PRESENTATION OF MULTIPLE MYELOMA IN GHANAIAN PATIENTS

College of Basic and Applied Sciences

MPhil thesis submitted by:

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE AWARD OF DEGREE OF MASTERS OF PHILOSOPHY IN

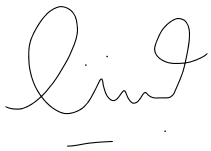
MOLECULAR BIOLOGY

Department of Biochemistry, Cell and Molecular Biology

April 2022

Declaration

I, Lambert Ato Essiaw, declare that except for references to other people's works that have been duly cited, all the experimental works presented in this thesis were undertaken by me at the Department of Biochemistry, Cell and Molecular Biology, under the supervision of Dr. Lily Paemka of the Biochemistry, Cell and Molecular Biology Department in University of Ghana.



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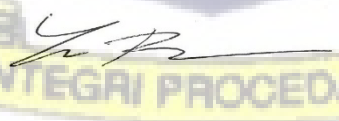

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Dr. Linda Baughn

(Co-supervisor)

Dedication

I dedicate this work to my future self- remember to push hard!

Acknowledgement

Thank you, God, for helping me. I am grateful to my parents and my family for the support. I would like to acknowledge my supervisor, Dr Lily Paemka for the immeasurable support and for guidance, and for breaking through barriers to help me complete this work. I am grateful to Professor Gordon Awandare and West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) for providing me with the ACE fellowship for my MPhil research project. I would like to acknowledge all the members of Paemka lab, especially Gertrude and Joshua, who I could count on irrespective of the time. I am grateful to Dr. Linda Baughn and Dr. Neeraj Sharma of Mayo Clinic, Rochester, Minnesota, United States, for their research support and advice. I also owe Dr. Dei-Adomakoh and Aunty Aimee of Korle Bu Teaching Hospital Haematology Department, lots of gratitude for their support. Finally, I would like to thank Fatima, Nelson, Ivan and my fellow MPhil colleagues of the 2021/2022 graduating class for all the support during my 3-year study at the University of Ghana.

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Abbreviations

AA- African Americans

MM- Multiple myeloma

SMM- smouldering multiple myeloma

MGUS- Monoclonal Gammopathy of Undetermined Significance

MYNN- Myoneurin

PCR- polymerase chain reaction

bp- base pairs

nt – nucleotides

LB- Luria-Bertani

DMEM- Dulbecco's Modified Eagle Medium

HEK293T cells- Human embryonic kidney 293 cells

Abstract

Multiple myeloma (MM) is an incurable plasma cell dyscrasia, which notably has an age of onset which varies among different races. Several risk factors have been implicated in the disease development and progression including genetics and environmental factors such as pesticide exposure. Despite the population-specific observations in MM presentation, this disease remains largely unexplored in the Ghanaian population. There is very little known about cytogenetic aberrations mediating MM in the African population. The growing prevalence of MM in Ghana necessitates investigating the cytogenetic and environmental risk factors mediating the disease development and presentation. This study therefore, sought to define MM presentations by comparing key clinical metrics at presentation between different racial groups. The study also investigated cytogenetic abnormalities which were present in Ghanaian patients with multiple myeloma. One hundred and six patients had their clinical data at presentation recorded and were age and sex matched with American Caucasian and African American patients. Twenty-two suspected MM patients scheduled for bone marrow aspirate collection were recruited upon receipt of informed consent. Bone marrow aspirates were obtained and demographic details collected using a structured questionnaire. From this study, a significant difference was observed in the M-component, total protein, plasma cell percentage and lactate dehydrogenase levels, with the African cohorts having elevated values. Contrary to earlier reports, females were more affected. Few patients showed indications of kidney damage and there was no observed association between pesticide exposure the disease presentation. Lastly, based on cytogenetic analyses, *MYC* was the gene found to be predominantly altered in majority of the Ghanaian patients. Also, there were trisomies of odd-numbered chromosomes in 2 out of 5 of the patients.

Chapter One

1.0 Introduction

Multiple Myeloma (MM) is a haematological dyscrasia which is characterized by abnormal accumulation of plasma cells (Palumbo and Anderson, 2011). The symptoms range from bone pain, fatigue and fever to severe anaemia and kidney damage. Multiple myeloma has been found to predominantly affect older people with a study in Ghana identifying multiple myeloma patients having a median age of 58 years (Acquah et al., 2019). Studies have shown that multiple myeloma has a higher prevalence in African Americans (AA), and this portends that being of African heritage is a significant risk factor. Although the genetics of African Americans has been shown to be similar to Africans to varying degrees, and a study that was carried out in Ghanaians confirmed that just like African Americans, Ghanaians were twice more susceptible to Multiple Myeloma than Caucasians (Landgren et al., 2007; Baughn et al., 2018), there has been no study to determine the particular cytogenetic abnormalities prevalent in African Multiple Myeloma patients. Most genetic studies that have been done on Black Multiple Myeloma patients have been done on African Americans, but they have been shown to be admixed with varying levels of genetical similarity as Africans and therefore not fully representative of Africans (Baughn et al., 2018). Also, most studies have been done using self-reported race (Baughn et al., 2018). There is therefore a gap in knowledge, as to what causes multiple myeloma in African people, in terms of the unique cytogenetic abnormalities that lead to its development. Pesticides have been shown to exacerbate the risk of development of multiple myeloma, but it is unclear the extent to which it has affected Africans.

A well characterized disease is not only easier to diagnose, but also easier to treat (Kumar and Rajkumar, 2018). Many cancers including lymphomas (which are also haematological

dyscrasias) have well established cytogenetic classification (Kumar and Rajkumar, 2018). Classification helps in stratification of the disease with respect to prognosis and staging. Multiple myeloma has traditionally been considered a single disease even though the causative cytogenetic abnormalities may differ and therefore the treatment regimens and outcome too should differ accordingly (Kumar and Rajkumar, 2018). This single disease classification has been attributed in part to the lack of cytogenetic characterization which is important in its classification. In the pathogenesis of multiple myeloma, the development of cytogenetic abnormalities is a significant event. Generally, there are two categories: the cytogenetic abnormalities that cause multiple myeloma: primary and secondary cytogenetic abnormalities. The primary abnormalities, which are the disease initiating events can be grouped into trisomies and translocations. The translocation usually involves chromosome 14, which has the gene for the immunoglobulin heavy chain. The trisomies usually involves the odd numbered chromosomes (Kumar and Rajkumar, 2018). The prognosis for translocations tends to be better than that of the trisomies (Palumbo et al., 2015). Even though these abnormalities rise in a particular clonal population making it exclusive for individuals, there is an estimate that about 10% of multiple myeloma patients have both kinds of abnormalities (Kumar and Rajkumar, 2018). These abnormalities are peculiar to the development of multiple myeloma. However, the cytogenetic aberrations mediating multiple myeloma development in Africans has not been studied and therefore those of Ghanaian multiple myeloma patients are unknown. Therefore, cytogenetic and abnormal plasma cell characterization will aid in understanding the causes of multiple myeloma in Ghanaian patients especially in a population at risk of early development of multiple myeloma. It is thus very likely that Ghanaian multiple myeloma patients will benefit from molecular characterization of the aberrant multiple myeloma cells and a more comprehensive diagnosis. This information is likely to lead to better health outcomes for patients owing to the fact that the type of cytogenetic abnormality determines the disease

progress and therefore, provide information on the best therapy. Since the range of underlying causes may be diverse, determining the cytogenetic profile will give an idea of the abnormalities in the Ghanaian population, and also inform clinicians on the effectiveness of their current treatment of the patients. This understanding can also inform future screening and treatment approaches.

Several genes have been implicated in multiple myeloma pathogenesis with some risk alleles found to be enriched in African Americans (Du et al., 2020; Went et al., 2018). One of which rs10936599, a mutation in the myoneurin (*MYNN*) gene. The role this SNP in the *MYNN* gene plays in the development of multiple myeloma is however unknown. Therefore, characterisation of the role this mutation plays, especially in the development of multiple myeloma will help us better understand the development of multiple myeloma in Africans.

1.1 Rationale

Multiple myeloma is a complex genetic disease characterised by key cytogenetic aberrations. These cytogenetic abnormalities determine the character of the disease. Therefore, study of the cytogenetic abnormalities in multiple myeloma patients will help in developing a better understanding of the carcinogenesis of multiple myeloma in Ghanaian multiple myeloma patients.

Furthermore, studying the effect of the rs10936599, a SNP in myoneurin gene, which has been seen to be enriched African Americans, will enhance our understanding of the development of multiple myeloma in Ghanaians and Africans. The role that this SNP in the *MYNN* gene plays in the development of multiple myeloma is unknown. Using PolyPhen, a protein function prediction tool, this SNP was predicted to have a deleterious effect on the protein.

1.2 Hypotheses

The clinical presentation of Ghanaian multiple myeloma patients differs from American Caucasian patients.

Cytogenetic aberrations mediate MM in Ghanaian patients.

1.3 Aim

To describe the presentation of multiple myeloma in Ghanaians compared to Americans and cytogenetic aberrations underpinning the phenotype.

1.4 Objectives

1. To compare key multiple myeloma health metrics among Ghanaian (African), African American and Caucasian patient cohorts.

2. To elucidate *in-silico* the possible mechanism by which the single nucleotide polymorphism rs10936599 mutation in the myoneurin gene increases susceptibility to multiple myeloma.

3. To describe multiple myeloma presentation as well as the cytogenetic abnormalities associated with its development in Ghanaian patients

Chapter 2

2.0 Literature Review

2.1 Multiple Myeloma is a plasma cell malignancy

Multiple myeloma (MM) is a plasma cell lymphoma which results from the increased proliferation of plasma cells in the bone marrow, leading to plasma cell accumulation in the bone marrow (Baker et al., 2013). Plasma cells are specialised B-lymphocyte immune cells which function to produce antibodies. Plasma cells begin life in the bone marrow as hematopoietic stem cells (HSCs) (Corre et al., 2015). The HSCs that will eventually become plasma cells differentiate to pro-B cells then through to mature B cells in the bone marrow, with intermediate stages where there is recombination of the genes of the heavy chain immunoglobulins protein and then the light chain immunoglobulins (Lutz et al., 2011; Soediono, 1989). The mature B cells migrate to the secondary lymphoid organs, where they will be transformed into plasma cells or memory B cells after binding to their cognate antigen (Corre et al., 2015; Cyster & Allen, 2019). Plasma cells function to produce immunoglobulins. These 4-chain immunoglobulins (2 light and 2 heavy chains) form antibodies, which function as part of the humoral immune system to fight diseases and infections. The antibodies are the main proteins responsible for humoral immunity, as they are capable of neutralisation of toxins, agglutination and opsonisation of pathogens (Forthal, 2014).

In multiple myeloma, the plasma cells, which are anomalous, produce M proteins (also known as paraproteins or Bence-jones proteins) instead of antibodies (Kumar & Rajkumar, 2018). The M-proteins produced are abnormal antibody-like proteins and thus, do not aid in immune function. However, the myeloma cancer cells multiply quickly which leads to decreased rates of production of normal haematopoietic cells. This resultant deficiency leads to recurrent infections, thrombocytopenia and anaemia. The platelet-deficient patient is also prone to

excessive bleeding, while accumulation of M-proteins in organs like the kidney also leads to organ damage (Perazella & Finkel, 2016).

2.2 Primary Cytogenetic Abnormalities

The early stages of Multiple myeloma are usually asymptomatic (Korde et al., 2011). The risk and rate of progression however largely depends on the cytogenetic abnormalities that birthed the precursor clones (Kumar & Rajkumar, 2018). As seen in figure 2.2.1, multiple myeloma progresses through the pre-malignant phases of: Monoclonal Gammopathy of Undetermined Significance (MGUS), and Smouldering Multiple Myeloma (SMM), then end stage Multiple Myeloma (MM) and finally, Plasma Cell Leukemia (PCL) (Prideaux et al., 2014). For staging, the current standard is the revised International Staging System (R-ISS) (table 2.1), which supplements the preceding International Staging System (ISS) with patient Chromosomal Abnormalities (CA) and serum Lactate Dehydrogenase (LDH) levels (Palumbo et al., 2015).

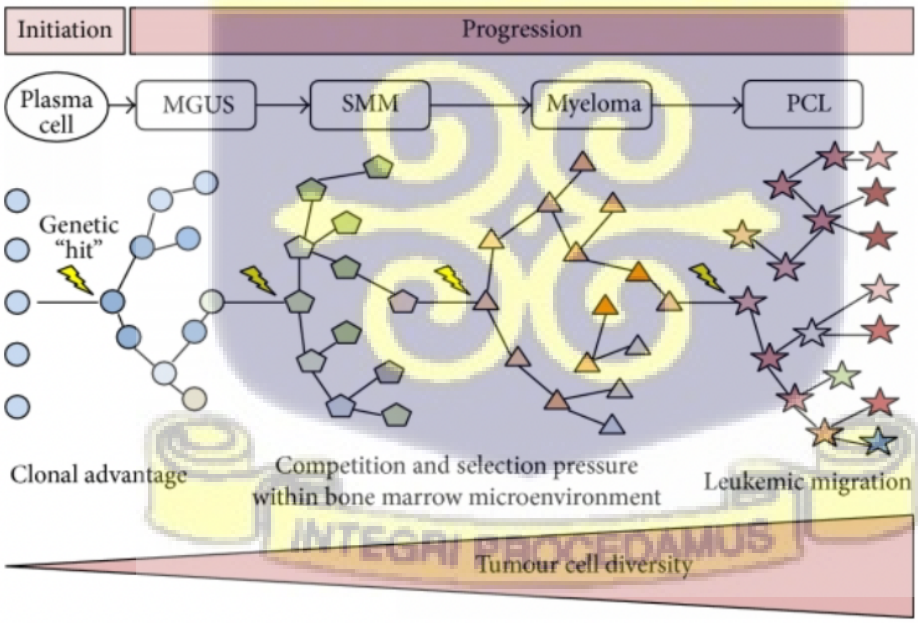


Figure 2.2.1: Prideaux, S. M., Conway O'Brien, E., & Chevassut, T. J. (2014). The genetic architecture of multiple myeloma. *Advances in hematology*, *2014*.

Table 2.1: Criteria for multiple myeloma staging

Stage	Criteria
I	ISS stage I (serum ß$_2$ -microglobulin < 3.5 mg/L, serum albumin ≥ 3.5 g/dL) & standard-risk CA by iFISH (No high-risk CA) & normal LDH (Serum LDH < 723 units/L)
II	Not ISS stage I or III
III	ISS stage III (serum ß$_2$ -microglobulin ≥ 5.5 mg/L) **either** high-risk CA by iFISH (Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)) **or** high LDH (Serum LDH > 723 units/L)

Adapted from Palumbo et al., 2015

There are different kinds of cytogenetic abnormalities, which have an effect on the health outcomes (Kumar & Rajkumar, 2018). Diagnosis, development, prognosis and response to therapy are affected by the cytogenetic abnormalities (Pertesi et al., 2020).

