This would mean more patients receiving the required care and monitoring and potentially reduce the number of children born with significant haemoglobinopathy. The follow-on effect of this would be fewer patients requiring lifelong treatment for these, therefore less money and resources required. And with the changing patterns in immigration these diseases have the potential to be a serious burden on our health care system.[15]

The initial stages (CBC and HbA1c) are routine tests and do not require further education to interpret. A microcytic, hypochromic anaemia with no obvious cause should be flagged by haematology staff if this is from an antenatal screen. Likewise for an abnormal peak detected while testing for HbA1c for all antenatal screens. This information then needs to be given to a speciality trained haematology medical laboratory scientist to decide what further testing is required. While this is currently carried out by Pathlab, permission from the patient, GP or midwife, and a haematologist is required to then add on a thalassaemia screen. If haemoglobinopathy was explained and accepted as part of the antenatal screen then this lengthy process could be skipped, and a thalassaemia screen immediately added.

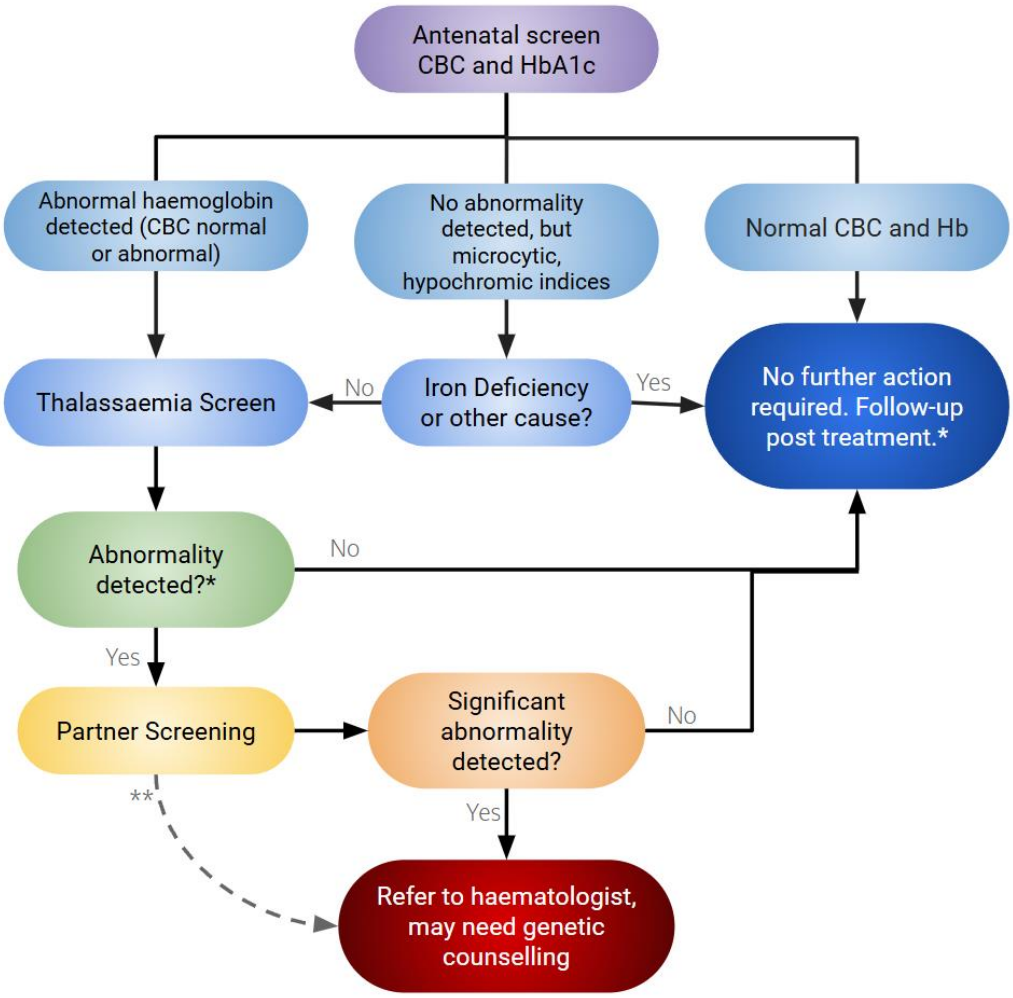


Figure 5.1: Proposed process for haemoglobinopathy screening in New Zealand.

**Follow up with CBC post iron replacement therapy or other cause of anaemia has subsided. However, it is important to note that many patients experience iron deficiency during pregnancy, therefore further testing may still be appropriate if there is a strong suspicion of thalassaemia such as a history of microcytic hypochromic anaemia with normal iron stores or family history of haemoglobinopathy.*

*** Pregnant patients who have a significant haemoglobin disorder such as HbS or β thalassaemia will be referred to a haematologist upon diagnosis without waiting for partner haemoglobinopathy results.*

Previously discussed in the literature review was the cost to treat. TDT patients will cost a healthcare system a significant amount for a lifetime of treatment. A blood transfusion can cost up to $1,200 NZD depending on the product and handling, according to a 2024 pricing list from New Zealand Blood Service. The previously mentioned Danish study by Gravholt et al.(2024)[40] found that the cost of setting up a national screening service would be offset if every two years they could prevent a single TDT child from being born through parental screening and advice. This is because the cost to treat one patient over their lifetime may be in the millions.[17] Based on the increase in the number of β thalassaemia major children being born, I consider that this minimum could be met in New Zealand.

The number of hospitalisations for sickle cell related illnesses in New Zealand is depicted in Figure 5.2. The graph shows that many of the people seeking treatment were children, and there is far more of them than expected given that only 4 homozygous HbS patients were in our database, and there are 49 patients in the data shown below. This is an example of the cost that occurs when a significant haemoglobinopathy is undiagnosed.

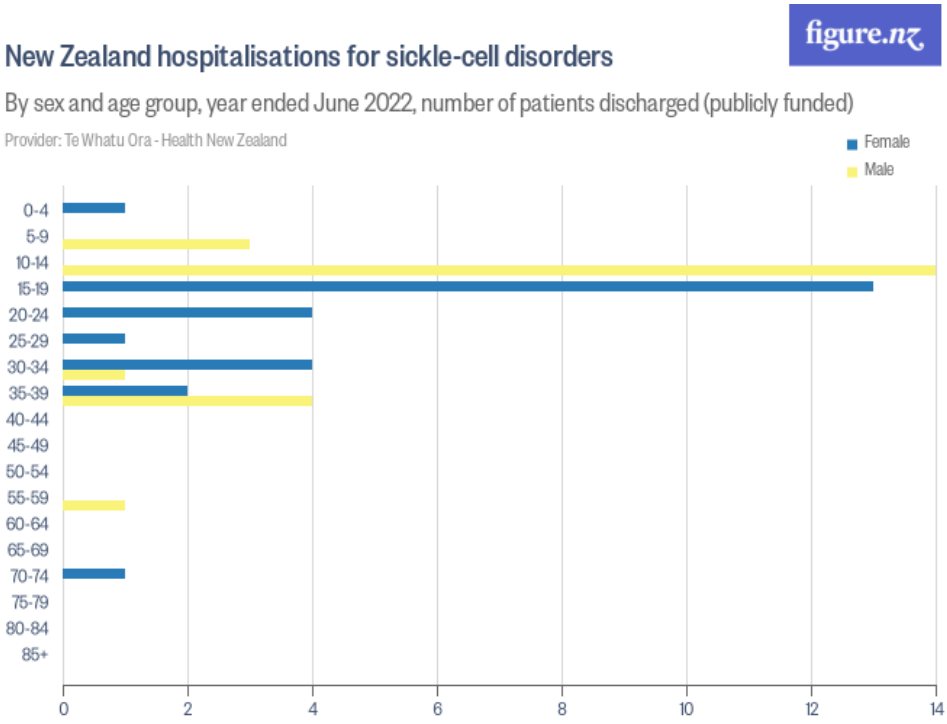


Figure 5.2: New Zealand hospitalisations for sickle-cell disorders.[52]

This shows the number of patients who were hospitalised due to illness related to sickle cell disease. Data is given by age and sex and can be found here: https://tewhatuora.shinyapps.io/hospitals-web-tool/

5.5 Next steps

While nationwide screening may take a large amount of time and effort to implement there are four recommended steps that can be taken first to improve our collective approach to haemoglobinopathy. Steps suggested from the literature review on screening services in other countries are to first provide more information and training for healthcare professionals, and secondly to provide more information in a more accessible way to patients.

5.5.1 Screening service requirements

To coordinate testing for a nation-wide screening service, the first step should be to educate clinicians, nurses and midwives so that they understand when testing is appropriate, who is at risk, and what the result means for the patient and their family. An increase in screening would also require more laboratory resources to offer this service. Currently, screening for haemoglobinopathies is performed at only seven laboratories throughout the country, both public and private. All these laboratories use the Sebia CAPY machine for haemoglobinopathy screening, so setting up a streamlined service between labs should theoretically be the easiest place to start. Creating a national database should therefore be easier if we are all producing the same results. However, this would also require more staff at these facilities who are capable of operating the machines and interpreting the results, as well as more haematologists available for patient care. Having designated experts in haemoglobinopathy at these testing centres is important not only for training other health professionals, but also as a point of contact if any questions or issues arise during testing, either from the clinician or patient. Finally, an antenatal screening service would also require an increase in genetic counsellors and clinical geneticists for familial testing.