There are two main types of cytogenetic abnormalities: primary and secondary. Most clonal populations of a multiple myeloma patient will usually have one primary cytogenetic abnormality, but may have several secondary cytogenetic abnormalities; even though rare, in about 10% of patients, two primary abnormalities have been reported (Kumar & Rajkumar, 2018).

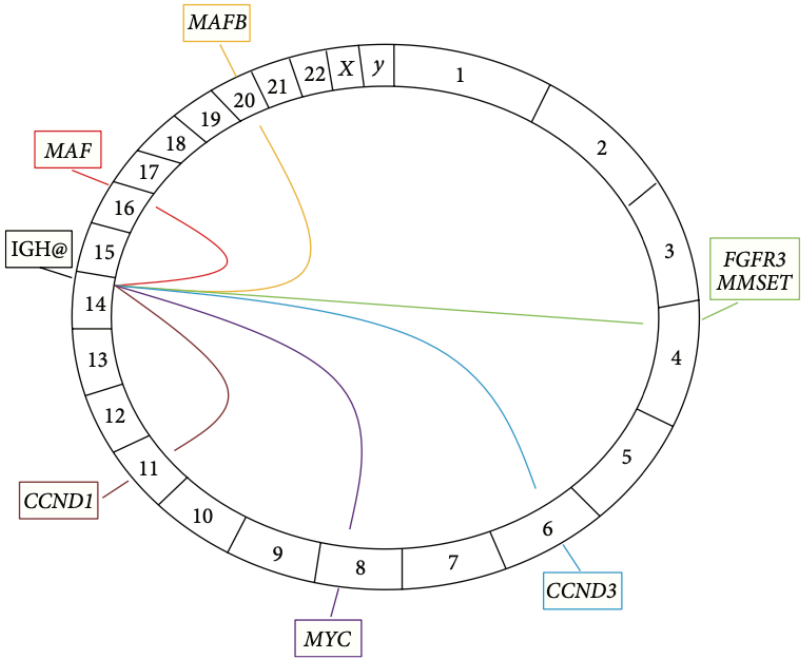


Figure 2.2.2: Prideaux, Conway O'Brien, & Chevassut (2014). The genetic architecture of multiple myeloma. *Advances in hematology*, *2014*.

Primary cytogenetic abnormalities are the mutagenic initiating events of multiple myeloma (Prideaux et al., 2014). They usually occur during class switch recombination at the post-geminal site (Prideaux et al., 2014). The primary cytogenetic causes of multiple myeloma vary but generally they can be divided into two: hyper-diploid and non-hyper-diploid causes (Prideaux et al., 2014). The main hyper-diploid cytogenetic abnormality is the trisomy. Most non-hyper-diploid abnormalities are translocations, but there are few conditions involving monosomies.

In the hyper-diploid condition of trisomy, there is an extra copy of one of an odd-numbered chromosomes. The chromosomes usually involved are chromosome: 5, 7, 9, 11, 13, and 15 (Kumar & Rajkumar, 2018). For the non-hyper-diploid condition, there is a translocation usually involving the 14q32 arm of the human chromosome 14, which has the gene for IgH

(the heavy chain of an antibody) (Prideaux et al., 2014). The common translocations are t(11:14), t(4:14), t(6:14), t(14:16) and t(14:20). This translocation leads to a transfer of an oncogene to the q arm of chromosome 14 which triggers the dysregulation mostly of cyclin genes (Kumar & Rajkumar, 2018). The translocations and trisomies may affect NF-kB regulation, MAPK signalling and MYC regulation, leading to TP53 inactivating mutations, and consequent dysregulation of cell proliferation (Corre et al., 2015). There are other primary abnormalities like monosomy 14, and translocations involving chromosomes 2 and 22, the two of which code for the two antibody light chains (lambda and kappa) (Nutting, 2002).

Abnormalities have different effects in each stage: trisomies can hasten the progress from SMM to multiple myeloma, but at the multiple myeloma stage, they portend low risk to end-stage. Different abnormalities are associated with different levels of risk. Some abnormalities like t(4;14) (translocation from chromosome 4 to chromosome 14), t(14;16), t(14;20), del(17p) and dup(1q) are associated with poor prognosis (Greenberg et al., 2015). Out of these, del (17p), t(4;14), and 1q21 gain were found to be independent predictors of adverse effects in a study done in Chinese patients (Jian et al., 2016). The secondary cytogenetic abnormality Del(17p) is linked with increased progression rate through the stages of multiple myeloma. Treatment may also be dependent on the kind of abnormality present. Abnormalities like t(4;14) and del(17p) can be treated with protease inhibitors (Binder et al., 2017).

One factor that determines which cytogenetic abnormality that develops may be race. As multiple myeloma both develops and presents differently in individuals of different races, there may be differences in cytogenetic abnormalities in multiple myeloma in different races. For example, the incidence of hyperdiploidy in Caucasians with multiple myeloma is about 50% but it was found to be about 10% in a Chinese cohort (Jian et al., 2016).

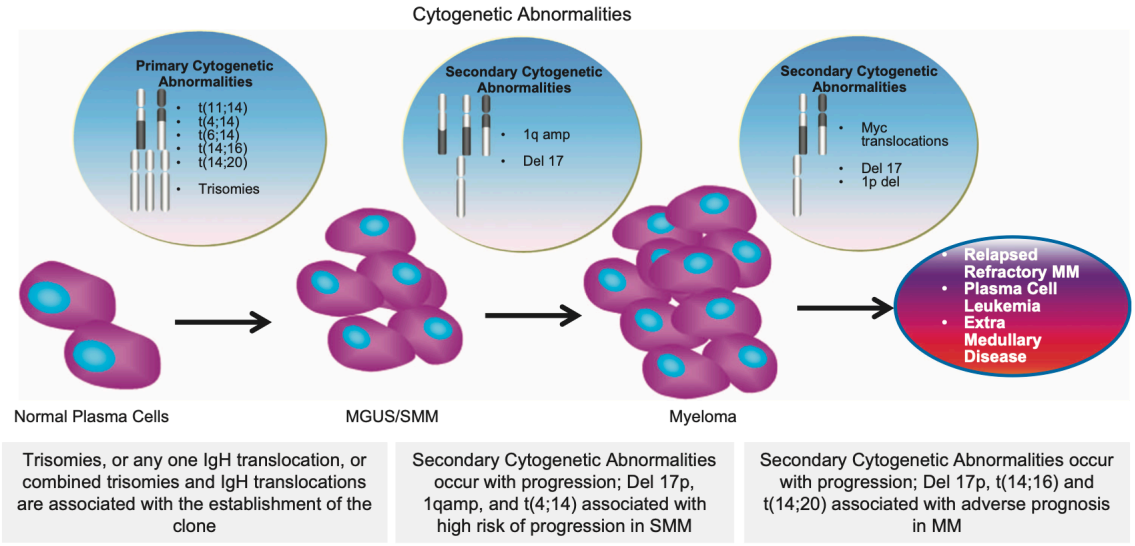


Figure 2.2.3: Rajan, & Rajkumar (2015). Interpretation of cytogenetic results in multiple myeloma for clinical practice. *Blood cancer journal*, *5*(10), e365-e365.

2.3 Secondary Cytogenetic abnormalities

Secondary cytogenetic abnormalities are class switch recombination-independent events, which are usually less pronounced and present in only a subset of the cancer clones (Kumar & Rajkumar, 2018). They usually develop after the primary cytogenetic abnormalities and are ideal for gauging the progression and prognosis of the disease. There may be more than one secondary cytogenetic abnormality in a clonal population. The secondary abnormalities which are usually monosomies, deletions or amplifications are accumulated in particular clones as the disease progresses (Rajan & Rajkumar, 2015). Some secondary cytogenetic effects are monosomy 13, a deletion in the q arm of chromosome 13 (Del 13q), a deletion of the p arm of chromosome 17 (Del 17), an amplification of the 1q arm (1q amp) or a deletion in the 1p arm and MYC translocation (Rajan & Rajkumar, 2015). A 1q amp and Del 17, in the MGUS or SMM stage indicate a tumour which is likely to progress to multiple myeloma (Rajan & Rajkumar, 2015). Given that Africans have lower progression rates than other races, it is likely that the incidence of these mutations will be lower than that of Caucasians.

Erstwhile, other secondary cytogenetic abnormalities that are usually associated with relapsing, plasma cell leukaemia and extramedullary multiple myeloma are: MYC translocations 1p deletions and Del 17 (Abdallah et al., 2020; Rajan & Rajkumar, 2015).

2.4 Symptoms of multiple myeloma

Worldwide, MM accounts for about 1% of all cancers and 13% of all haematological cancers (Palumbo & Anderson, 2011). In Ghana, diagnosis of multiple myeloma is done both clinically and by using lab results. Clinically, a patient's history is taken, with the classic symptoms looked for. The symptoms associated with multiple myeloma is abbreviated as CRAB, which stands for hypercalcaemia (C), renal insufficiency (R), anaemia (A) and bone disease (B) (Prideaux et al., 2014).

Anaemia due to MM is caused by the decreased production of red blood cells (RBCs), which is caused by competition in the bone marrow by the now increased production of plasma cells. This competition also results in decreased megakaryocytes and therefore decreased platelet production, which can result in excessive bleeding. All these contribute to the severe anaemic state of the patient. A full blood count can be done to monitor the haemoglobin levels of the patient- which will give an idea of the anaemic state of the patient.

Using lab results, the abnormal antibodies produced by the cancerous plasma cells which are called M proteins, can be monitored. Myeloma cells, after they have migrated to the bone marrow, may give a bone marrow aspirate a granular appearance. Also, the increased production of M proteins deposited in the blood causes damage to the kidney, leading to renal failure (Dimopoulos et al., 2008). The production of M proteins also has an effect on the

immune system, as the body channels resources into cells that are not functioning to help fight diseases.

The problematic plasma cells also release bone-dissolving chemicals which causes the breakdown of calcium-containing bone tissue and leads to hypercalcaemia, an increased plasma calcium concentration. This also leads to lytic bone lesions as a result of weakened bones which will manifest as bone pains to the patient (Hameed et al., 2014).

However, an asymptomatic early stage, coupled with non-specific symptoms during development makes diagnosis difficult. Therefore, many patients report at the late stage, and a study finding that majority of patients in Ghana reported with stage III disease (Acquah et al., 2019). This is especially problematic, given that the population structure of Ghana has changed over the past thirty years, with an increase of over 200% in the population of people over 50 years (World Bank, 2020). Therefore, diseases associated with ageing can no longer be ignored, especially diseases that Ghanaians may be at a higher genetic predisposition to, like multiple myeloma.

Even though the exact prevalence in the Ghanaian population is not clear, it is known that Africans are 2- 4 times more susceptible to multiple myeloma compared to Asians, Caucasians and Europeans (Lewis et al., 1994; Landgren et al., 2007).

2.5 Pathogenesis of multiple myeloma

The key distinctive feature of multiple myeloma is uncontrolled proliferation of plasma cells. Random mutations are normal occurrences in cells. However, cells have DNA repair genes which they use to combat these mutations. Mutagens can tilt the balance by increasing the rate

of mutations, but when DNA repair fails, the cell copes by triggering apoptosis. There is a perpetual flux between perfectly normal cells and cells that have accumulated acquired mutations. There is an element analogous to natural selection, where the mutated cells best adapted for survival outcompete the other abnormal cells, leading their daughter cells becoming the dominant population of cells.

In the development of B-cells, the B lymphocyte acquires mutations in the lymph node geminal centre. These purposefully acquired mutations in the genes of the B lymphocyte are useful in that they help the antibody in affinity maturation, which enhances its immune function. A key mediator of this process is the activation-induced deaminase. These events happen in the geminal centre, also where somatic hypermutation and class switching occurs. This is when the mutations that usually lead to the development of multiple myeloma happen. Generally, a higher age has a positive corelation with the development of multiple myeloma, with most incidents of multiple myeloma happening in people advanced in age (Kristinsson et al., 2007). In the western world, the median age of diagnosis was found to be 66- 70 years, but studies in Lima, Peru and Ghana have found median ages of 61 and 58 respectively (Acquah et al., 2019b; Kristinsson et al., 2007; Vasquez et al., 2020). This suggests that despite age being a major risk factor, other factors may play significant roles in the development of multiple myeloma. Increased incidence has been seen in males, in studies of different populations (Acquah et al., 2019b; Kristinsson et al., 2007; Vasquez et al., 2020). The African race has also been associated with increased risk as well as family history.

2.6 Pesticides and Multiple Myeloma

Pesticides is an umbrella term for insecticides, herbicides, fungicides, rodenticides and some disinfectants (Horsak et al., 1964)(Nicolopoulou-Stamati et al., 2016). In many societies, they

are primarily used in commercial agriculture to improve yield and reduce crop loss. However, there are pesticides like the rodenticides which are primarily used in domestic settings (Horsak et al., 1964). Pesticides are inherently toxic, as they are chemical agents meant to destroy or control pests. Nonetheless, their toxicological effect on humans has been well-documented. The major classes of pesticides: carbamates, organophosphates and organochlorides (of which DDT is an example) have all been shown to also have adverse effects on humans.

In multiple myeloma, carbamates and organochlorides have been shown to be associated with increased incidence of MGUS and also end-stage multiple myeloma (Landgren et al., 2009; Pahwa et al., 2012). Exposed individuals are believed to be at a 2-fold to 5-fold risk of MGUS, and therefore, multiple myeloma development (Packard et al., 2019; Presutti et al., 2016). It is likely that the chemicals in these pesticides possess carcinogenic properties. One such chemical is Captan, which is a phthalimide- and is used as an anti-fungus for fruit-bearing trees was shown to increase threefold the incidence of multiple myeloma in exposed individuals (Presutti et al., 2016).

In Ghana, despite regulations demanding the registration and distribution of pesticides to enable monitoring, lack of qualified laboratories means not only is this not done, but there is also pesticide misuse, misapplication and pollution of the environment (Onwona Kwakye et al., 2019). Also, the high burden of malaria necessitates the use of insecticides to get rid of the insect vector- the mosquito. Many of these insecticides contain carbamates and organochlorides (Baffour-Awuah et al., 2016). The exposure of individuals to these chemicals may increase their risk of multiple myeloma development.

In the survival dynamics, the most significant factor in multiple myeloma survival is the access to healthcare (Fillmore et al., 2019). This is because healthcare helps in both the diagnosis and treatment of multiple myeloma. Multiple myeloma requires specialist diagnosis and also, a bone marrow aspirate which also helps with staging.

2.7 Genetic Risk Factors

Risk factors and genetics of multiple myeloma

Multiple myeloma like any cancer is a genetic disease, and therefore it has genetic factors which are associated with increased susceptibility for development as well as environmental conditions that may accelerate its development. African Americans are more prone to multiple myeloma because of increased susceptibility to the premalignant condition (MGUS) (Greenberg et al., 2015). The risk of an individual developing multiple myeloma given a person is of African American descent is twice that of European and American descents (Du et al., 2020).

African Americans have also been shown to develop multiple myeloma at an earlier age relative to Caucasians and Asians (Greenberg et al., 2015). The cytogenetic abnormalities predominant in African Americans were also found to be different to those in Caucasians, with African Americans less likely to have IgH translocations (Baker et al., 2013). Some families with variants of MYC genes which cause deregulation of the production of the transcription factor were found to be two to four times as likely to develop MGUS (Barwick et al., 2019). First degree relatives of patients with multiple myeloma are two to six times as likely to develop multiple myeloma than the general population and there is evidence for a stronger familial relationship in Blacks (Brown et al., 1999; VanValkenburg et al., 2016). However, even though Blacks have a higher chance of developing multiple myeloma, no study has been done to

characterise the cytogenetic abnormalities present in the most genetically diverse African population. Most studies have been done with self-reported race data in African Americans, who are admixed and of African origin (Baughn et al., 2018). A study using samples from Blacks in the USA found a link between having high percentage of African genetic similarity and the occurrence of the IgH translocations (t(11;14), t(14;16), and t(14;20)), which are abnormalities of multiple myeloma (Baughn et al., 2018). The t(14;16), and t(14;20) translocations which are associated with poor prognosis may therefore be found in the Ghanaian population, owing to the common ancestry with African Americans.

Like many cancers, aging is a risk factor. In the case of multiple myeloma, males are also more likely to develop multiple myeloma. Exposure to pesticides has also been linked with development of multiple myeloma (Korde et al., 2011). There are three categories of genes of special interest in multiple myeloma: Cyclin genes, Myc genes and genes that influence the NF-KB pathway (Dehghanifard et al., 2018). Single Nucleotide Polymorphisms (SNPs) in some of these genes or pathways related to them have been shown to have a correlation with developing multiple myeloma. There are 17 multiple myeloma risk loci, but their contribution to the development of multiple myeloma is not clear (Mitchell et al., 2016). The development of multiple myeloma in Blacks has been associated with seven risk alleles: 3p22.1, 3q26.2, 7p15.3, 8q24.21, 11q13.3, 16p13.11 and 17p11.2. Of these, the 11q13.3 encoding the cyclin D1 protein CCND1 870G>A has been associated with increased risk of t(11;14) development (Baughn et al., 2020).

These seven SNPs were found to be enriched in multiple myeloma patients in African Americans: *CCND1* (rs9344), *ULK4* (rs1052501), *MYNN* (rs10936599), *DNAH11* (rs57104699), *CCAT1* (rs1948915), *FOPNL* (rs10656021) and *TNFRSF13B* (rs4273077).

In a study done by Bolli *et al*. to characterize the genomic landscape of multiple myeloma, it was found that copy number alterations and translocations had a greater influence in the prognosis and genotype of multiple myeloma, compared to mutations (Bolli et al., 2014). This buttresses the point that translocation which may cause increased expressions and polyploidy may be more pathogenic in the pathogenesis of multiple myeloma.

Myoneurin- MYNN

Myoneurin (MYNN), a BTB/POZ and zinc finger protein, was originally characterised as a developmental regulator of *Drosophila melanogaster* (Alliel et al., 2000). In humans, the myoneurin gene is found at the Cytogenetic location: 3q26.2. MYNN was found to be involved in both activation and repression of the expression of genes in various species (Alliel et al., 2000). In humans, it was found to be predominantly expressed in the neuromuscular system (Alliel et al., 2000). However, it also has been found to play a role in the differentiation and effector function of T-cells.

There are 2 major variants of MYNN seen in mammals, marked by different sized coding sequences and therefore protein size. Variant 1 (the predominant one) is 1833 nt long, whilst variant 2 is 1746 nt long. Variant 1 is translated into a protein which is 610 amino acids long. The difference between the two proteins is that variant 2 is missing 29 amino acids in the 496-524 region.

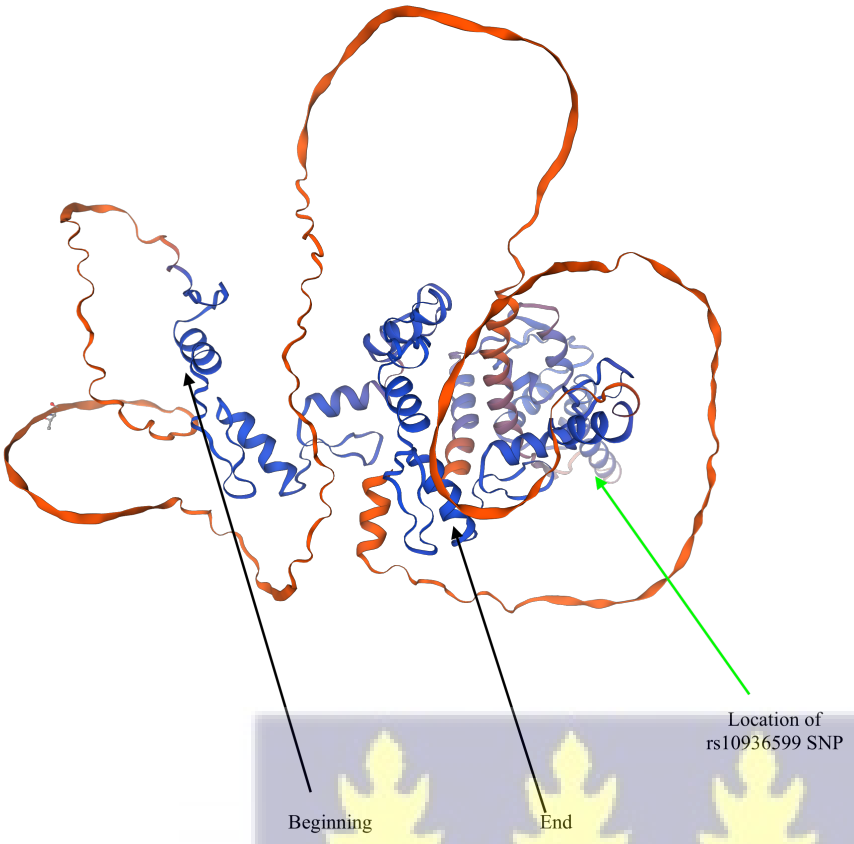


Figure 2.7.0- Myoneurin structure predicted by Alphafold: The beginning shows where the zinc finder domains begin from and the end shows where it ends. The rs10936599 SNP affects His6, which is shown by the green arrow (generated by Alphafold)

The structure of MYNN predominantly contains alpha helices as well as 8 zinc finger domains. As a transcription factor, MYNN has to be able to bind to DNA as well as proteins. The eight zinc finger domains, which span residue 302 to 527 function for DNA binding. Using EBI's interpro protein function prediction tool, it is estimated that the protein binding domain may start anywhere from His6 to residue Leu144. Like many transcription factors, it is also predicted to interact with a variety of corepressor proteins.

MYNN is described as having C_2H_2 zinc finger motifs (Alliel et al., 2000). C_2H_2 is a domain of 28-30 amino acids which fold into a hairpin of 2 antiparallel beta pleated sheets followed by a left-handed ββα α-helix (Fedotova et al., 2017). They contain 2 Cysteine, 2 Histidine (Cys2-

His2) and are the most common domains found in transcription factors (Fedotova et al., 2017). One of the most conserved transcription factors across species is the C_2H_2 zinc factor CTCF (CCCTC-binding factor), which is a transcriptional repressor that functions fundamentally to mould the architecture of chromosomes (Fedotova et al., 2017). It works by mediating long-distance interactions between chromosomal regions (Fedotova et al., 2017).

The protein binding domain of MYNN is the BTB or POZ domain, which is a conserved N-terminal domain in zinc finger transcription factors (Ahmad et al., 1998). This domain has been associated with transcriptional repression of proto-oncogenes in non-Hodgkin's lymphoma (Seyfert et al., 1996). Furthermore, the repression was seen to be effectively a function of the protein (the BTB) domain (Seyfert et al., 1996).

The rs10936599 SNP which was found to be associated with multiple myeloma patients and enriched in African Americans with multiple myeloma causes a cytosine to thymine substitution which results in change in histidine 6 to glutamine. Another closely related mutation is the substitution of cytosine to guanine. These missense mutations happen in the protein binding domain. Using Polyphen2, it was predicted that this mutation is probably damaging with a perfect score of 1.000.

The MYNN and TERC (a gene that helps in the maintenance of the telomere) genes are in linkage disequilibrium at the 3q26.2 chromosomal region (Polat et al., 2019). A study in Turkey, on bladder cancer, found an association between the rs10936599 SNP on MYNN, the rs2293607 SNP on TERC and the development of bladder cancer. Interestingly enough, like multiple myeloma, being male, having family history and ageing are risk factors for the development of bladder cancer as well (Polat et al., 2019). The rs2293607 SNP has been

associated with longer telomeres, so there may be a possibility that the African patients with multiple myeloma (who already have a higher chance of possessing the rs10936599 SNP) may also have the rs2293607 SNP, and therefore have longer telomeres.

To understand the possible mechanism by which the rs10936599 SNP in *MYNN* stimulates the development of multiple myeloma, the function and therefore the downstream genes regulated by the myoneurin transcription factor have to be found. Firstly, the effect of the mutation on forming a functional protein must be investigated, then the function integrity- especially if there are any alterations which may accelerate the development of cytogenetic abnormalities may be investigated.

For a transcriptional factor like MYNN, there are 2 nuclear localisation signals in the amino acid sequence. A possibility is that the mutation negates the localisation signal's effect, stopping it from translocating to the nucleus after protein synthesis, therefore impeding its ability to carry out its function. Another possibility is that the amino acids switch will cause protein misfolding which may not be seen in the localisation of the protein, but as a functional difference.

Ideally, to find the functional integrity of the protein, RNAseq could be used to find the genes which have both a positive or negative corelation with MYNN. Using proteinatlas.com, which contains data based on large scale cell line RNAseq- the genes which had the highest association with myoneurin expression were: *GFM1, ZNF639, CDV3, C4orf19, TRPV6, FAM214A, ACAP2, OTUD6B, GGT6, PPM1K, RBM27, LRRIQ4 and ZNF800*. Another possibility is that the rs10936599 SNP in *MYNN* causes and exaggerated effect in one of the major pathways associated with multiple myeloma development. Using next generation

sequence, about 65% of patients with multiple myeloma were found to have mutations which affected the genes: *BRAF, DIS3, DNAH11, DNAH9, FAM46C, FAT1, FAT3, FAT4, IRF4, KRAS, NRAS, PCLO, SP140, TP53 and TRAF3*.

2.8 Diagnosis

For diagnosis, a patient is first assessed to have multiple myeloma, then the stage is ascertained. Laboratory diagnosis in Ghana, is usually done using bone marrow aspirates, blood and urine samples (Acquah et al., 2019). Diagnosis uses bone marrow aspirates to demonstrate percentage plasma cells; and also assesses serum protein electrophoresis, serum free light chains, skeletal survey. ß2-microglobulin and lactate dehydrogenase (LDH) is done to help with staging- which is useful in predicting survival (Palumbo et al., 2015). In multiple myeloma there is increased proliferation of plasma cells, so detection of elevated levels of these cells and their related proteins in urine or blood are the mode of diagnosis. One such protein is the Bence-Jones protein (M proteins), which are the light chain subunits of the immunoglobulin proteins that are found in high concentrations in the urine of multiple myeloma patients (Edelman & Gally, 1962). In the blood, there is also a high concentration of monoclonal lambda proteins (M proteins), with an associated suppressed IgA, IgG and IgM concentrations. Lambda proteins are detected in urine via urine electrophoresis (Katzmann et al., 2002). In multiple myeloma patients, there will be low albumin percentage and high M-protein percentage. An M-protein level greater than 4 g/dL has been shown to be an indicator of risk of progression from smouldering to active multiple myeloma (Katzmann et al., 2002). Full blood counts are also done along with tests to demonstrate rouleaux, any circulating plasma cells and determine levels of platelets and other blood cells.

Even though M-protein level is used in diagnosis, care must be taken as the very similar condition of amyloidosis also leads to excessive light chains of antibodies circulating in the bloodstream. Consequently, a bone marrow biopsy is increasingly becoming the more favoured and sensitive way of detecting multiple myeloma. With the biopsy, Fluorescent in-Situ Hybridization (FISH), is done to detect chromosomal abnormalities (Kumar et al., 2010).

In Ghana, most patients with multiple myeloma present late (Acquah et al., 2019). This may be because of the asymptomatic early stage and also the non-specific symptoms of multiple myeloma.

Diagnosis is done primarily by demonstrating bone marrow plasma cell percentage, with a positive result being above 10-15%. Electrophoresis is also done to determine paraprotein levels. For multiple myeloma stage determination, it is done using the revised international staging system which includes checking the levels of ß2 microglobulin, serum albumin, cytogenetics and Lactate dehydrogenase (LDH).

Flow cytometry has emerged as a valuable tool in multiple myeloma diagnosis due to the unique phenotypic characteristics of both normal plasma cells and myeloma cells (Olteanu, 2016). Owing to its sensitivity, it is used in assessing the prognosis and therapeutic efficiency of the disease by assessing biomarkers (like CD38, CD138, CD45, CD19 and CD56) related to minimal residual disease (Olteanu, 2016). Conventionally, bone marrow aspirates are used in multiple myeloma diagnosis, but the sensitivity of flow cytometry, which allows sensitive analysis of high number of cells has expanded the sample base (San Miguel et al., 2006). The detection of abnormal circulating plasma cells (CPCs) has been shown to be very effective at multiple myeloma diagnosis, with a study showing about 75% detection at presentation and 92% diagnosis at relapse (Rawstron et al., 1997). CPCs have also proved valuable in prognosis

as a predicter of survival of multiple myeloma patients (Nowakowski et al., 2005). This prediction is independent of the established metrics of albumin, beta-2-microglobulin (ß2M) and age (Nowakowski et al., 2005). The detection of CPCs also may serve as an indicator of extramedullary multiple myeloma- a condition where the abnormal plasma cells reside outside the bone marrow.