5.5.2 Sharing of information

The next hurdle is to begin sharing and discussion between testing centres, specifically between private laboratories and Te Whatu Ora laboratories. This is already being worked on, and a big step forward was taken in 2024 when a special interest group hosted by the New Zealand Institute of Medical Laboratory Science was held in Wellington. This was attended by scientists involved in haemoglobinopathy testing all over the country and created an important networking opportunity between laboratories. It was the hope of the organiser Alan Neal from Pathlab that this would create an environment of cooperation and distribution

of information which is still being worked on. This was also progress toward identifying the ‘experts’ in this discipline and an example of making information more accessible through knowledge sharing, as outlined in the steps discussed in the paper by van Vliet et al. (2022).[45] The next step from this would be to create a national database for all diagnoses of haemoglobinopathy. This may propose a challenge as Te Whatu Ora has extremely stringent information restrictions, therefore this may need to be created and curated within public health and access provided to relevant private health workers and institutions. For the past five years Pathlab Waikato and Waikato hospital have been locally sharing haemoglobinopathy databases, and we are in regular contact regarding certain patients. This cooperation is largely because all additional testing required for Pathlab Waikato patients is processed through Te Whatu Ora Waikato. Therefore, the scientists and haematologists involved in haemoglobinopathy testing are often sharing information and guidance. This is the kind of working relationship we hope to foster between all laboratories in New Zealand, and we are proof that it is always beneficial for our patients and community.

One way to improve the accessibility of information provided to patients, is to have this material translated into different languages, as mentioned in the results. Upon diagnosis, a leaflet is sent to the requesting clinician and the patient. An example of this information can be found in Appendix 1 (Document 7.3). This seems an obvious step to take as most thalassaemia or haemoglobinopathies are more common in non-English speaking countries, so it makes sense to provide information with diagnosis that can be completely understood by the patient. This suggestion has been brought up to higher management and plans are in process to provide this. Suggestions for languages into which to translate are Te Reo Māori, Mandarin, and Hindi. However, further discussions may also include Punjabi, Tagalog, Samoan, Tongan, Cantonese, and Spanish. This is based on language census data from Stats NZ and the prevalence data in the literature review. With this we aim to cover most individuals who do not speak English as a first language to make sure they better understand what the diagnosis of haemoglobinopathy means to them and their families.

5.5.3 Machine Learning

The benefit of a nationwide database containing large quantities of data will also become a hindrance if there is no capacity to utilise this information. With technology moving at the current pace and artificial intelligence (AI) being integrated into most workspaces it is worth

investigating how we might best embrace this to our advantage. Machine learning is the creation and employment of modelling and computer algorithms that are able to learn off a data set and adapt to give a desired output given new data. Simply put, a computer is able to learn from a task that has already been completed, to perform the same task in future. This learning may be supervised, unsupervised, semi-supervised or reinforced depending on the data provided for the task required. The most common of these, and the only one I think worth discussing here is supervised machine learning. This is when clearly labelled data is presented to the algorithm so the input and output is precisely mapped.[53] This kind of machine learning can be further divided into two types: classification or regression. Regression is used when the desired response is numerical, and it is the role of the machine to make predictions based on this. Classification is used for yes/no responses to assign a type or 'classification' to every data point.

Supervised machine learning could be utilised to diagnose haemoglobinopathy via decision tree classification. This is because our database already contains the data to teach the machine such as CBC results, capillary electrophoresis results and haemoglobinopathy diagnosis. This provides a clear, already established path to the desired output which is haemoglobinopathy diagnosis. Supervised machine learning is extremely useful in healthcare as all it requires is the model data. With everything in healthcare now being digitalised, there are vast amounts of data available to build robust and accurate machine learning processes. This technology is already in use in many places, and the application in healthcare has been well researched, and as described by Agrawal, R. (2020), has been used to aid diagnosis and prognosis of many diseases including Parkinson's disease and cancer.[53]

A basic decision tree for the current method of haemoglobinopathy diagnosis has been created and can be found in Appendix 1 (Figure 7.4), roughly following the flow chart above (Figure 5.1) and based on data and numbers currently used in diagnosis at Pathlab. If we apply machine learning and provide enough data, this process can be automated to increase efficiency and decrease human input, therefore producing a more standard diagnosis, particularly if used by every lab that currently runs the Sebia CAPY electrophoresis system. This would cut down significantly on analysis time and would result in very few haemoglobinopathies requiring human intervention for further testing or diagnosis.

5.6 Conclusion

By collating and analysing the data for this thesis, it is my hope that we may continue to provide a successful haemoglobinopathy screening service to our community, while working toward improving coverage and detection levels through a coordinated and cooperative national screening service. It is recommended that this be accomplished by including a haemoglobinopathy screen as part of the antenatal screening service already provided to pregnant women in New Zealand. From there, family members and other high-risk individuals can be identified through the methods already carried out, such as CBC and HbA1c. Through this, it is also recommended that a collective effort be made to increase awareness and offer more information about haemoglobinopathy, not only to healthcare workers, but also to the general public. Increasing the number of health professionals with specialised knowledge on this subject would not only improve our current service, but it would also be a step towards setting up a national screening service for haemoglobinopathies in New Zealand.

6. References

1. Mclaughlin, K. (2020). *To Determine the Validity of the Sebia HbA1c Assay for Identifying Variant Haemoglobin Disorders* Auckland University of Technology]. https://hdl.handle.net/10292/13189
2. Thein, S. L. (2011). Milestones in the History of Hemoglobin Research (In Memory of Professor Titus H.J. Huisman). *Hemoglobin*, *35*(5-6), 450-462. https://doi.org/10.3109/03630269.2011.613506
3. Bain, B. J., Daniel, Y., Henthorn, J., de la Salle, B., Hogan, A., Roy, N. B. A., Mooney, C., Langabeer, L., & Rees, D. C. (2023). Significant haemoglobinopathies: A guideline for screening and diagnosis: A British Society for Haematology Guideline. *British Journal of Haematology*, *201*(6), 1047-1065. https://doi.org/10.1111/bjh.18794
4. Vichinsky, E. (2008). Sickle Cell Disease and Thalassemia. In. ASH 50 Years in Hematology: Research that Revolutionised Patient Care: American Society of Hematology.
5. Bain, B. J. (2020). *Haemoglobinopathy Diagnosis* (3rd ed.). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/waikato/detail.action?docID=351173
6. Ingram, V. M. (1956). A Specific Chemical Difference Between the Globins of Normal Human and Sickle-Cell Anæmia Hæmoglobin. *Nature (London)*, *178*(4537), 792-794. https://doi.org/10.1038/178792a0
7. Whipple, G. H., & Bradford, W. L. (1936). Mediterranean disease-thalassemia(Erythroblastic anemia of cooley). Associated pigment abnormalities simulating hemochromatosis. *The Journal of pediatrics*, *9*(3), 279-311. https://doi.org/10.1016/S0022-3476(36)80021-3
8. Faye, F. B. K. (2009). *Malaria Resistance or Susceptibility in Red Cells Disorders*. Nova Science Publishers, Incorporated. http://ebookcentral.proquest.com/lib/waikato/detail.action?docID=3019453
9. Li, C.-K. (2017). New trend in the epidemiology of thalassaemia. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *39*, 16-26. https://doi.org/10.1016/j.bpobgyn.2016.10.013
10. Database, G. T. H. G. (2024). *HBA1 Gene - Haemoglobin Subunit Alpha 1*. Retrieved 23/09/2024 from https://www.genecards.org/cgi-bin/carddisp.pl?gene=HBA1&keywords=Hemoglobin,Subunit,Zeta
11. Leung, Y. T., & Lao, T. T. (2012). Thalassaemia in pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *26*(1), 37-51. https://doi.org/10.1016/j.bpobgyn.2011.10.009
12. Giardine B, B. J. V. E. P. C. M. K. J. P. B. M. R. C. M. W. T. G. W. H. H. R. C. (2024). *Updates of the HbVar database of human hemoglobin variants and thalassemia mutations*. https://globin.bx.psu.edu/hbvar/menu.html
13. Brown, T. S., Lakra, R., Master, S., & Ramadas, P. (2023). Sickle Cell Trait: Is It Always Benign? *J Hematol*, *12*(3), 123-127. https://doi.org/10.14740/jh958
14. Fucharoen, S., & Weatherall, D. J. (2012). The hemoglobin E thalassemias. *Cold Spring Harbor perspectives in medicine*, *2*(8), a011734-a011734. https://doi.org/10.1101/cshperspect.a011734
15. Tuo, Y., Li, Y., Li, Y., Ma, J., Yang, X., Wu, S., Jin, J., & He, Z. (2024). Global, regional, and national burden of thalassemia, 1990–2021: a systematic analysis for the global burden of disease study 2021. *eClinicalMedicine*, *72*, 102619. https://doi.org/https://doi.org/10.1016/j.eclinm.2024.102619