When using flow cytometry in multiple myeloma diagnosis, the goal is to identify plasma cells with aberrant phenotypes (Olteanu, 2016). All plasma cells express CD 38 and CD 138, although myeloma cells express CD 138 at higher levels. Normal plasma cells are CD 19 and CD 45 positive. Normal plasma cells are also CD 20 and CD 56 negative with a polytypic cytoplasmic light chain expression ratio; kappa: lambda ratio ranging from 1 to 4 (Olteanu, 2016). Therefore, myeloma cells have an immune-phenotype of CD 38+, CD 138+, CD 19-, CD 45-, CD 20+ (weakly), CD 56+ (Olteanu, 2016). Also, multiple myeloma patients who have been found to be CD81- and CD117+ have better prognosis (Chen et al., 2018). Thus, flow cytometry or the alternative of checking the level of mRNA expression of these immunoglobulins in peripheral blood can be used as a non-evasive way of evaluating the prognosis of multiple myeloma patients.

The utility of Lactate Dehydrogenase and beta-2-microglobulin

The serum levels of Lactate Dehydrogenase (LDH) and ß2M have been shown to have a positive correlation with the stage of multiple myeloma. High serum level of LDH has been linked with advanced disease and low chance of survival (Terpos et al., 2010). In the revised ISS staging system, an LDH level below the upper bound is categorised as stage I and a level above the upper bound will be categorised as stage III (Palumbo et al., 2015). The upper limit for the Ghanaian population has been found to be 723 units/L (Dosoo et al., 2012). ß2M which

is the light chain of the HLA histocompatibility complex (Avilés et al., 1992). Multiple myeloma patients with elevated B_2M levels have been shown to have worse prognosis than those with lower levels (Avilés et al., 1992). A serum ß2M level greater than 5.5 mg/L is categorised as stage III multiple myeloma (Palumbo et al., 2015).

2.9 Treatment of Multiple Myeloma

Multiple Myeloma is incurable, however, the late stage which is characterised by symptomatic end-organ damage is remedied using therapy. Multiple myeloma is caused by the proliferation of aberrant plasma cells so it can be treated by the destruction of those cells. The method of destruction may vary; chemotherapy, radiation therapy or chimeric antigen receptor T-cell therapy may be used. Immunomodulatory drugs, like thalidomide and lenalidomide triggers caspase-8-mediated apoptosis (Robak et al., 2018). Corticosteroids, like dexamethasone, which will induce apoptosis via reduction of mitochondrial potential, may be used to increase the efficiency of the response against the cells. Chemotherapeutic agents (like doxorubicin), proteasome inhibitors, monoclonal antibodies and oncolytic viruses are all ways used in treatment of multiple myeloma patients (Robak et al., 2018). A bone marrow transplant, which is the most effective treatment, can also be used to treat myeloma (Korde et al., 2011; Robak et al., 2018).

Both the canonical and non-canonical NF-κB signaling pathways are essential to the development and survival of multiple myeloma. Hence, some drugs like the proteasome inhibitor bortezomib works by inhibiting the 26s proteasome and thereby reversibly inhibiting the NF-κB pathway, leading to apoptosis by caspase 8 activation.

3.0 Methodology

3.1 Study design and Area

This was a cohort study conducted in Ghana. The Republic of Ghana is in the West Africa and occupies a land mass of 238,500 square kilometre with a rising population of about 30 million. Samples were collected from Korle Bu Teaching Hospital after ethical approval from Korle Bu teaching hospital Institutional Review Board and Ethics Committee of School of Basic and Applied Sciences.

3.2 Clinical data

For the retrospective study, Clinical records of patients in the haematological unit of Korle Bu Teaching hospital diagnosed with multiple myeloma were retrieved from their files to get date of birth, age, sex, occupation, date of first diagnosis, race, residence and tribe records. The clinic notes were then looked through for the metrics: haemoglobin levels, lactate dehydrogenase levels, serum-sodium, serum-potassium, serum chloride, urea, creatinine, the estimated glomerular filtration rate (eGFR), serum-calcium, serum-ca adjusted, albumin, m component, gamma globin, beta 2 globin, beta 2 microglobulin, lambda free chain, kappa/lambda ratio, kappa free light chains, plasma cell percentage, total proteins, uric acid and last date of follow-up. Matching data was obtained from a cohort of multiple myeloma patients reporting at Mayo Clinic (Minnesota, USA).

3.3 Sampling (Prospective study)

Consent was obtained from the patients suspected to have multiple myeloma. The questionnaire was then administered, after which 1 ml of the bone marrow aspirate collected by the clinician was obtained and stored in an EDTA tube on ice. The expected sample size (calculation in appendix) was 600 to be collected over the 4 sampling sites over 12 months.

3.4 CD138 isolation

CD138 (marker for myeloma cells) positive cells were isolated using the StraightFromTM WholeBlood kit and Bone Marrow &CD138 MicroBeads kit (Miltenyi Biotech, Cat No: 130-105-961) following the manufacturer's instructions. Briefly, 50 uL of beads were added per 1 ml of blood and incubated at 4°C. The mixture was then washed by adding 5 ml of PBS buffer (with 0.5% BSA and 2 mM EDTA). After, it was spun at 445 g for 10 minutes. The supernatant was aspirated and the pellet resuspended in the PBS buffer. The resuspended mixture was then decanted into a conditioned column which had been placed on the magnet. The eluent was collected and labelled as the CD 138 negative cells (rest of blood cells). After washing 2 times with the separation buffer, the column was taken from the magnet and placed in a falcon tube. The column was then flushed with 4 ml of the elution buffer and labelled CD 138 positive cells (myeloma cells).

3.5 DNA extraction and sequencing

Using Quick-DNA extraction kits from Zymo (cat. No: D3024), DNA extraction was done on both the myeloma cells and myeloma-depleted white blood cells. Briefly, after adding 400 µl of Genomic Lysis Buffer to 100 µl of blood, the mixture was vortexed and made to stand at room temperature for 10 minutes. After transferring the mixture to a zymo spin column, it was spun at 10,000 x g for 1 minute after which the flow through was discarded. The column was transferred to a new collection tube and 200 µl of Prewash buffer was added after which it was centrifuged at 10,000 x g for another minute. A volume of 500 µl of genome Wash buffer was then added to the spin column and then centrifuged at 10,000 x g for one minute. The column was then transferred to a collection tube, where 100 µl of nuclease free water was added. After incubation at room temperature for 5 minutes, the column was spun for 30 seconds at 10,000 x g and the eluent was quantified for DNA concentration and purity using a nanodrop. The

genomic DNA was sent for long-read whole genome sequencing using the Novaseq 6000D, after DNA quality, quantity and purity were assessed using Qubit 3.0 Fluorometer.

3.5.1 Whole genome sequencing (WGS) Library preparation and sequencing

DNA was extracted using the Qiagen Puregene extraction protocol. Paired-end libraries were prepared with 500 ng of genomic DNA using the modified NEB Ultra II (New England Biolabs, Ipswich, MA) and the Nextera Flex systems (Illumina, San Diego, CA). For the NEB Ultra II, DNA fragmentation was performed using the Covaris LE220 and a 0.7X SPRIselect bead ratio was used to purify the sheared DNA post-fragmentation while a 0.6X bead ratio was used for the post-adaptor ligation clean-up and for the post-PCR clean-up. For the Nextera Flex systems, fragmentation and adapter ligation were performed with an on-bead tagmentation step with subsequent purification and amplification according to the manufacturer's instructions. The concentration and size distribution of the completed libraries were determined using the Fragment Analyzer (Agilent, Santa Clara, CA) and Qubit fluorometry (Invitrogen, Carlsbad, CA). Whole genome sequencing was then performed on the Illumina NovaSeq 6000 sequencer using paired-end sequencing and reads from libraries were combined and analyzed.

3.5.2 Bioinformatics and visualization

The sequencing FASTQ files were aligned to GRCh38 reference genome using BWA-MEM 0.7.17 (Li, 2013). The output BAM was processed to determine coverage (reads/Kb) across the genome and normalized using a GC content and mappability (Umap k24; (Karimzadeh et al., 2018)) correction and then binned to 30 Kb windows. Regions of similar copy-number level were segmented using a sliding window method and then the copy-number value of each region was normalized by the mode of the coverage probability distribution function to center the

values around the expected 2N level. Copy number aberrations (CNA) regions were calculated as any region that deviated from this expected 2N level by >10% (loss ≤ −10% deviation, gain ≥ +10% deviation). For SV detection, reads that mapped to locations ≥5 Kb bp apart or to different chromosomes were considered discordant. These discordant fragments were clustered by both fragment size (absolute difference in genomic positions) and midpoint (sum of genomic position). Clustering was done on both parameters using a cutoff of 5 Kb. The clusters from the normal peripheral blood WGS samples were used to create a 5 Kb mask to eliminate likely false positive from the clustering results. A further filtering was applied that required junction calls to have ≥3 supporting fragments to be called and have a genomic footprint of ≥50 bp on both sides of the junction. These SV and CNA calls were combined and visualized in a genome U-plot(Gaitatzes et al., 2018).

3.6.0 Cloning of WT and mutant MYNN

To check the effect of the SNP on myoneurin, the protein accession number from uniprot.org was retrieved and entered into poyphen2. The amino acid change was also entered and the results were viewed.

The myoneurin cDNA sequence was ordered from Integrated DNA Technologies (IDT), Inc. The cDNA sequence of *MYNN* (accession number: NM_018657.5) was obtained from the National Center for Biotechnology Information (NCBI) database, and the restriction sites HindIII and KpnI were introduced upstream and downstream of the sequence, respectively. Site-directed mutagenesis was done using PCR primers (see Table 3.5.1) with nucleotide changes to create two variants of the *MYNN* coding sequence. Mutant sequences were cloned into the pEGFP-N plasmid to be used for downstream characterization in cell lines.

Table 3.5.1 Primers used for site-directed mutagenesis

	Forward Primer	Reverse Primer
WT MYNN	*CAA GCT TCG AAT GCA GTA TTC GCA CCA **C**TG*	*TCG GTA CCT CTT TGT ATA ATT GTT G*
MYNN- mutation 1	*CAA GCT TCG AAT GCA GTA TTC GCA CCA **G**T*	*TCG GTA CCT CTT TGT ATA ATT GTT G*
MYNN- mutation 2	*CAA GCT TCG AAT GCA GTA TTC GCA CCA **T**T*	*TCG GTA CCT CTT TGT ATA ATT GTT G*

Shown in bold are altered bases

3.6.1 MYNN cloning

Restriction digest of *MYNN*, and the 2 variants was carried out using KpnI and HindIII (FastDigest enzymes from Thermo Scientific™) enzymes. A restriction digest of the pEGFP-N plasmid was also done under the same conditions.

The buffer (2 uL) and the enzymes (0.5 uL each) were added to 7 uL of nuclease free water and 10 uL the DNA (x ng). The mixture was incubated at 37ºC for 15 minutes and then incubated at 80ºC for 5 minutes to inactivate the enzymes. The digest was confirmed on 1% agarose gel and the bands were cut out and gel purified using the Monarch DNA Gel Extraction Kit (New England Biolabs). Briefly, the bands were excised and dissolved using 4 volumes of Monarch Gel Dissolving Buffer. The mixture was left to incubate at 50ºC for 10 minutes, after which the mixture was poured into a collection tube and spun at 16,000 g for 1 minute. The flow through was discarded, and 200 uL of DNA wash buffer was added and spun again at the same speed for 1 minute. After the previous step was repeated, the column was transferred to a new tube, and 20 uL of nuclease free water was used to elute the DNA.

Using T4 ligase, the purified plasmid was then ligated with each of the three purified inserts. Each construct, made of plasmid plus insert, was then used to transform X-10 *E. coli* competent cells in order to replicate the plasmids. Briefly, after thawing the competent cells on ice, 5 uL of each DNA was added to 50 uL of competent cells. The mixture was incubated on ice for 30

minutes and heat shocked at 42°C for 30 seconds. After, the mixture was gently placed on ice for 30 minutes. A volume of 950 uL of LB broth was then added to the bacteria cells, after which they were incubated at 37°C for 1 hour spinning at 180 rpm. A volume of 25 uL was then used to inoculate an LB agar plate supplemented with 10% kanamycin.

After growing overnight, single colonies were selected and bacteria culture were started using Kanamycin as selective marker. Plasmid isolation was done after 48 hours later using a Monarch midi prep. Briefly, 600 uL of bacteria culture was added to a tube and 100 uL of cell lysis buffer was added. A volume of 350 uL of neutralisation solution was added and spun down. The supernatant was transferred to a PureYield column and spun for 15 seconds. The flowthrough was discarded and 200 uL of Endotoxin Removal Wash was used to wash the column. After, 400 uL of Column Wash Solution was added to the minicolumn and spun. A volume of 50 uL of nuclease free water was then used to elute the DNA.

To verify the insertion, EcoR1 was used to linearize the plasmids and a gel was run to confirm the size and using primers targeting the sequence, PCR was used to confirm insertion of sequence into plasmid.

3.7 Transfection protocol

HEK293T cells were transfected using the calcium phosphate precipitation method as described by Yanling Chen (Chen, 2012). Briefly, solution A, made of 100 uL 2x HBS and solution B which was made up of 5 ug of plasmid DNA, 12.2 uL of 2 M $CaCl_2$ and topped to 100 uL using nuclease free water was prepared in a sterile hood. Solution B was added to solution A dropwise and mixed. The mixture was incubated at room temperature for 30 minutes. After incubation, the mixture was added gently dropwise to HEK293T cells which had been seeded on a 6-well plate. After tilting to mix, the plate was incubated at 37°C in 5% CO_2 for 48 hours.

4.0 Results

4.1 Comparison of median values of clinical data obtained from 3 cohorts

The clinical data obtained from patients that had reported to Korle Bu Teaching Hospital diagnosed with multiple myeloma over the past twelve years (2010 to 2022) was obtained from patient folders. A total of 106 observations of patient data was recorded. The data was then compared to the CoMMpass data, which was a large longitudinal observational study, which monitored newly diagnosed multiple myeloma patients in the United States of America (USA). Ghanaian (African) patients were age and sex matched to that of the CoMMpass data- matching each patient to Caucasian (CoMMpass White) and African American (CoMMpass African) counterparts. For each patient, not all the data values were available.

Out of 13 variables investigated, 7 variables showed a pattern which involved having a significant difference among the cohorts. The rest of the 6 variables showed no significant difference among the groups.

Figure 4.1.1 showed pattern 1. With a median value of 17.5 g/L, Ghanaian patients were shown to present with significantly higher M component levels than both Caucasian (2.76 g/L) and African Americans (2.35 g/L) at their first presentation.

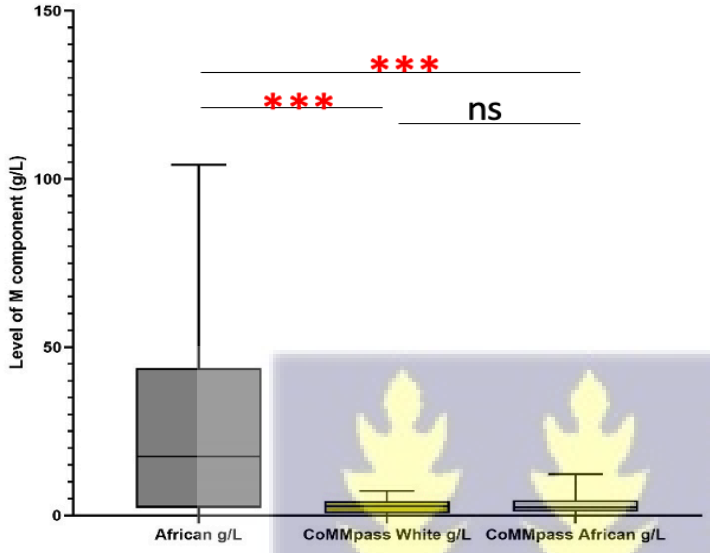


Figure 4.1.1: Box and whisker plot showing the median values of the M component of the 3 groups Plot shows median with bars showing the interquartile range. The sample size was 76 for the African patients, 105 for the CoMMpass White and 101 for the CoMMpass Africans. The p-values for the significant differences were both<0.0001.

Figure 4.1.2 The median value for the total protein was significantly lower for African patients compared to CoMMpass White and CoMMpass African patients. The African patients had a median value of 7.7 g/L compared to 8.85 g/L and 8.7 g/L for CoMMpass White and CoMMpass African patients, respectively.

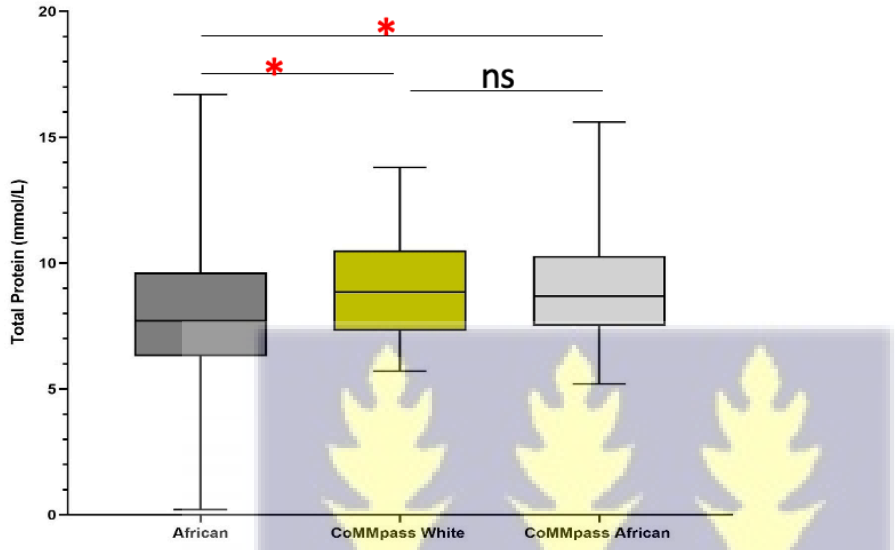


Figure 4.1.2: Box and whisker plot showing the values of total protein for the 3 groups: Plot shows median with bars showing the interquartile range. The sample size was 90 for the African patients, 104 for the CoMMpass White and 100 for the CoMMpass Africans. The p-value for the significant difference was <0.0367 for the African vs. CoMMpass White and 0.01 for the African vs. CoMMpass African.

Figure 4.1.3, where there was a significant different among all three groups. The Africans had the lowest values with a median value of 5.585 g/dL, whilst the CoMMpass White and CoMMpass African patients had values of 6.758 g/dL and 6.076 g/dL, respectively. The CoMMpass White patients had the highest median value.

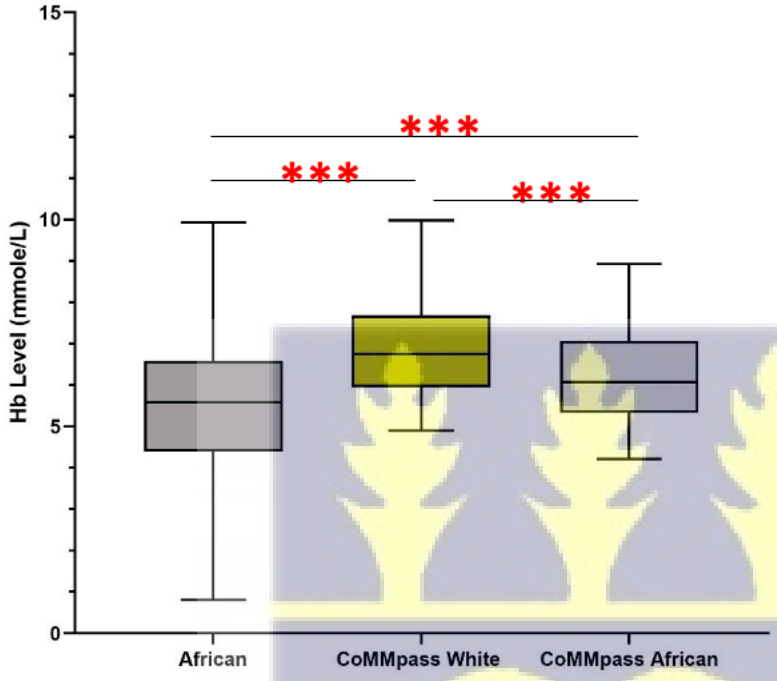


Figure 4.1.3: Box and whisker plot showing the values of haemoglobin level for the 3 groups; Plot shows median with bars showing the interquartile range. The sample size was 106 for the African patients, 106 for the CoMMpass White and 106 for the CoMMpass Africans. The p-value for the significant differences were all <0.0001.

Figure 4.1.4, where the median value for the total protein was significantly higher for African patients compared to CoMMpass White and CoMMpass African patients. The Africans had a median value of 3.734 IU/L whilst the CoMMpass White and CoMMpass African patients had values of 2.801 IU/L and 2.917 IU/L, respectively.

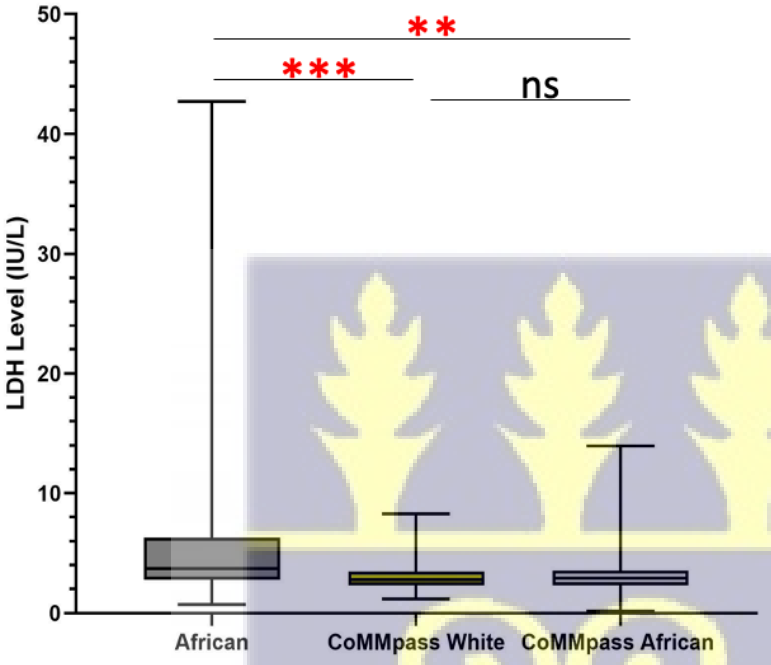


Figure 4.1.4: Box and whisker plot showing the median values of lactate dehydrogenase (LDH) levels for the 3 groups; Plot shows median with bars showing the interquartile range. The sample size was 56 for the African patients, 103 for the CoMMpass White and 91 for the CoMMpass Africans. The p-value for the significant difference was <0.0005for the African vs. CoMMpass White and 0.0085 for the African vs. CoMMpass African.

Figure 4.1.5 there was no significant difference between the Africans and the CoMMpass White as well as the Africans and the CoMMpass African patients, however, there is a significant difference between the CoMMpass White and CoMMpass African patients.

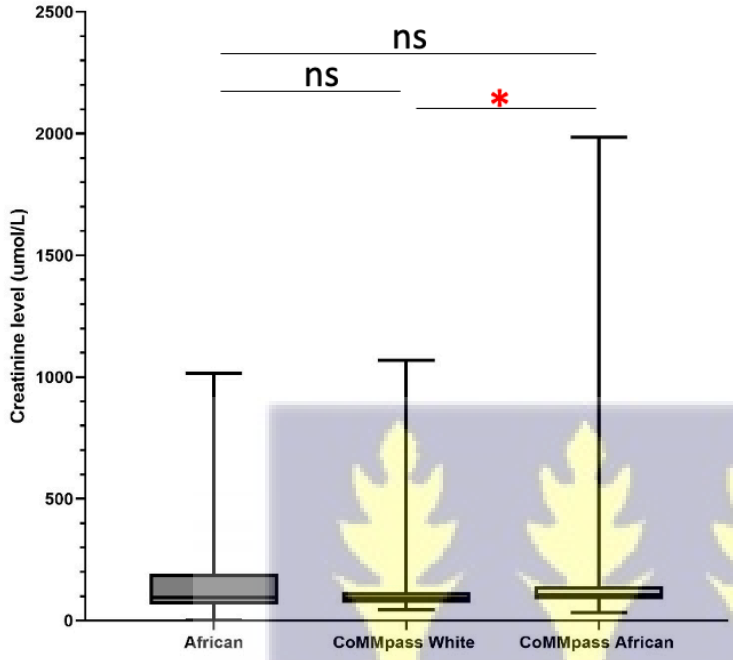


Figure 4.1.5: Box and whisker plot showing the values of median creatinine levels for the 3 groups; Plot shows median with bars showing the interquartile range. The sample size was 102 for the African patients, 106 for the CoMMpass White and 106 for the CoMMpass Africans. The p-value for the CoMMpass White vs. CoMMpass African was 0.023.

Figure 4.1.6 there was no significant difference between the Africans and the CoMMpass African patients, but there was a significant difference between the both the Africans and CoMMpass White and CoMMpass White and CoMMpass African patients. The median values for the Africans was 33.3 g/L. The CoMMpass White had a median of 38.0 g/L and the CoMMpass African had a median value of 36.0 g/L. So generally, the CoMMpass White patients had the highest median value, followed by the CoMMpass Africans and the Africans had the lowest median value.

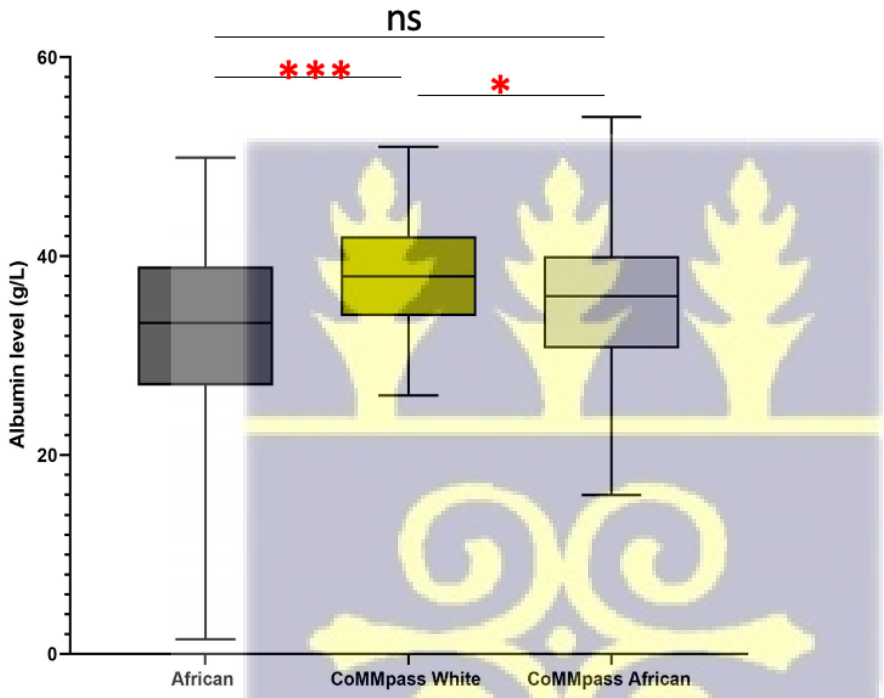


Figure 4.1.6: Box and whisker plot showing the median albumin levels for the 3 groups; Plot shows median with bars showing the interquartile range. The sample size was 100 for the African patients, 105 for the CoMMpass White and 106 for the CoMMpass Africans. The p-value for the CoMMpass White vs. CoMMpass African was 0.019 and that for the African vs. CoMMpass White, it was <0.0001.

Figure 4.1.7 The median plasma cell percentage for the African patients was 32 % whilst the CoMMpass White had a median of 9% with the CoMMpass African having a median of 9.5 %. The African cohort had the highest median value, followed by the CoMMpass Africans, and the lowest median value was seen in the CoMMpass African patients.

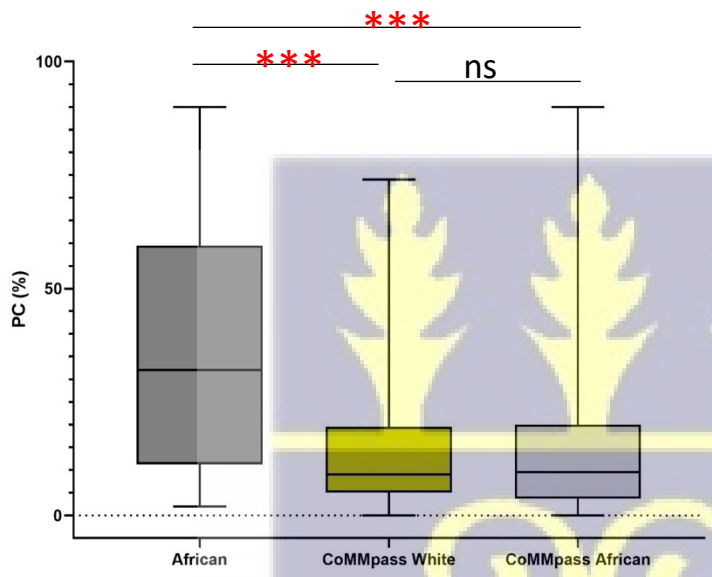


Figure 4.1.7: box and whisker plot showing the median plasma cell percentage levels for the 3 groups; Plot shows median with bars showing the interquartile range. The sample size was 29 for the African patients, 106 for the CoMMpass White and 106 for the CoMMpass Africans. The p-value for the African vs CoMMpass White was 0.0004 and that for the African vs. CoMMpass African, it was 0.0002.