16. Sinha, S., Seth, T., Colah, B. R., & Bittles, H. A. (2020). Haemoglobinopathies in India: estimates of blood requirements and treatment costs for the decade 2017–2026. *Journal of Community Genetics*, *11*(1), 39-45. https://doi.org/10.1007/s12687-019-00410-1
17. Crighton, G., Wood, E., Scarborough, R., Ho, J. P., & Bowden, D. (2016). Haemoglobin disorders in Australia: where are we now and where will we be in the future? *Internal Medicine Journal*, *46*(7), 770-779. https://doi.org/10.1111/imj.13084
18. Acharya, B., Mishra, D. P., Barik, B., Mohapatra, R. K., & Sarangi, A. K. (2023). Recent progress in the treatment of sickle cell disease: an up-to-date review. *Beni-Suef University journal of basic and applied sciences*, *12*(1), 38-18. https://doi.org/10.1186/s43088-023-00373-w
19. Moore, R. D., Charache, S., Terrin, M. L., Barton, F. B., & Ballas, S. K. (2000). Cost-effectiveness of hydroxyurea in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. *Am J Hematol*, *64*(1), 26-31. https://doi.org/10.1002/(sici)1096-8652(200005)64:1<26::aid-ajh5>3.0.co;2-f
20. Grech, L., Borg, K., & Borg, J. (2022). Novel therapies in β-thalassaemia. *British Journal of Clinical Pharmacology*, *88*(6), 2509-2524. https://doi.org/10.1111/bcp.14918
21. bluebird bio Releases Details of Its U.S. Commercial Infrastructure to Enable Patient Access to ZYNTEGLO. (2022). *Manufacturing Close - Up*.
22. Malay, J., Salama, R. A. A., Alam Qureshi, G. S., Ammar, A., Janardhan, G., Safdar, M., & Elshamy, H. A. H. (2024). Gene Therapy: A Revolutionary Step in Treating Thalassemia. *Hematol Rep*, *16*(4), 656-668. https://doi.org/10.3390/hematolrep16040064
23. The gene-therapy revolution risks stalling if we don't talk about drug pricing. (2023). *Nature (London)*, *616*(7958), 629-630. https://doi.org/10.1038/d41586-023-01389-z
24. *CAPILLARYS 3 TERA & OCTA SERVICE MANUAL*. (2019). Retrieved 16/02/2025 from https://www.scribd.com/document/686257521/Capillarys-3-TERA-Service-Manual-v1-15P1-22-03-2019
25. Kenndler, E., & Minárik, M. (2021). Capillary Electrophoresis and its Basic Principles in Historical Retrospect Part 1. The Early Decades of the "Long Nineteenth Century": The Voltaic Pile, and the Discovery of Electrolysis, Electrophoresis and Electroosmosis. *Substantia*, *5*(1). https://doi.org/10.36253/substantia-1018
26. Jabeen, R., Payne, D., Wiktorowicz, J., Mohammad, A., & Petersen, J. (2006). Capillary electrophoresis and the clinical laboratory. *Electrophoresis*, *27*(12), 2413-2438. https://doi.org/10.1002/elps.200500948
27. Hempe, J. M., & Craver, R. D. (1994). Quantification of hemoglobin variants by capillary isoelectric focusing. *Clinical Chemistry*, *40*(12), 2288-2295. https://doi.org/10.1093/clinchem/40.12.2288
28. Celia Henry, A. (2016). 50 years of HPLC. *C & EN global enterprise*, *94*(24), 28-33. https://doi.org/10.1021/cen-09424-cover1
29. Griffiths, J. (2008). A Brief History of Mass Spectrometry. *Analytical Chemistry*, *80*(15), 5678-5683. https://doi.org/10.1021/ac8013065
30. Hjelm, L. N., Chin, E. L., Hegde, M. R., Coffee, B. W., & Bean, L. J. (2010). A simple method to confirm and size deletion, duplication, and insertion mutations detected by sequence analysis. *J Mol Diagn*, *12*(5), 607-610. https://doi.org/10.2353/jmoldx.2010.100011
31. Modell, B. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization*, *2008*(6), 480-487. https://doi.org/10.2471/BLT.06.036673

32. Thalassaemia and Other Haemoglobinopathies. (2006). In (Vol. 118th Session, Agenda item 5.2). World Health Organisation.
33. Wang, W.-D., Hu, F., Zhou, D.-H., Gale, P. R., Lai, Y.-R., Yao, H.-X., Li, C., Wu, B.-Y., Chen, Z., Fang, J.-P., Chen, S.-J., & Liang, Y. (2023). Thalassaemia in China. *Blood Reviews*, *60*, 101074. https://doi.org/10.1016/j.blre.2023.101074
34. Williams, T. N., & Weatherall, D. J. (2012). World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harbor perspectives in biology*, *4*(9), a011692-a011692. https://doi.org/10.1101/cshperspect.a011692
35. Musallam, K. M., Lombard, L., Kistler, K. D., Arregui, M., Gilroy, K. S., Chamberlain, C., Zagadailov, E., Ruiz, K., & Taher, A. T. (2023). Epidemiology of clinically significant forms of alpha- and <scp>beta-thalassemia</scp>: A global map of evidence and gaps. *American Journal of Hematology*, *98*(9), 1436-1451. https://doi.org/10.1002/ajh.27006
36. Parker, L. S., Williamson, D., Shepherd, C. S., & Boswell, D. R. (1989). Alpha thalassaemia in the Maori: a family study. *N Z Med J*, *102*(868), 245-246.
37. Kattamis, A., Forni, G. L., Aydinok, Y., & Viprakasit, V. (2020). Changing patterns in the epidemiology of β-thalassemia. *Eur J Haematol*, *105*(6), 692-703. https://doi.org/10.1111/ejh.13512
38. Ha, J., Martinson, R., Iwamoto, S. K., & Nishi, A. (2019). Hemoglobin E, malaria and natural selection. *Evolution, Medicine, and Public Health*, *2019*(1), 232-241. https://doi.org/10.1093/emph/eoz034
39. Colah, B. R., Nadkarni, H. A., Gorakshakar, C. A., Sawant, M. P., Mehta, R. P., Gorivale, S. M., Hariharan, P., Mohanty, D., & Ghosh, K. (2021). The Changing Trends in Prenatal Diagnosis of Hemoglobinopathies in India: The Quest of a Single Center to Reduce the Burden of Disease over Three Decades. *Hemoglobin*, *45*(2), 112-118. https://doi.org/10.1080/03630269.2021.1908346
40. Gravholt, E. A. E., Petersen, J., Mottelson, M., Nardo-Marino, A., Rathe, M., Olsen, M., Holm, C., Jørgensen, S. F., Birgens, H., & Glenthøj, A. (2024). The Danish national haemoglobinopathy screening programme: Report from 16 years of screening in a low-prevalence, non-endemic region. *British Journal of Haematology*, *204*(1), 329-336. https://doi.org/10.1111/bjh.19103
41. National Screening, C. (2006). NHS sickle cell and thalassemia screening programme. *London: NHS Sickle Cell and Thalassaemia Screening Programme*.
42. Mitton, J., & Jeffry, B. M. (2002). Hardy-Weinberg Equation. In (1 ed., Vol. 1, pp. 459-461): Oxford University Press.
43. Angastiniotis, M., Eleftheriou, A., Galanello, R., Harteveld, C. L., Petrou, M., Traeger-Synodinos, J., Giordano, P., Jauniaux, E., Modell, B., & Serour, G. (2013). In J. Old (Ed.), *Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 1: Principles*. Thalassaemia International Federation. https://www.ncbi.nlm.nih.gov/books/NBK190485/
44. Li, Y., Zhang, H., & Jiang, Y. (2023). Research on the changes and predictions of the burden of type 2 diabetes mellitus in Pacific Island countries from 1990 to 2019. *PLOS ONE*, *18*(12), e0293681-e0293681. https://doi.org/10.1371/journal.pone.0293681
45. van Vliet, M. E., Kerkhoffs, J. L. H., Harteveld, C. L., & Houwink, E. J. F. (2022). Hemoglobinopathy prevention in primary care: a reflection of underdetection and difficulties with accessibility of medical care, a quantitative study. *European journal of human genetics : EJHG*, *30*(7), 790-794. https://doi.org/10.1038/s41431-022-01051-8
46. Lee, J. S., Rhee, T. M., Jeon, K., Cho, Y., Lee, S. W., Han, K. D., Seong, M. W., Park, S. S., & Lee, Y. K. (2022). Epidemiologic Trends of Thalassemia, 2006-2018: A

Nationwide Population-Based Study. *J Clin Med*, *11*(9). https://doi.org/10.3390/jcm11092289

47. Ngim, F. C., Ibrahim, H., Lai, M. N., & Ng, S. C. (2015). A single centre study on birth of children with transfusion-dependent thalassaemia in Malaysia and reasons for ineffective prevention. *Prenatal Diagnosis*, *35*(1), 51-59. https://doi.org/10.1002/pd.4484
48. Weil, L. G., Charlton, M. R. M., Coppinger, C., Daniel, Y., & Streetly, A. (2020). Sickle cell disease and thalassaemia antenatal screening programme in England over 10 years: a review from 2007/2008 to 2016/2017. *Journal of clinical pathology*, *73*(4), 183-190. https://doi.org/10.1136/jclinpath-2019-206317
49. Sánchez, M. M. J., Cancho, B. J. E., Benéitez, D., Payán-Pernía, S., Gimbert, C. A., Ruiz-Llobet, A., Salinas, A. J., Sebastián, E., Argilés, B., Bermúdez, M., Vázquez, Á. M., Ortega, J. M., Rubio, L. M., Gondra, A., Uriz, J. J., Morado, M., Coll, T. M., Duarte, L. M., Baro, M.,…Cela, E. (2024). Haemoglobinopathies and other rare anemias in Spain: ten years of a nationwide registry (REHem-AR). *Annals of Hematology*, *103*(8), 2743-2755. https://doi.org/10.1007/s00277-024-05788-8
50. Aramayo-Singelmann, C., Halimeh, S., Proske, P., Vignalingarajah, A., Cario, H., Christensen, M. O., Yamamoto, R., Röth, A., Reinhardt, D., Reinhardt, H. C., & Alashkar, F. (2022). Screening and diagnosis of hemoglobinopathies in Germany: Current state and future perspectives. *Scientific reports*, *12*(1), 9762-9762. https://doi.org/10.1038/s41598-022-13751-8
51. van Vliet, M. E., Kerkhoffs, J. L. H., Harteveld, C. L., & Houwink, E. J. F. (2023). Hemoglobinopathy screening in primary care in the Netherlands: exploring the problems and needs of patients and general practitioners. *European journal of human genetics : EJHG*, *31*(4), 417-423. https://doi.org/10.1038/s41431-022-01156-0
52. *New Zealand hospitalisations for sickle-cell disorders*. (2021). Figure.nz. https://figure.nz/chart/UBzueIrTPNHu0QJQ
53. Agrawal, R. (2020). *Machine learning for healthcare : handling and managing data* (1st ed.). CRC Press. https://books.google.co.nz/books?id=3ZUBEAAAQBAJ&lpg=PP1&ots=p6zfpswky_&dq=Machine%20Learning%20for%20Healthcare%20%3A%20Handling%20and%20Managing%20Data&lr&pg=PP1#v=onepage&q=Machine%20Learning%20for%20Healthcare%20:%20Handling%20and%20Managing%20Data&f=false

7. Appendix 1

Table 7.1: Literature Review Summary. An overview table of all the common haemoglobinopathies and their incidence as discussed in the literature review.