Figure 4.1.8 there was no significant difference between the patients from the 3 groups. The median urea levels for the Africans were 5.3 mM/L, whilst the CoMMpass White patients had a median value of 6.426 mM/L and the CoMMpass Africans presented with 5.712 mM/L.

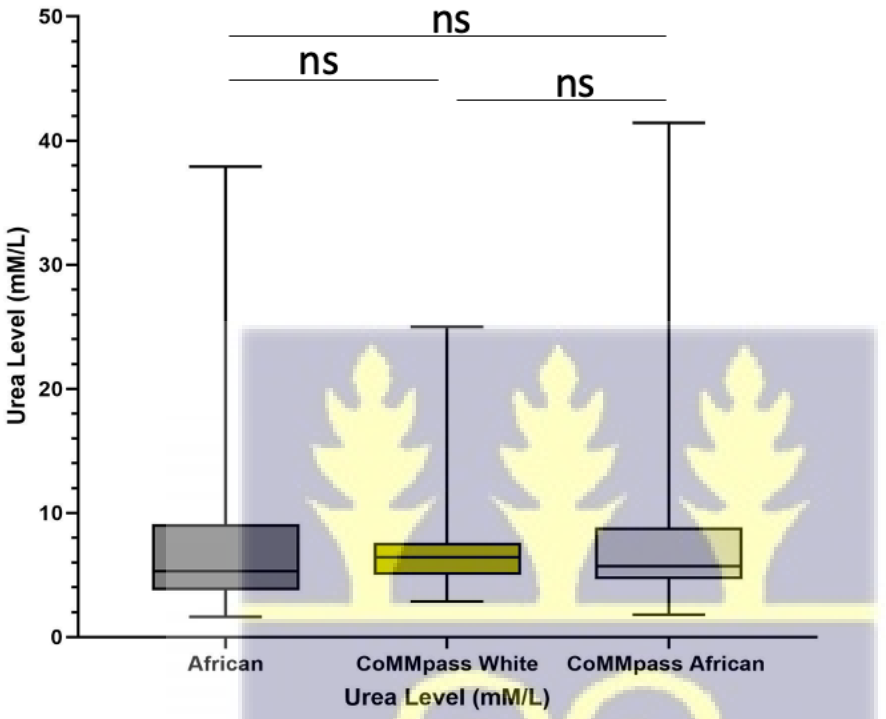


Figure 4.1.8: box and whisker plot showing the median urea levels for the 3 groups; Plot shows median with bars showing the range. The sample size was 101 for the African patients, 106 for the CoMMpass White and 104 for the CoMMpass Africans. There was no significant difference between the urea levels for the 3 groups.

Figure 4.1.9 shows Africans presented with a median value of 2.32 mmol/L. The CoMMpass White presented with a median value of 2.363 mmol/L and the CoMMpass Africans 2.35 mmol/L. Statistically, there was no difference between the three groups even though the variability between the African patients was larger than all the other patients.

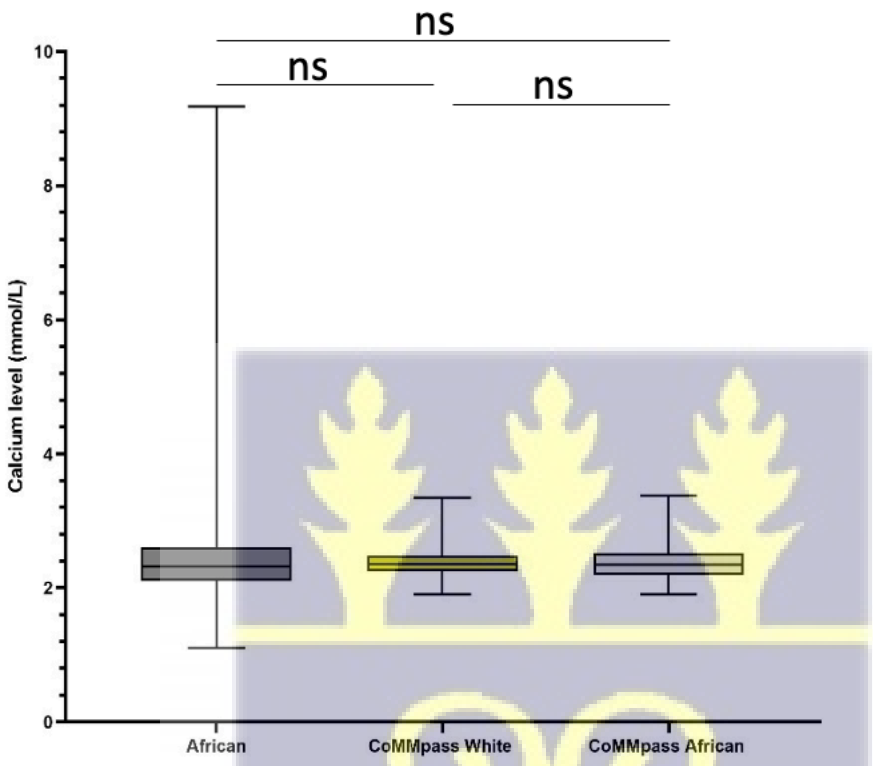


Figure 4.1.9: box and whisker plot showing the median calcium levels for the 3 groups; Plot shows median with bars showing the range. The sample size was 92 for the African patients, 106 for the CoMMpass White and 105 for the CoMMpass Africans. There was no significant difference between the levels for the 3 groups.

The median ß$_2$ microglobulin level for African patients was 4.6 g/L, which was higher than that of both the CoMMpass White (3.23 g/L) and CoMMpass Africans (3.22 g/L).

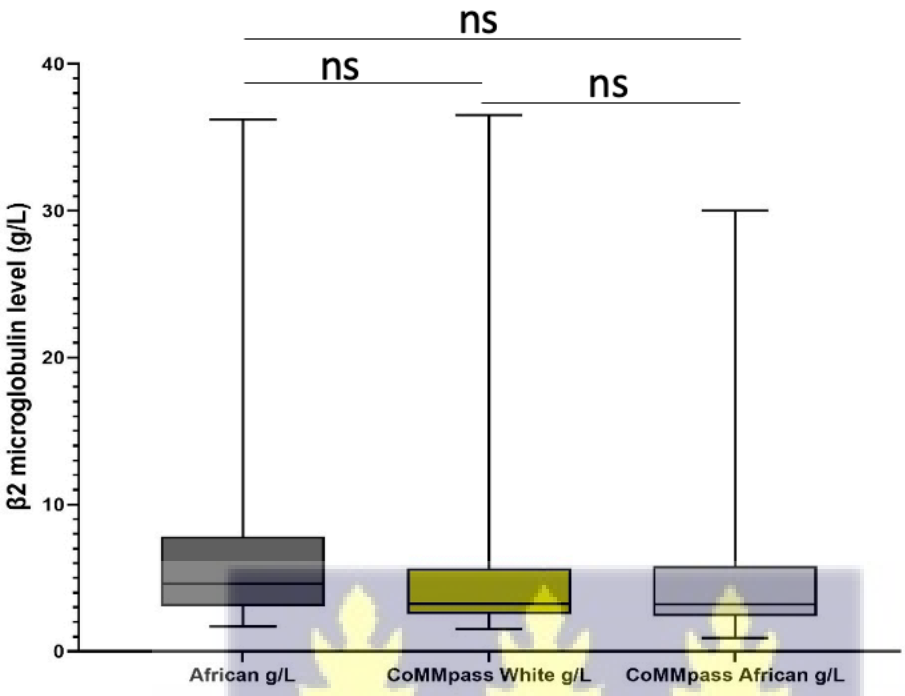


Figure 4.1.10: box and whisker plot showing the median B_2-microglobulin levels for the 3 groups: Plot showing median with bars showing the range. The sample size was 32 for the African patients, 105 for the CoMMpass White and 103 for the CoMMpass Africans. There was no significant difference between the levels for the 3 groups.

Figure 4.1.11 which compared the median values of platelet count between the different groups showed that there was no significant difference between the groups. The median value for the African group was 229 x10^9 cells/L. whilst the CoMMpass White patients had a median value of 216 x10^9 cells/L and the CoMMpass Africans presented with 209.5 x10^9 cells/L.

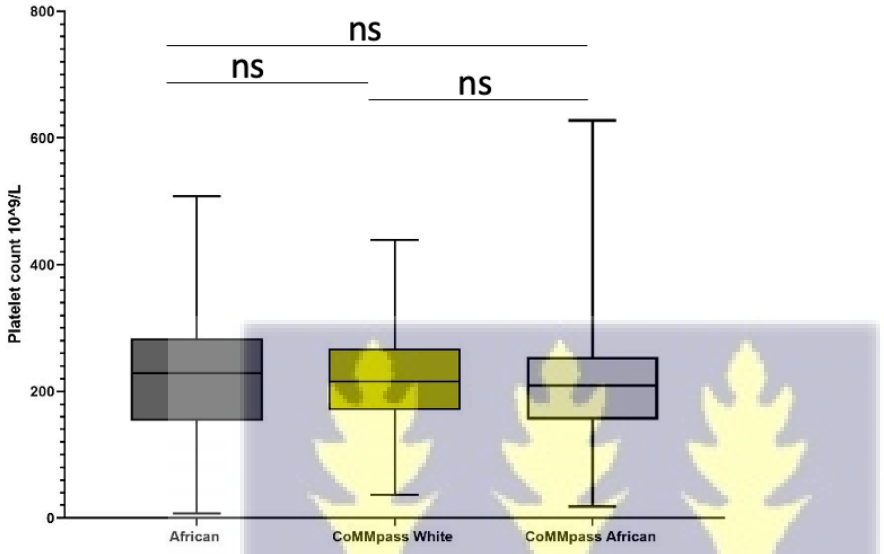


Figure 4.1.11: box and whisker plot showing the median platelet count levels for the 3 groups Plot showing median with bars showing the range. The sample size was 99 for the African patients, 106 for the CoMMpass White and 106 for the CoMMpass Africans. There was no significant difference between the levels for the 3 groups.

Figure 4.1.12 which compared the median values of white blood cell count between the different groups and showed there was no significant difference between the groups. The median value for the African group was 5.78 x10^9 cells/L. whilst the CoMMpass White patients had a median value of 5.85 x10^9 cells/L and the CoMMpass Africans presented with 5.95 x10^9 cells/L.

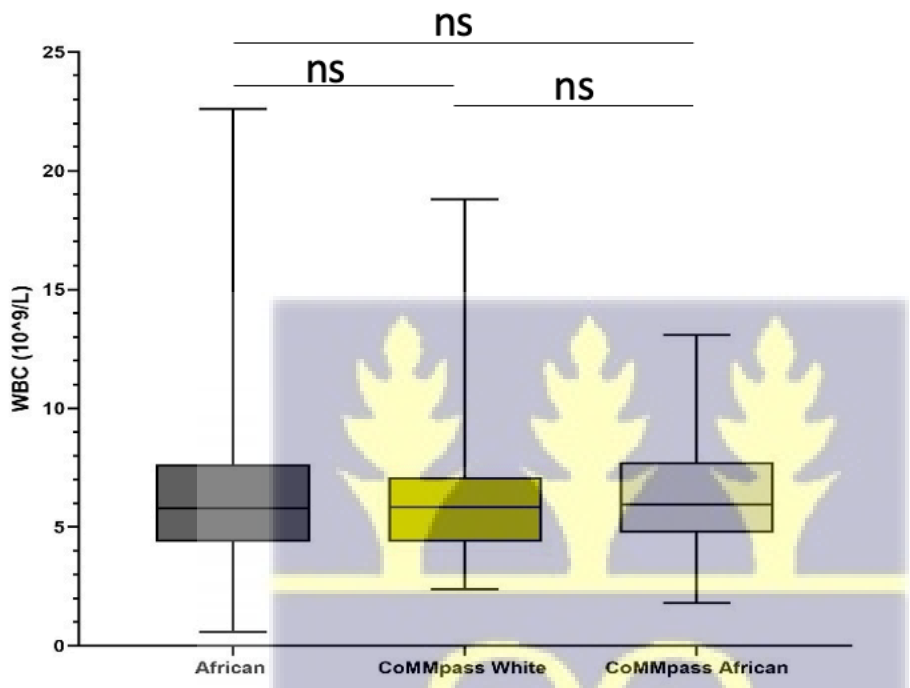


Figure 4.1.12: box and whisker plot showing the median white blood cell count for the 3 groups; Plot showing median with bars showing the range. The sample size was 100 for the African patients, 106 for the CoMMpass White and 106 for the CoMMpass Africans. There was no significant difference between the levels for the 3 groups.

Figure 4.1.13 which compared the median values of lambda chain levels between the 3 groups. There was no significant difference between the groups. The median value for the African group was 71.12 mg/dL whilst the CoMMpass White patients had a median value of 95.99 mg/dL and the CoMMpass Africans presented with 74.05 mg/dL.

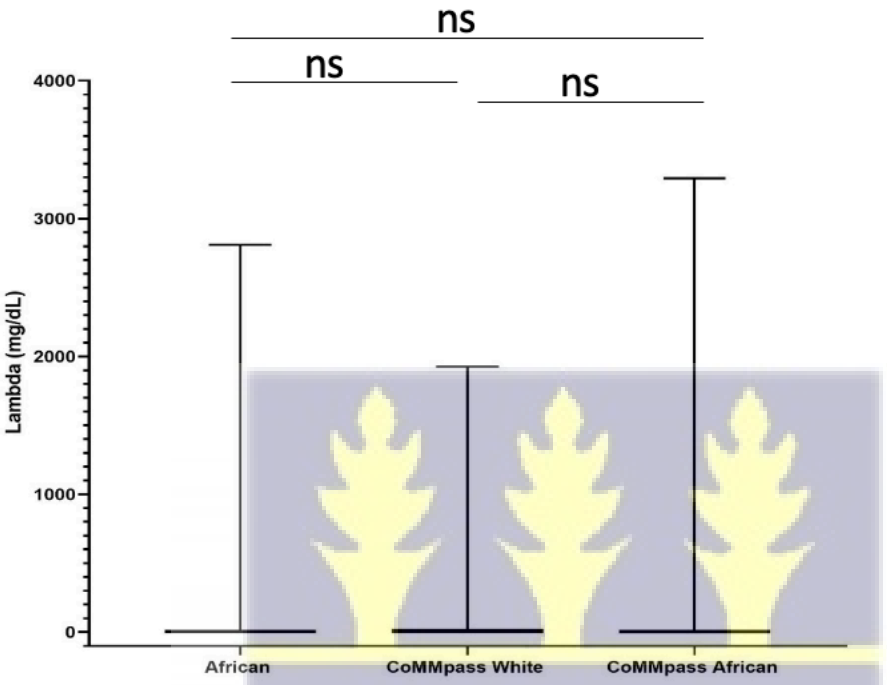


Figure 4.1.13: box and whisker plot showing the median lambda chain levels for the 3 groups;
Plot showing median with bars showing the range. The sample size was 71 for the African patients, 105 for the CoMMpass White and 105 for the CoMMpass Africans. There was no significant difference between the levels for the 3 groups.