**Prevalence from Haemoglobinopathy Diagnosis by Bain BJ, Published 2020[5]*

Type	Subtype	Possible Genotype	Common mutations	Phenotype	Common ethnicities (percentage where available) *
Alpha thalassaemia	α^+	-α/αα -α/-α	$-\alpha^{3.7}$, $-\alpha^{4.2}$	Undetectable to mild anaemia	• Greece 7-10% • Cyprus 26% • Turkey 6% • Italy (10% Sicily, 28% Sardinia) • Spain 2% • Portugal 10% • Middle east <1-23% (overall 9%) • North Africa 5-8% • West Africa 8-58% • Central Africa 36-40% • East Africa 2-34% • Southern Africa 7-39% • Pakistan 15-20% • India 5-33% (up to 99%) • Sri Lanka 15-16% • Nepal 6-14% (up to 97%) • Southern China <1-6% • Thailand 3-17% • Cambodia 12-28% • Laos 14% • Vietnam 8-22% • Malaysia up to 29% • Philippines 5% • Indonesia 6% • Solomon Islands and Vanuatu 45% • Australia 6% • New Zealand Māori 5-10
	α^0	--/αα --/--	$--^{SEA}$, $--^{FIL}$, $--^{THA}$, $--^{MED}$	Mild anaemia to Barts Hydrops Fetalis	• Southeast Asia, Southern China 3-9% • Greece 1.5% • Middle East
	Constant Spring (non-deletional)	αα/αα^{CS} αα^{CS}/αα^{CS}		Undetectable to mild anaemia	• Thailand 1-8% • Cambodia 0-2% • Laos 9% • Vietnam 0-4% • Malaysia <1-6%
Beta thalassaemia	β^+	β/β^+ β^+/β^+		Undetectable to mild anaemia	• Greece 6-28% (overall 8%) • Cyprus 14-18% • Turkey and Italy 1-37% (overall 3%) • Spain and Portugal 8% • France 3% • Eastern Europe 2-20% • Middle East 2-20% (highest in Arabic populations) • North Africa 2-4% Overall • West Africa 1-14% Overall • East Africa – up to 2% (rare) • Central Africa <1% (very rare) • Afghanistan 3% • Pakistan 5% • Nepal 13% • India 1-16% Overall (average 3% but up to 40%) • Bangladesh 3% • Sri Lanka 1-5% • Maldives 16% • China: North 0.5, South 2-6% • Myanmar 0.5-6% • Thailand 4-11% • Cambodia 1-5% • Laos 1-9% • Vietnam 1-25% • Malaysia 1-5% • Philippines 1-2% • Indonesia 0-11% • Papua New Guinea 1-25%
	β^0	β/β^0 β^0/β^0		Moderate anaemia to transfusion dependent anaemia or foetal demise.	

Beta haemoglobinopathy	HbS	β/β^S β^S/β^S		Undetectable in heterozygous state Significant haemolytic anaemia with organ damage in homozygous state	• North Africa <1-17% (highest in Nigeria) • East Africa <1-34% • West Africa <1-41% • Central Africa <1-46% (highest in the DRC) • Southern Africa <1-40% • United States of America 1.6%	• Central America <1-25% • South America <1-22% • Europe 0-34% (highest in Greece and Turkey) • Middle East<1-34% (highest in Syria and Saudi Arabia) • Asia 0-35% (highest in India and Nepal)
	HbC	β/β^C β^C/β^C		Undetectable in heterozygous state. Mild but chronic haemolytic anaemia in homozygous state	• North Africa 0-13% • West Africa <1-50 (highest in Burkina Faso and Côte d'Ivoire) • Central Africa <1% • Southern Africa <0.1%	• United States of America 1-3.5% (in African Americans) • Central America <1% • South America 0-7% (highest in Suriname and French Guiana) • Europe 0.5-1% (highest in Turkey)
	HbD	β/β^D β^D/β^D		Undetectable in heterozygous state. Mild haemolytic anaemia in homozygous state	• India 2-3% (in the Punjab region) • Pakistan • Afghanistan • Iran	• Italy (Sicily 0.5%) • Greece • Eastern Europe • Sri Lanka 0-1.3%
	HbE	β/β^E β^E/β^E		Undetectable in heterozygous state. Mild MHA in homozygous state	• India 0-3.5% • Pakistan 0.5-1% • Bangladesh 4% • Bhutan 1.5-6.5% • Myanmar 1-33% • Thailand 8-40%	• Laos 20-40% • Cambodia 15-30% • Vietnam 2-4% • Southern China 1-2.5% • Malaysia 1-40% • Indonesia 1-13%
	HbO	β/β^O β^O/β^O		Undetectable in heterozygous state. Very mild haemolytic anaemia in homozygous state	• Greece • Italy • Eastern Europe (Particularly Bulgaria) • Israel • Saudi Arabia	• Yemen • Egypt • Kenya • Sudan • Morocco • Tunisia

7.1 List of WHO countries by region

Original data can be found here: https://data.who.int/countries

7.1.1 African Region (AFR)

Algeria, Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Ivory Coast, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Namibia, Niger, Nigeria, Republic of the Congo, Rwanda, São Tomé and Príncipe, Senegal, Seychelles, Sierra Leone, South Africa, South Sudan, Eswatini, Togo, Uganda, Tanzania, Zambia, Zimbabwe.

7.1.2 Region of the Americas (AMR)

Antigua and Barbuda, Argentina, Bahamas, Barbados, Belize, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Ecuador, El Salvador, Grenada, Guatemala, Guyana, Haiti, Honduras, Jamaica, Mexico, Nicaragua, Panama, Paraguay, Peru, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Suriname, Trinidad and Tobago, Uruguay, Venezuela.

7.1.3 South-East Asian Region (SEAR)

Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, Timor-Leste.

7.1.4 European Region (EUR)

Albania, Andorra, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Malta, Moldova, Monaco, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkey, Turkmenistan, Ukraine, United Kingdom, Uzbekistan.

7.1.5 Eastern Mediterranean Region (EMR)

Afghanistan, Bahrain, Djibouti, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Qatar, Saudi Arabia, Somalia, Sudan, Syria, Tunisia, United Arab Emirates, Yemen.

7.1.6 Western Pacific Region (WPR)

Australia, Brunei, Cambodia, China, Cook Islands, Fiji, Japan, Kiribati, Laos, Malaysia, Marshall Islands, Micronesia, Mongolia, Nauru, New Zealand, Niue, Palau, Papua New Guinea, Philippines, Samoa, Singapore, Solomon Islands, South Korea, Taiwan, Tonga, Tuvalu, Vanuatu, Vietnam.

Document 7.1: Ethics approval letter from the University of Waikato

Full ethics approval application available upon request.

The University of Waikato
Private Bag 3105
Gate 1, Knighton Road
Hamilton, New Zealand

Human Research Ethics Committee
Roger Moltzen
Telephone: +64021658119
Email:humanethics@waikato.ac.nz

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

21 November 2023

Sally Annan
HECS
By email: sallyannan@hotmail.com

Dear Sally

HREC(Health)2023#45 : Antenatal Thalassaemia Screening in the Waikato and Bay of Plenty Regions

Thank you for your detailed responses to the Committee feedback.

We are now pleased to provide formal approval for your project.

Please contact the Committee by email (humanethics@waikato.ac.nz) if you wish to make changes to your project as it unfolds, quoting your application number with your future correspondence. Any minor changes or additions to the approved research activities can be handled outside the monthly application cycle.

We wish you all the best with your research.

Regards,

Emeritus Professor Roger Moltzen MNZM
Chairperson
University of Waikato Human Research Ethics Committee

Document 7.2: Authorisation letter from Pathlab

pathlab

11th September 2023

The Secretary
Human Research Ethics Committee
Private Bay 3105
Hamilton 3240

Via email: humanethics@waikato.ac.nz

To whom it may concern

We hereby confirm that Sally Annan is authorised to access information related to her MSc project. This information / data is routinely collected & accessed for diagnostic purposes, with usual patient applied consent by requesting clinician (With informed consent for any investigation suggested by laboratory). The data collected should be de-identified in the MSc project report - as per IANZ Specific criteria (Information previously provided by Sally).

If you have any further queries on this matter, please do not hesitate to contact me.

Kind regards

Alan Neal
Lead of Specialty, Haematology

Document 7.3: (2 pages) Example of a letter given to patients and clinicians upon diagnosis of haemoglobinopathy. This example is of the letter that accompanies the diagnosis of Sickle Cell Disease or homozygous HbS.

Sickle cell disease

Sickle cell disease is a disorder of haemoglobin production. Haemoglobin is a protein in red blood cells that carries oxygen around our bodies. Haemoglobin is made up of haem groups containing iron and globin chains. The main globin chains are call alpha (α) and beta (β). Sickle cell disease or sickle cell anaemia results from a genetic mutation/alteration affecting the β globin gene. The variant haemoglobin (haemoglobinopathy) is referred to as Haemoglobin S.