4.2 Cloning Myoneurin (*MYNN*)

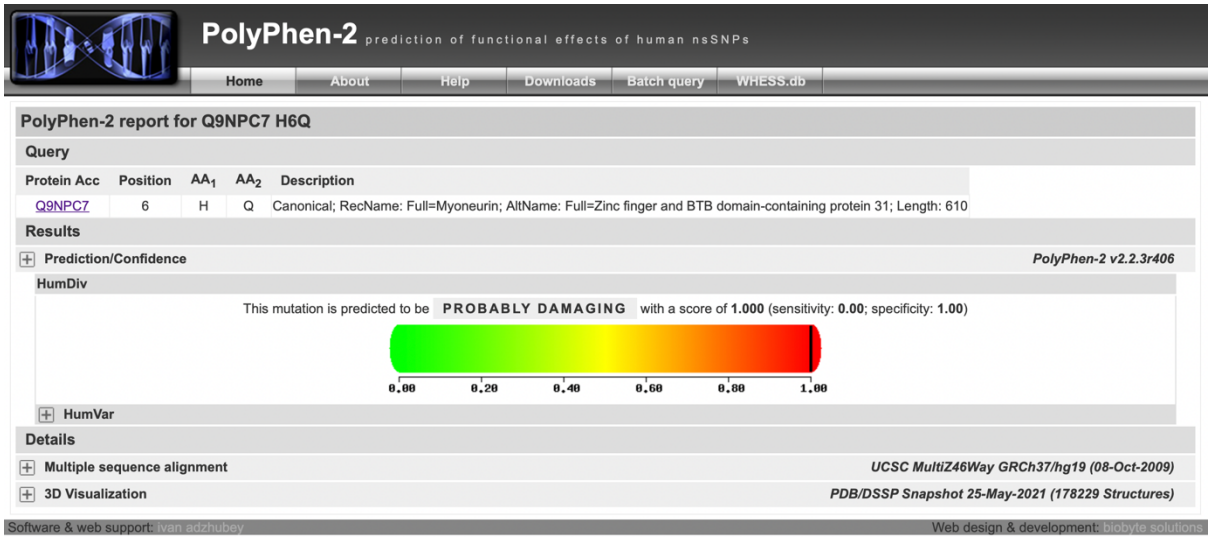


Figure 4.2.0: Result of polyphen-2 SNP in protein prediction tool: The result for the amino acid substitution caused by the SNP is a mutation that is probably damaging, with a perfect result of 1.0 given by PolyPhen-2.

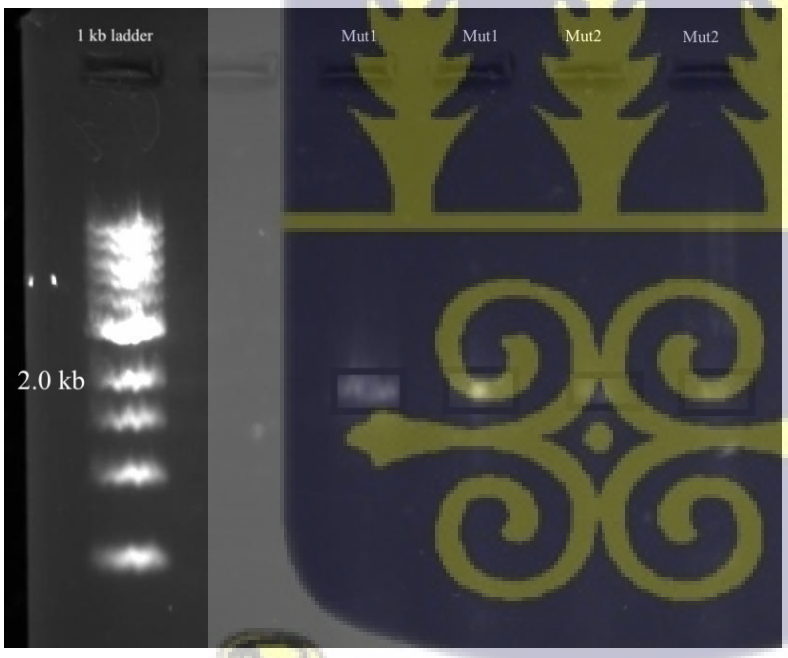


Figure 4.2.1: Gel showing result for site directed mutagenesis

Using 2 forward primers which contained the mutated base, PCR was done on the cDNA to create the 2 variants flanked by restriction sites. The PCR products from well 3 was designated Mut1 and the one from well 5 was Mut2. The size was approximately 1800 bases.

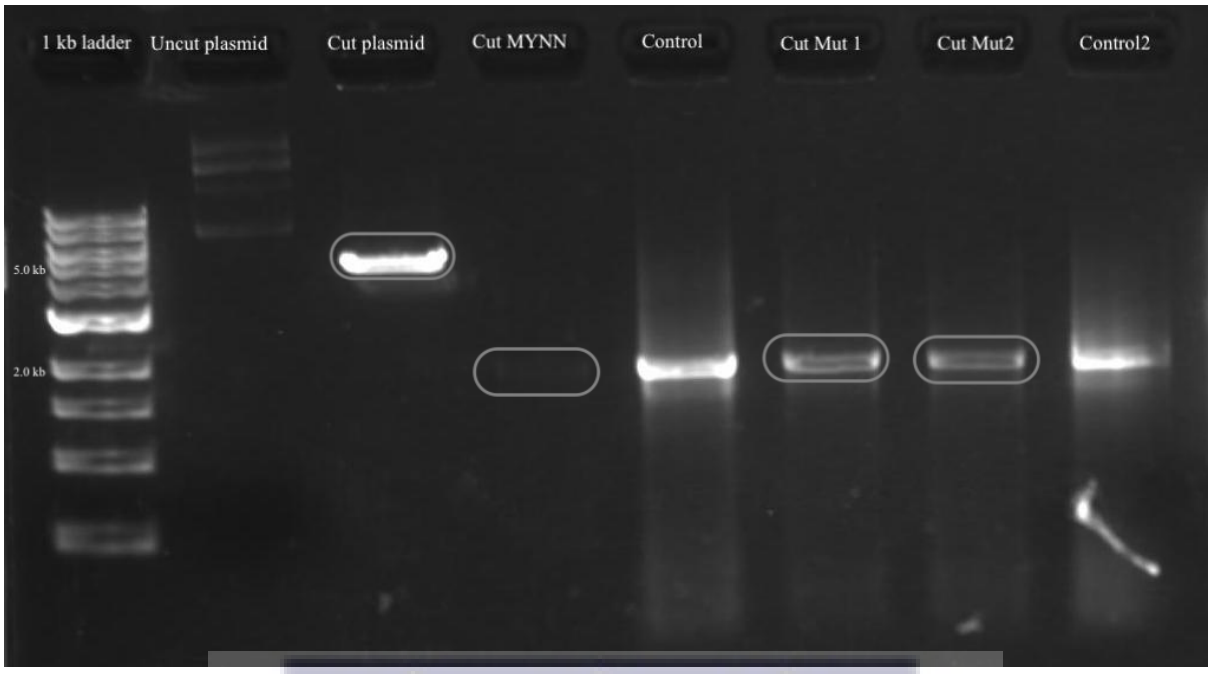


Figure 4.2.2 Gel image showing result for restriction digest

Using HindIII and KpnI, a double digest was done on the pEGFP-N plasmid and the 3 inserts (wild type MYNN, MYNN with mutation 1- Mut1 and MYNN with mutation 2-Mut2) of the gene. Control-1 and control 2 are undigested mut1and Mut2 amplicons, respectively. The cut Mut1 and Mut2.

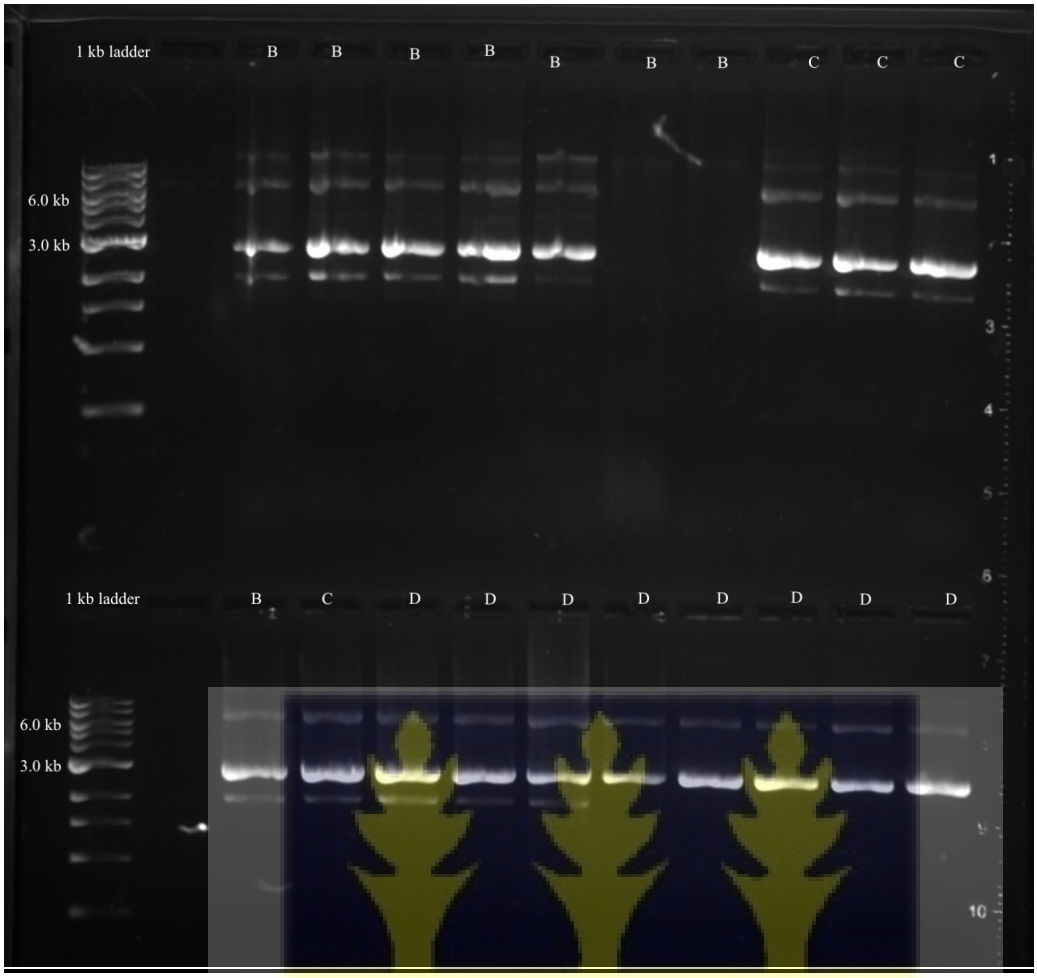


Figure 4.2.3: Gel image showing the result for plasmid isolation from the transformed *E.coli* cells

After ligation, the plasmids were used to transform *E. coli* competent cells. The single colonies, which were used to start a bacterial culture were subjected to plasmid extraction. The plasmids were extracted from bacteria named after their insert- with B- *MYNN* wild type gene, C- *MYNN* (C>G) with the first mutation and D- *MYNN* (C>T) with the second mutation. Two main bands were seen with each plasmid extraction. The bands had sizes 3.0 kb and 6.0 kb. In addition, some clones had a smaller band around 2 kb and others had a larger band around 10 kb long.

4.3 Prospective study patient demographic data

Currently, 22 patients have been recruited. Majority of the patients are female (13 female patients). The bone marrow aspirates were successfully separated into CD138 positive and CD138 negative cells, DNA isolated and sent for long-read sequencing which will provide information on the cytogenetic abnormalities present in the patients.

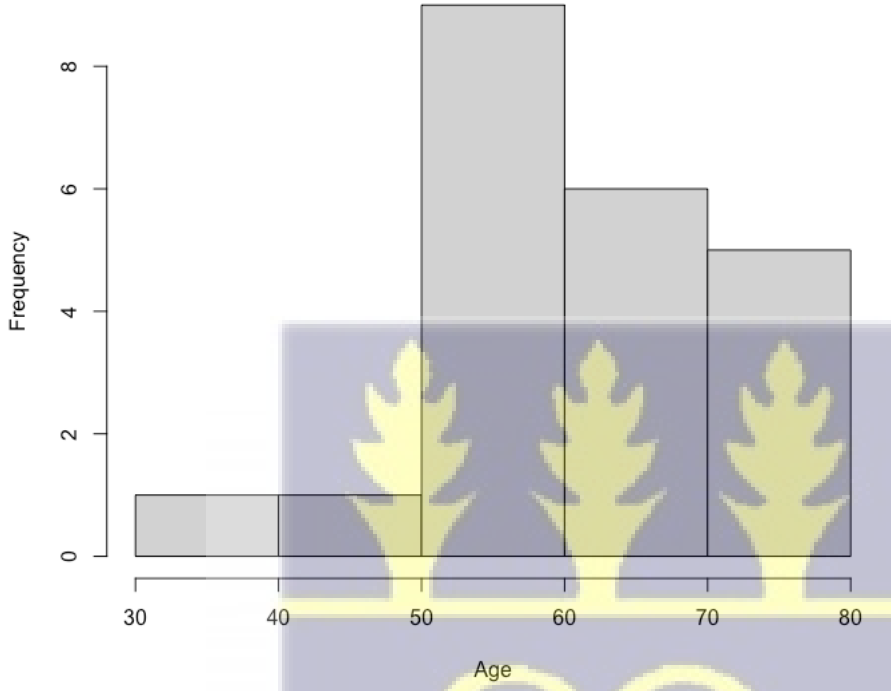


Figure 4.3.1: showing the age distribution of the patients: The median age of the current cohort is 59.5 years, with a range from 37 years to 90 years.

Figure 4.4.2 showing the number of patients who live close to commercial farms: Majority (95%) of the patients have never lived closed to commercial farms.