Sickle cell disease is an inherited condition meaning it is passed from parent to child in genes. Genes carry information about human characteristics such as hair colour. Sickle cell disease is **NOT** contagious and **NOT** transmitted by germs.

Inheritance

- A person normally inherits two β globin genes for the production of the β globin protein in haemoglobin.
- A person may have the haemoglobin S mutation in one of their two β globin genes. This person is referred to as a sickle cell carrier and is generally healthy. Doctors may use the terms heterozygous haemoglobin S or sickle cell trait, but they mean the same thing.
  - Carriers may be at risk of having a child with sickle cell disease if their partner is also a sickle cell carrier.
- When both β globin genes have the sickle cell mutation, this person has sickle cell disease. Instead of the patient's red blood cells being round, they become sickle or crescent shaped. These sickle cells are fragile and break down more easily.
  - In sickle cell disease, the amount of haemoglobin and the number of red bloods cells is less, resulting in anaemia.
  - The sickle cells can cause blockages in small blood vessels. If this occurs in the bones, it can cause severe pain and is called a sickle cell 'crisis'. Damage can also occur to the kidneys, lungs, and eyes.
- Haemoglobin S can be inherited with other haemoglobin variants or thalassaemia's resulting in severe conditions. Examples of other variant haemoglobins/ thalassaemia's to be aware of include:
  - β thalassaemia (beta thalassaemia)
  - Haemoglobin C
  - Haemoglobin D^{Punjab}
  - Haemoglobin O^{Arab}
  - δβ thalassaemia (delta-beta thalassaemia)
  - Haemoglobin Lepore
  - HPFH (Hereditary Persistence of Foetal Haemoglobin)

Treatment for sickle cell disease

Patients with sickle cell disease require ongoing treatment to prevent and manage the disease.

Sickle cell disease and family planning

The genes for sickle cell disease are common in people of African, Middle eastern, Southern European, Indian, Pakistani, and Caribbean descent.

Couples planning a pregnancy should have a blood test to determine whether they are carriers if:

- They or their partner are carriers for haemoglobin S or have sickle cell disease, or
- There is a family history of thalassaemia or variant haemoglobin, or
- If their family origin is an area listed above.

Testing can be arranged by your local doctor. By testing we are able to determine whether there is a risk of having a child with sickle cell disease.

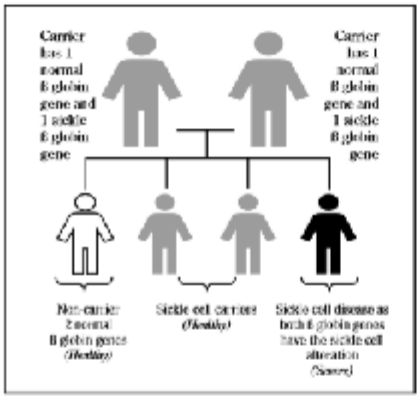
Where there is a risk of having an affected child, several options are available. This condition can be diagnosed as early as the 12th week of pregnancy so termination of pregnancy can be considered, if appropriate. Couples can adopt or can consider assisted reproductive techniques (such as pre-implantation genetic diagnosis, the use of donor eggs or donor sperm). Others may choose to take the chance of having an affected child. All of these options should be discussed with a Genetic Counsellor.

Important information for your family

If you are a sickle cell carrier or have sickle cell disease, other members of your family should be tested as they may also be carriers and at risk of having children with a sickle cell disease. It is recommended that other family members and their partners are screened before having children of their own.

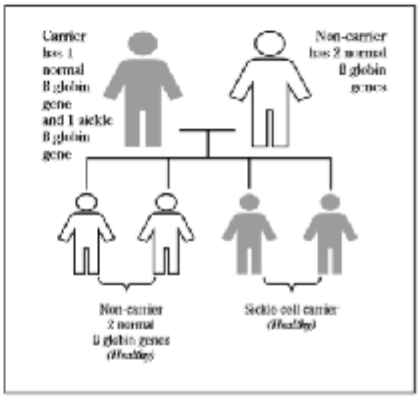
Chances of having a child affected with Sickle Cell Disease

Figure 1:
Both parents are sickle cell carriers



With each pregnancy, this couple has a:
- 1 in 4 chance of having a child with 2 normal β globin genes.
- 2 in 4 chance of having a sickle cell carrier.
- 1 in 4 chance of sickle cell disease.

Figure 2:
Only one parent is a sickle cell carrier



With each pregnancy, this couple has a:
- 2 in 4 chance of having a child with 2 normal β globin genes.
- 2 in 4 chance of having a sickle cell carrier.

References:

https://www.tasca.org.au

https://hematology.org/education/patients/anemia/sickle-cell-disease

For Medical Professionals seeking further information or advice, please contact Pathlab to discuss with one of our Haematologists: **07 858 0795**

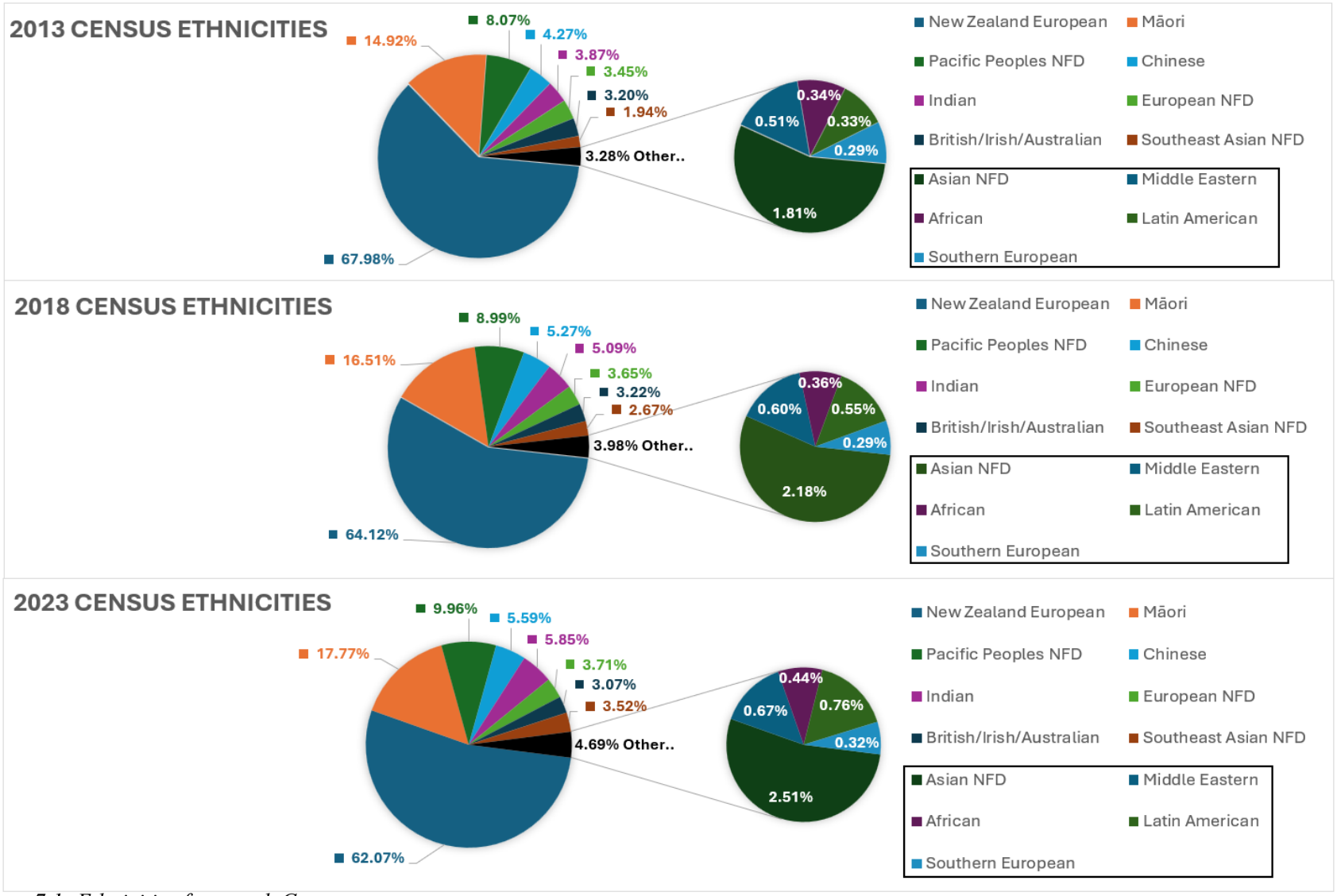


Figure 7.1: Ethnicities from each Census.

NFD= not further defined. The ethnicity groups were created by combining the following census categories:

Pacific Peoples NFD: Samoan, Cook Island Māori, Tongan, Niuean, Fijian, Tokelauan, Other Pacific Peoples, and Pacific Peoples NFD.

European NFD: Dutch, German, Polish, Other European, and European NFD

Southeast Asian NFD: Filipino, Cambodian, Vietnamese, and Southeast Asian NFD

Asian NFD: Korean, Japanese, Sri Lankan, Other Asian, Asian NFD

Southern European: South Slav., Italian, and Greek.

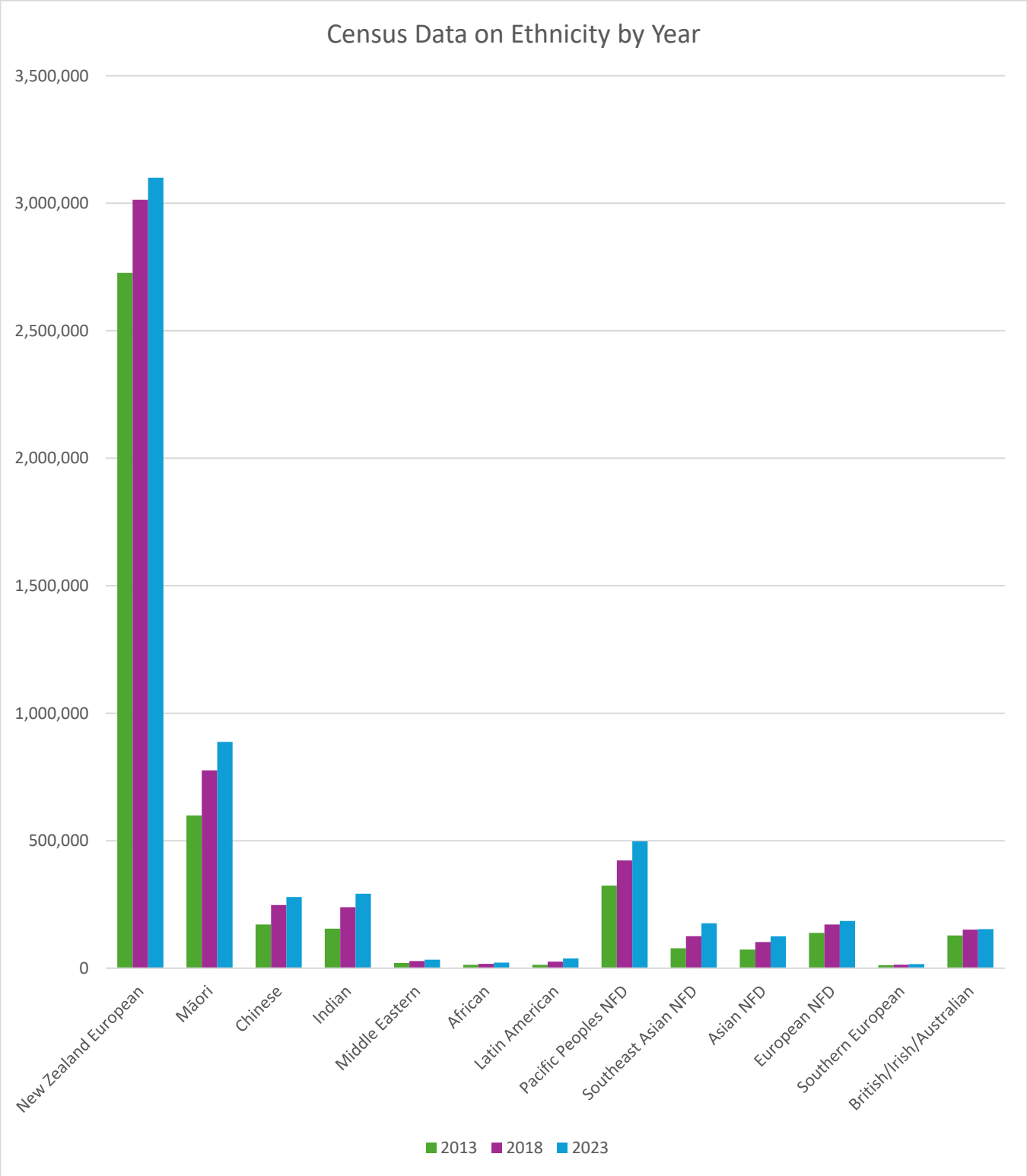


Figure 7.2: A comparison of each of the ethnic groups to show the changes between the census in 2013, 2018 and 2023.

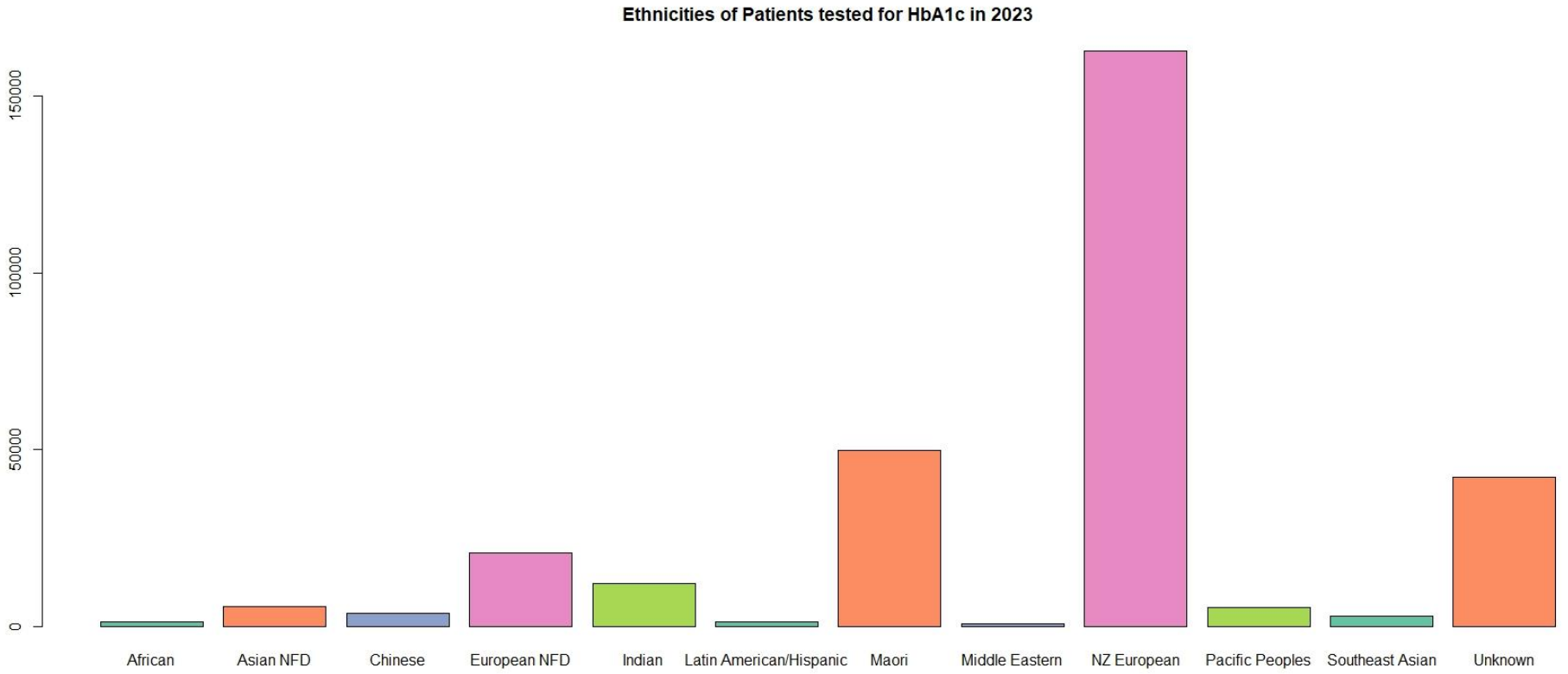


Figure 7.3: Ethnicities of the patients who were tested for HbA1c tested in 2023, produced in RStudio.

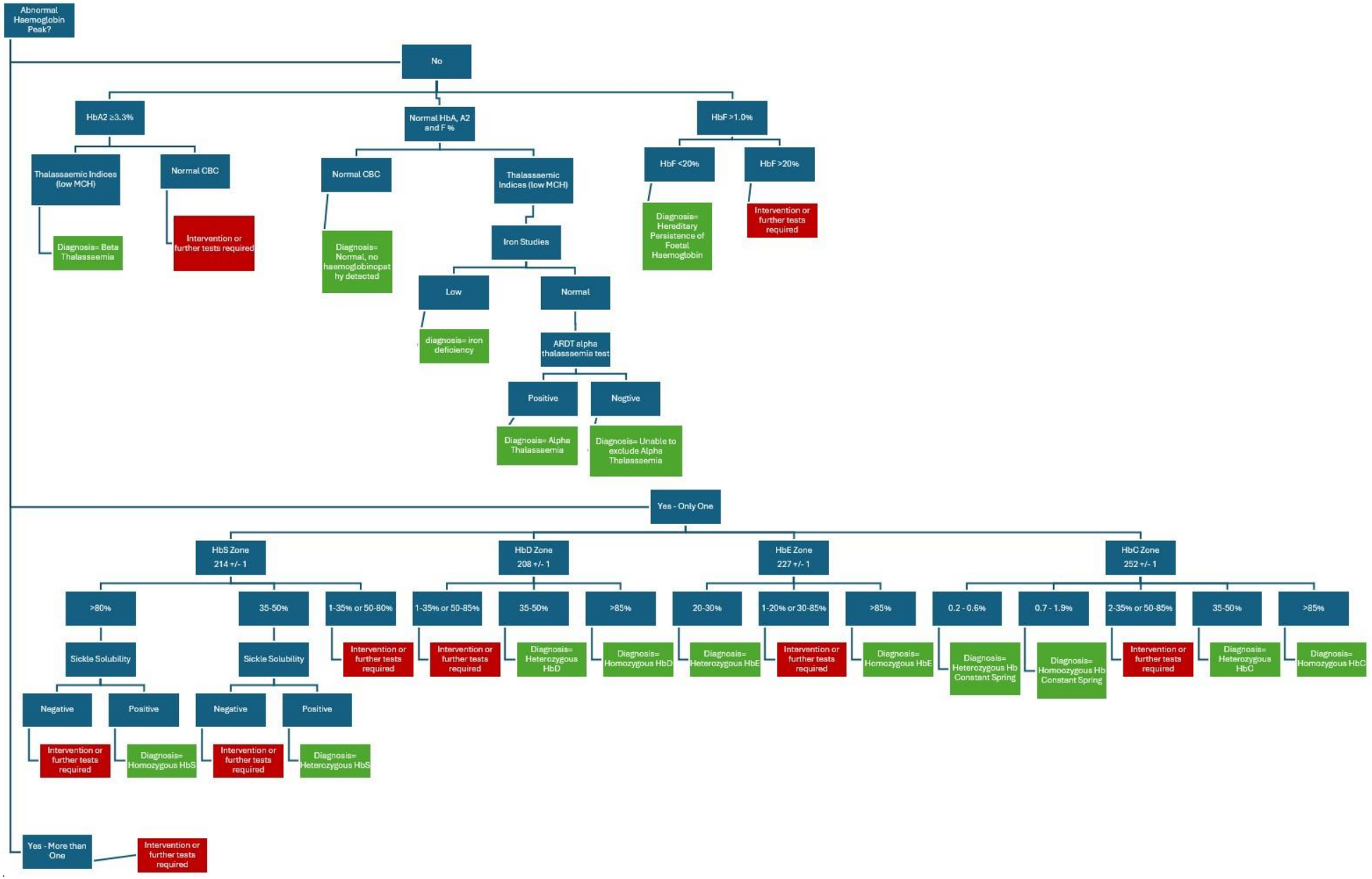


Figure 7.4: Decision tree for haemoglobinopathy diagnosis by machine learning.

8. Appendix 2

8.1 RStudio code:

```
#Download required packages
library(tidyverse)
library(plyr)
library(emmeans)

#Import data
HBAC <- read.csv("HBAC23data.csv", stringsAsFactors = T, header=T, sep=',')
View(HBAC)

#Remove duplicate rows based on NHI
HBAC <- HBAC %>% distinct(NHI, .keep_all = TRUE)

#Fix formats
HBAC$GENDER <- as.factor(HBAC$GENDER)
HBAC$ETHNICITY <- as.factor(HBAC$ETHNICITY)
HBAC$Diagnosis <- as.factor(HBAC$Diagnosis)
HBAC$Abnormal.Hb <- as.factor(HBAC$Abnormal.Hb)
HBAC$Significant <- as.factor(HBAC$Significant)
HBAC$Known <- as.factor(HBAC$Known)

#Create simplified ethnicity groups
HBAC$ETHNICITY <- revalue(HBAC$ETHNICITY, c("Cook Island Māori"="Pacific Peoples",
"Fijian"  = "Pacific Peoples", "Niuean" = "Pacific Peoples", "Other Pacific Peoples" = "Pacific
Peoples", "Pacific Peoples NFD" = "Pacific Peoples", "Samoan" = "Pacific Peoples", "Tokelauan" =
"Pacific Peoples", "Tongan" = "Pacific Peoples"))
HBAC$ETHNICITY <- revalue(HBAC$ETHNICITY, c("Do not know" = "Unknown", "Not Stated"
= "Unknown", "Other (code retired)" = "Unknown", "Refused to answer" = "Unknown", "Response
unidentifiable" = "Unknown", "NA" = "Unknown", "Other ethnicity" = "Unknown"))
HBAC$ETHNICITY <- revalue(HBAC$ETHNICITY, c("Other European" = "European NFD"))
HBAC$ETHNICITY <- revalue(HBAC$ETHNICITY, c("Other Asian" = "Asian NFD", "Asian not
further defined" = "Asian NFD"))
HBAC$ETHNICITY <- revalue(HBAC$ETHNICITY, c("New Zealand European/Pakeha" = "NZ
European"))

#Get information about ethnicity and Hb abnormalities
table(HBAC23$ETHNICITY, useNA = "ifany")
print(HBAC23 %>% count(ETHNICITY), n=28)
table (HBAC23$`Abnormal hb`, useNA = "ifany")
table (HBAC23$`Known`, useNA = "ifany")
table (HBAC23$`?Diag`, useNA = "ifany")


#Get the percentage of each ethnicity
```

```
ethn_counts <- table(HBAC23$ETHNICITY)
print.table(ethn_counts)
total_ethn <- sum(ethn_counts)
percentage_by_ethn <- (ethn_counts/total_ethn)*100
ethn_results <- data.frame(Ethnicity = names(percentage_by_ethn), Percentage =
percentage_by_ethn)
print(ethn_results)
ethn_percentages <- HBAC23 %>%
  group_by('ETHNICITY', `Abnormal hb`, 'Signif.') %>%
summarise(percentage = n()/nrow(HBAC23)*100)
print(ethn_percentages)

#Sort data by ethnicity
HBAC %>% arrange(factor(ETHNICITY, levels = c('NZ European', 'Māori', 'Pacific Peoples',
'European NFD', 'Indian', 'Chinese', 'Southeast Asian', 'Asian NFD', 'Latin American/Hispanic',
'Middle Eastern', 'African', 'Unknown')))

#Analyse relationship between ethnicity and abnormal haemoglobin
HBAC.BINOMIAL <- glm(Abnormal.hb ~ ETHNICITY, family = binomial, data=HBAC)
anova(HBAC.BINOMIAL, test='LRT')
summary(HBAC.BINOMIAL)

#Create an Estimated marginal means pairs table to compare ethnicities to each other
HBAC.emm <- emmeans(HBAC.BINOMIAL, "ETHNICITY")
pairs(HBAC.emm)
```

8.2 RStudio Output

	ETHNICITY	n	Percentage.Var1	Percentage.Freq
	<chr>	<int>		
1	African	1223		0.420257584
2	Asian not further defined	949		0.326103391
3	Chinese	3585		1.231907963
4	Cook Island Māori	997		0.342597556
5	Do not know	43		0.014776023
6	European NFD	2123		0.729523181
7	Fijian	1181		0.405825189
8	Indian	11986		4.118730499
9	Latin American/Hispanic	1190		0.408917845
10	Māori	49804		17.114070897
11	Middle Eastern	625		0.214767776
12	New Zealand European/Pakeha	162715		55.913501849
13	Niuean	174		0.059791349
14	Not Stated	24341		8.364259893
15	Other (code retired)	16		0.005498055
16	Other Asian	4607		1.583096230
17	Other European	18601		6.391832639
18	Other Pacific Peoples	556		0.191057413
19	Other ethnicity	877		0.301362143
20	Pacific Peoples NFD	129		0.044328069
21	Refused to answer	17		0.005841684
22	Response unidentifiable	24		0.008247083
23	Samoan	1363		0.468365566
24	Southeast Asian	2924		1.004769563
25	Tokelauan	176		0.060478606
26	Tongan	786		0.270091955
27	NA	16923		

Ethnicities with abnormal Hb

	N	Y
African	1203	20
Asian not further defined	937	12
Chinese	3563	22
Cook Island Māori	996	1
Do not know	43	0
European NFD	2121	2
Fijian	1170	11
Indian	11837	149
Latin American/Hispanic	1175	15
Māori	49767	37
Middle Eastern	619	6
New Zealand European/Pakeha	162455	260
Niuean	174	0
Not Stated	24249	92
Other (code retired)	16	0
Other Asian	4515	92
Other ethnicity	865	12
Other European	18548	53
Other Pacific Peoples	554	2
Pacific Peoples NFD	129	0
Refused to answer	17	0
Response unidentifiable	24	0
Samoan	1361	2
Southeast Asian	2856	68
Tokelauan	176	0
Tongan	783	3

Abnormality Not Significant

	N	Y
African	0	3
Asian not further defined	0	7
Chinese	0	8
Cook Island Māori	0	0
Do not know	0	0
European NFD	0	0
Fijian	0	3
Indian	0	52
Latin American/Hispanic	0	3
Māori	0	20
Middle Eastern	0	1
New Zealand European/Pakeha	0	201
Niuean	0	0
Not Stated	0	51
Other (code retired)	0	0
Other Asian	0	36
Other ethnicity	0	3
Other European	0	35
Other Pacific Peoples	0	2
Pacific Peoples NFD	0	0
Refused to answer	0	0
Response unidentifiable	0	0
Samoan	0	2
Southeast Asian	0	40
Tokelauan	0	0
Tongan	0	2

Significant Abnormality

	N	Y
African	0	17
Asian not further defined	0	5
Chinese	0	14
Cook Island Māori	0	1
Do not know	0	0
European NFD	0	2
Fijian	0	8
Indian	0	97
Latin American/Hispanic	0	12
Māori	0	17
Middle Eastern	0	5
New Zealand European/Pakeha	0	59
Niuean	0	0
Not Stated	0	41
Other (code retired)	0	0
Other Asian	0	56
Other ethnicity	0	9
Other European	0	18
Other Pacific Peoples	0	0
Pacific Peoples NFD	0	0
Refused to answer	0	0
Response unidentifiable	0	0
Samoan	0	0
Southeast Asian	0	28
Tokelauan	0	0
Tongan	0	1

	?Alpha Variant	?Beta	?Beta Variant	?Delta	?HbC	?HbD	?HbD can't excl. D Punjab	?HbH	?Hete E	?Hete E/Alpha
African	1	2	0	2	2	0	0	0	1	0
Asian not further defined	0	1	0	0	0	0	0	0	7	1
Chinese	1	13	4	0	0	1	0	0	1	0
Cook Island Maori	0	1	0	0	0	0	0	0	0	0
Do not know	0	0	0	0	0	0	0	0	0	0
European NFD	0	1	0	0	1	0	0	0	0	0
Fijian	0	4	0	0	0	0	0	0	1	0
Indian	9	80	3	2	0	1	42	1	3	0
Latin American/Hispanic	1	2	0	0	4	0	0	0	0	0
Maori	5	9	1	0	1	1	0	0	2	0
Middle Eastern	0	5	0	0	0	0	1	0	0	0
New Zealand European/Pakeha	47	30	50	0	10	19	3	0	7	0
Niuean	0	0	0	0	0	0	0	0	0	0
Not Stated	14	24	5	2	2	2	3	2	7	1
Other (code retired)	0	0	0	0	0	0	0	0	0	0
Other Asian	2	22	0	1	0	1	1	3	39	4
Other ethnicity	1	2	0	0	2	0	1	0	5	0
Other European	11	13	4	4	3	3	0	0	5	0
Other Pacific Peoples	1	0	1	0	0	0	0	0	0	0
Pacific Peoples NFD	0	0	0	0	0	0	0	0	0	0
Refused to answer	0	0	0	0	0	0	0	0	0	0
Response unidentifiable	0	0	0	0	0	0	0	0	0	0
Samoan	1	0	0	0	0	0	0	0	0	0
Southeast Asian	0	7	0	0	0	0	0	3	44	4
Tokelauan	0	0	0	0	0	0	0	0	0	0
Tongan	1	1	0	0	0	0	0	0	0	0

	?Hete E/others	?Hete S	?Hete S/Alpha	?Homo E	?HPFH	?IDA	?Split A2	Other	Raise A2	Raise F	Unknown
African	0	10	1	0	0	0	0	0	1	0	0
Asian not further defined	0	0	0	2	0	0	0	0	1	0	0
Chinese	0	0	0	0	0	0	0	0	1	1	0
Cook Island Maori	0	0	0	0	0	0	0	0	0	0	0
Do not know	0	0	0	0	0	0	0	0	0	0	0
European NFD	0	0	0	0	0	0	0	0	0	0	0
Fijian	0	2	2	0	1	0	0	0	1	0	0
Indian	0	2	0	1	0	0	0	2	1	1	1
Latin American/Hispanic	0	6	0	0	0	0	1	0	0	1	0
Maori	0	6	0	0	0	0	0	2	4	6	0
Middle Eastern	0	0	0	0	0	0	0	0	0	0	0
New Zealand European/Pakeha	0	5	1	0	7	1	1	18	27	33	1
Niuean	0	0	0	0	0	0	0	0	0	0	0
Not Stated	0	4	1	6	1	0	0	3	7	8	0
Other (code retired)	0	0	0	0	0	0	0	0	0	0	0
Other Asian	1	1	0	15	0	0	0	0	1	1	0
Other ethnicity	0	1	0	0	0	0	0	0	0	0	0
Other European	0	0	0	0	1	0	2	5	1	1	0
Other Pacific Peoples	0	0	0	0	0	0	0	0	0	0	0
Pacific Peoples NFD	0	0	0	0	0	0	0	0	0	0	0
Refused to answer	0	0	0	0	0	0	0	0	0	0	0
Response unidentifiable	0	0	0	0	0	0	0	0	0	0	0
Samoan	0	0	0	0	0	0	0	0	1	0	0
Southeast Asian	0	0	0	7	0	0	0	1	2	0	0
Tokelauan	0	0	0	0	0	0	0	0	0	0	0
Tongan	0	0	0	0	1	0	0	0	0	0	0

```
> anova(HBAC.BINOMIAL, test='LRT')

Analysis of Deviance Table
Model: binomial, link: logit
Response: Abnormal.hb
Terms added sequentially (first to last)

          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                    291011      11723
ETHNICITY 11    899.2    291000      10824 < 2.2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> summary(HBAC.BINOMIAL)

Call:
glm(formula = Abnormal.hb ~ ETHNICITY, family = binomial, data = HBAC)
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.2169  -0.0729  -0.0566  -0.0566   3.7960

Coefficients:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      -4.0968     0.2255 -18.171  < 2e-16 ***
ETHNICITYAsian NFD                0.1375     0.2462   0.558  0.57657
ETHNICITYChinese                 -0.9905     0.3108  -3.187  0.00144 **
ETHNICITYPacific Peoples         -1.5423     0.3219  -4.790 1.66e-06 ***
ETHNICITYUnknown                 -1.3939     0.2459  -5.668 1.45e-08 ***
ETHNICITYEuropean NFD            -1.8322     0.2628  -6.972 3.12e-12 ***
ETHNICITYIndian                  -0.2782     0.2401  -1.159  0.24650
ETHNICITYLatin American/Hispanic -0.2641     0.3440  -0.768  0.44262
ETHNICITYMāori                   -3.1073     0.2790 -11.137  < 2e-16 ***
ETHNICITYMiddle Eastern          -0.5395     0.4681  -1.153  0.24909
ETHNICITYNZ European             -2.3406     0.2338 -10.009  < 2e-16 ***
ETHNICITYSoutheast Asian          0.3592     0.2567   1.399  0.16173
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 11723  on 291011  degrees of freedom
Residual deviance: 10824  on 291000  degrees of freedom
  (16923 observations deleted due to missingness)
AIC: 10848

Number of Fisher Scoring iterations: 9
```

Table 8.1: Estimated marginal mean pairs table using data from the 2023 HbA1c testing. The presence of an abnormal haemoglobin is compared between the different ethnic groups.

Contrast		Estimate	SE	z. ratio	p. value
African	Asian NFD	-0.1375	0.246	0.558	1.0000
African	Chinese	0.9905	0.311	3.187	0.0638
African	Pacific Peoples	1.5423	0.322	4.790	0.0001
African	Unknown	1.3939	0.246	5.668	<.0001
African	European NFD	1.8322	0.263	6.972	<.0001
African	Indian	0.2782	0.240	1.159	0.9918
African	Latin American/Hispanic	0.2641	0.344	0.768	0.9998
African	Māori	3.1073	0.279	11.137	<.0001
African	Middle Eastern	0.5395	0.468	1.153	0.9922
African	NZ European	2.3406	0.234	10.009	<.0001
African	Southeast Asian	-0.3592	0.257	1.399	0.9640
Asian NFD	Chinese	1.1280	0.236	4.786	0.0001
Asian NFD	Pacific Peoples	1.6798	0.250	6.713	<.0001
Asian NFD	Unknown	1.5314	0.139	10.980	<.0001
Asian NFD	European NFD	1.9697	0.167	11.765	<.0001
Asian NFD	Indian	0.4157	0.129	3.227	0.0567
Asian NFD	Latin American/Hispanic	0.4016	0.278	1.444	0.9547
Asian NFD	Māori	3.2448	0.192	16.911	<.0001
Asian NFD	Middle Eastern	0.6770	0.422	1.604	0.9080
Asian NFD	NZ European	2.4781	0.117	21.210	<.0001
Asian NFD	Southeast Asian	-0.2217	0.158	1.406	0.9627
Chinese	Pacific Peoples	0.5518	0.314	1.758	0.8409
Chinese	Unknown	0.4034	0.235	1.714	0.8621
Chinese	European NFD	0.8417	0.253	3.328	0.0414
Chinese	Indian	-0.7123	0.229	3.108	0.0804
Chinese	Latin American/Hispanic	-0.7263	0.337	2.158	0.5802
Chinese	Māori	2.1169	0.270	7.848	<.0001
Chinese	Middle Eastern	-0.4510	0.463	0.975	0.9982
Chinese	NZ European	1.3502	0.223	6.063	<.0001
Chinese	Southeast Asian	-1.3496	0.247	5.474	<.0001
Pacific Peoples	Unknown	-0.1483	0.250	0.593	1.0000
Pacific Peoples	European NFD	0.2900	0.267	1.088	0.9952
Pacific Peoples	Indian	-1.2641	0.244	5.177	<.0001
Pacific Peoples	Latin American/Hispanic	-1.2781	0.347	3.684	0.0123
Pacific Peoples	Māori	1.5651	0.283	5.539	<.0001
Pacific Peoples	Middle Eastern	-1.0028	0.470	2.133	0.5991
Pacific Peoples	NZ European	0.7984	0.238	3.354	0.0382
Pacific Peoples	Southeast Asian	-1.9014	0.261	7.298	<.0001
Unknown	European NFD	0.4383	0.167	2.625	0.2656
Unknown	Indian	-1.1157	0.128	8.699	<.0001
Unknown	Latin American/Hispanic	-1.1298	0.278	4.067	0.0028
Unknown	Māori	1.7134	0.192	8.947	<.0001
Unknown	Middle Eastern	-0.8544	0.422	2.026	0.6759
Unknown	NZ European	0.9467	0.116	8.146	<.0001
Unknown	Southeast Asian	-1.7531	0.157	11.152	<.0001

European NFD	Indian	-1.5540	0.158	9.823	<.0001
European NFD	Latin American/Hispanic	-1.5681	0.293	5.355	<.0001
European NFD	Māori	1.2751	0.213	5.994	<.0001
European NFD	Middle Eastern	-1.2927	0.432	2.993	0.1103
European NFD	NZ European	0.5084	0.149	3.421	0.0307
European NFD	Southeast Asian	-2.1914	0.182	12.011	<.0001
Indian	Latin American/Hispanic	-0.0141	0.273	0.052	1.0000
Indian	Māori	2.8291	0.184	15.385	<.0001
Indian	Middle Eastern	0.2613	0.418	0.625	1.0000
Indian	NZ European	2.0624	0.103	19.987	<.0001
Indian	Southeast Asian	-0.6374	0.148	4.312	0.0010
Latin American/Hispanic	Māori	2.8432	0.307	9.247	<.0001
Latin American/Hispanic	Middle Eastern	0.2754	0.486	0.567	1.0000
Latin American/Hispanic	NZ European	2.0765	0.267	7.773	<.0001
Latin American/Hispanic	Southeast Asian	-0.6233	0.287	2.169	0.5723
Māori	Middle Eastern	-2.5678	0.442	5.811	<.0001
Māori	NZ European	-0.7667	0.176	4.364	0.0008
Māori	Southeast Asian	-3.4665	0.205	16.900	<.0001
Middle Eastern	NZ European	1.8011	0.415	4.341	0.0009
Middle Eastern	Southeast Asian	-0.8987	0.428	2.099	0.6237
NZ European	Southeast Asian	-2.6998	0.138	19.634	<.0001