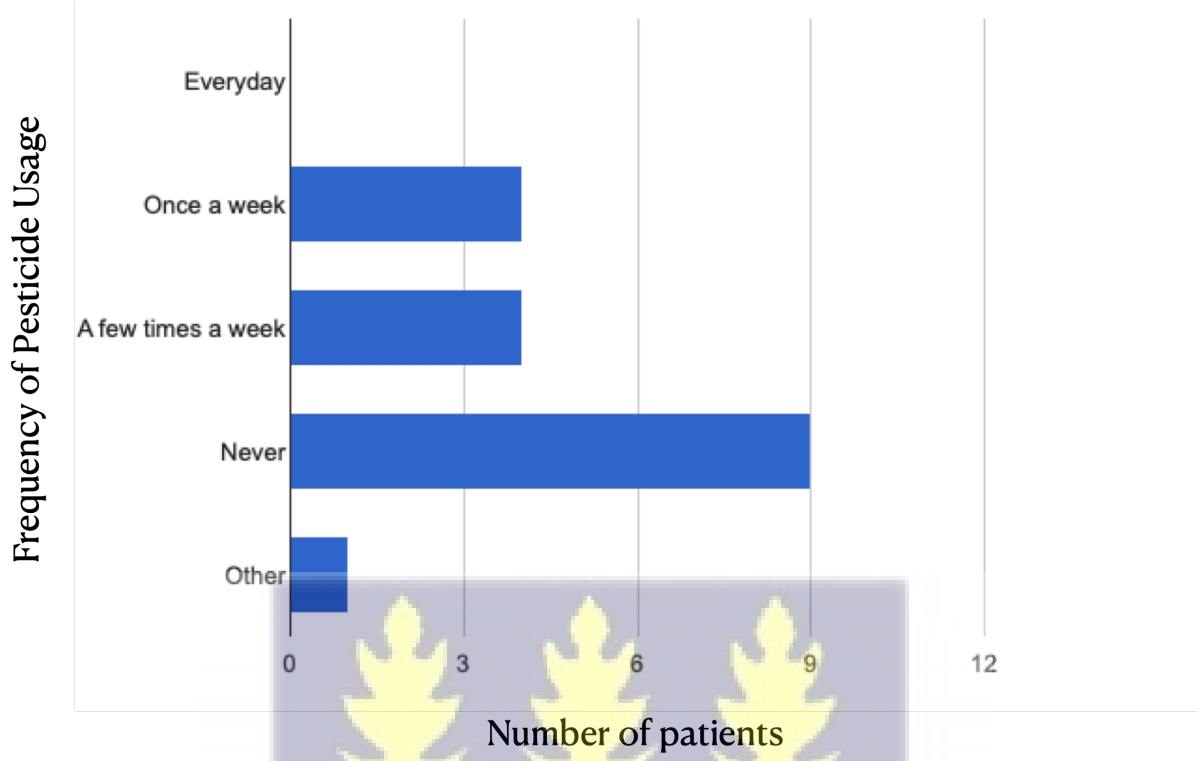


Figure 4.3.3: showing the frequency of pesticide usage among the patients.

Half of the patients never use insecticides, and a few 4/18 each come into contact with pesticides either once a week or a few times a week.

Figure 4.3.4 Showing the frequency of patients reporting with recurrent infections

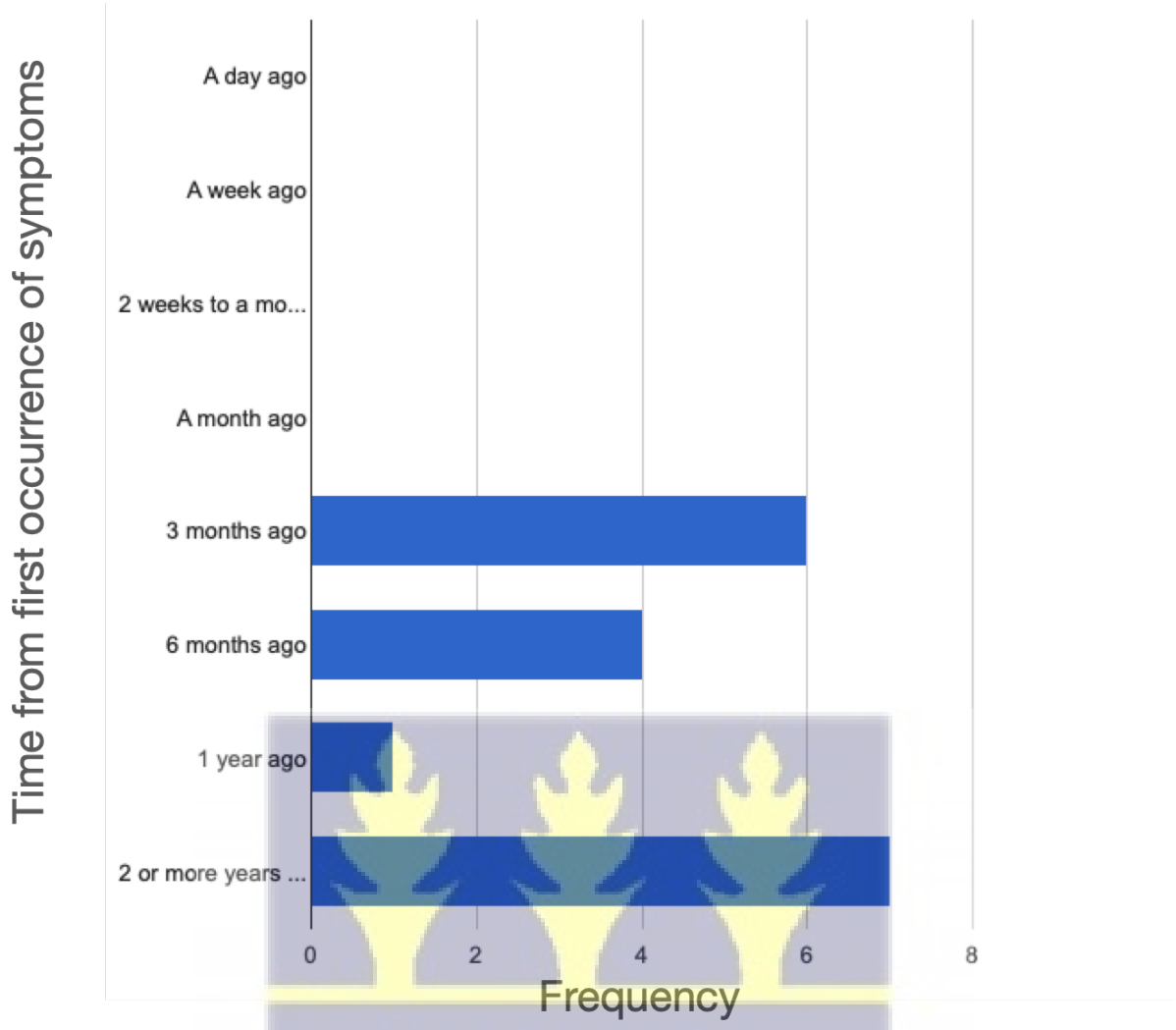


Figure 4.3.5: showing the time of occurrence of first symptoms to the time when the first bone marrow aspirate was being taken for diagnosis.

The largest proportion (7/18) of the patients reported Korle Bu Teaching Hospital over 2 years after they first begun having symptoms. A third of the patients reported 3 months after symptoms occurred and 4/18 reported approximately 6 months after they started having symptoms.

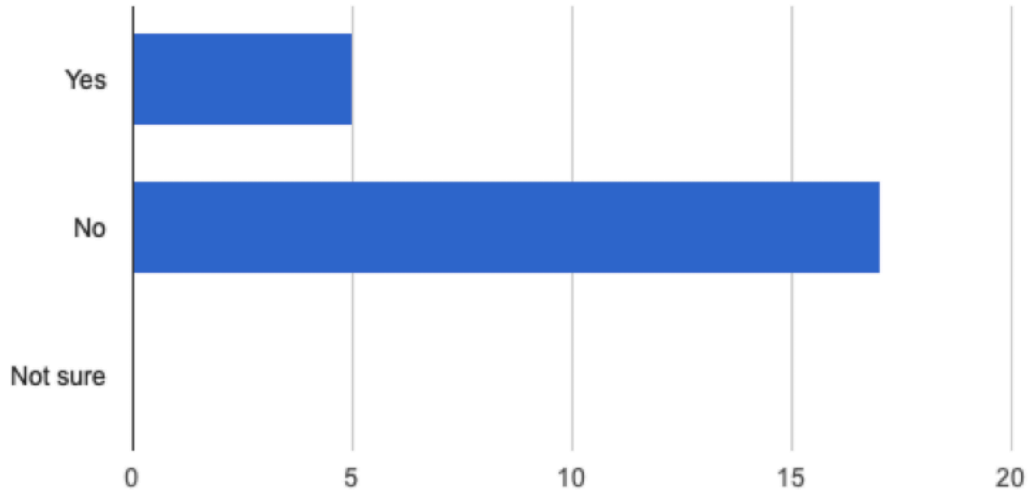


Figure 4.3.6: Showing the proportion of the patients who have been diagnosed with kidney disease.

Less than 25% of the patients recruited reported with symptoms of kidney disease. Out of 22 patients recruited, 5 had been diagnosed with having kidney problems and 17 had never had renal issues.

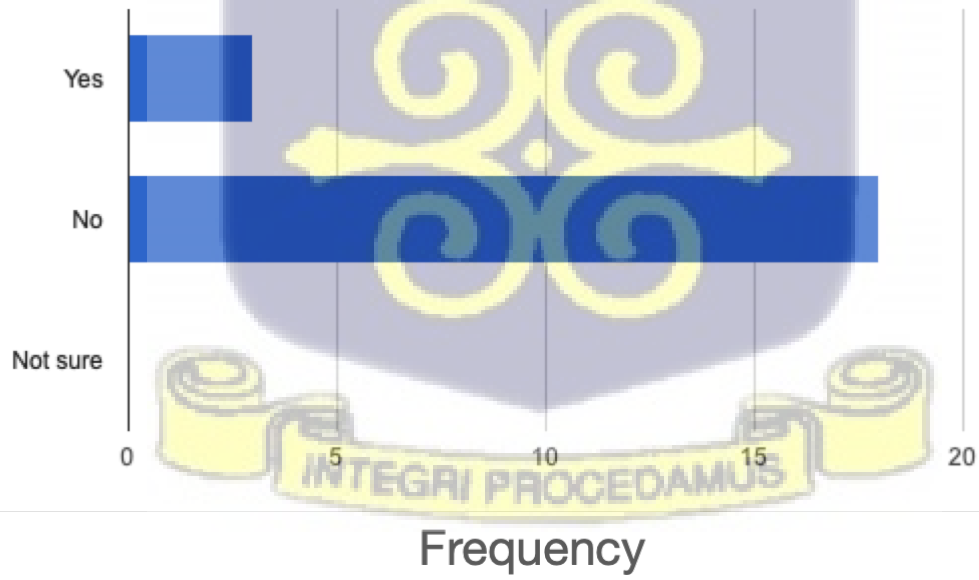


Figure 4.3.7: Showing the frequency of patients presenting with a history of bone fractures

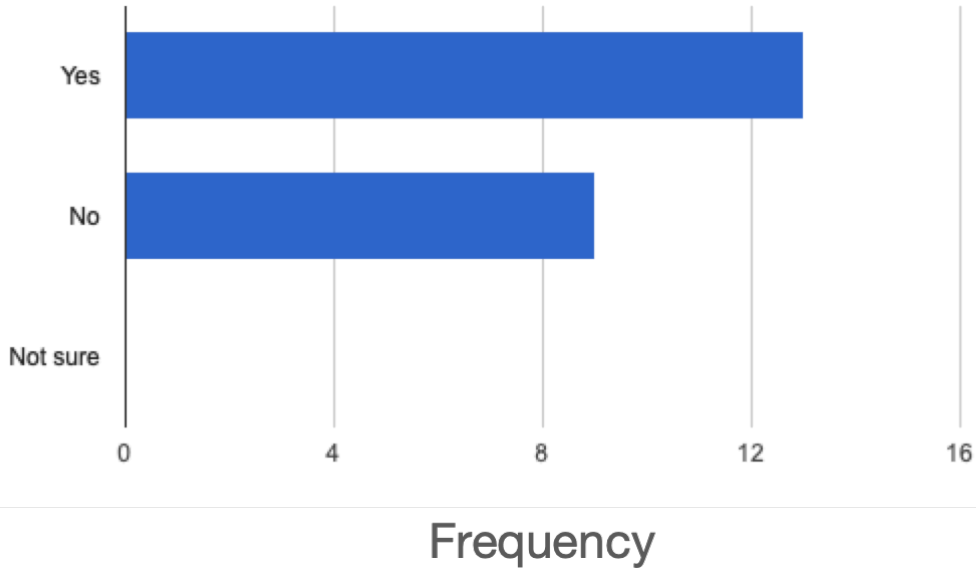


Figure 4.3.8: showing the frequency of patients presenting with anaemia

4.4 Cytogenetic Results

Out of the 22 samples collected, 9 (with the highest tumour DNA concentration) were sequenced and chromosomal abnormalities related to mm were identified. Out of the 9 sequenced samples, 4 did not have any known mm-related chromosomal abnormality. Samples 12 and 14 had only *C-MYC* translocations. Sample 8 A had hyperdiploidy (from trisomy 5, 6, 9, 15), monosomy 13, and a PSMD8 translocation. Sample 7 had the highest tumour percentage, with cytogenetic abnormalities of: 1q gain, trisomy 3,5,6,9,15,17,18,19 and *MYC/TENT5* complex. Sample 2 had a 1q gain, monosomy 13 and a 5p deletion.

The summary of the frequency of each cytogenetic abnormality can be seen in table 4.1. *MYC*-related disorders were the dominant cytogenetic abnormality seen, with 2 rearrangement and 1 *MYC/TENT5* complex. There was also a high occurrence of trisomies in the tumour samples.

Table 4.1: Cytogenetics Abnormalities of the multiple myeloma patients

Patient Sample	Abnormality	Type
2	1q gain Monosomy 13 5p deletion	Translocations and deletions
7	1q gain, Trisomy 3,5,6,9,15,17,18,19 MYC/TENT5 complex	Trisomy and translocations
8	Hyperdiploid (trisomy 5, 6, 9, 15), monosomy 13	Trisomy and monosomy
12	MYC rearrangement	Rearrangement (translocation)
14	MYC rearrangement	Rearrangement (translocation)

5.0 Discussion

Multiple myeloma is currently an incurable disease (Sonneveld et al., 2018). The single most important factor that determines the outcome of multiple myeloma is the standard of care the patient receives (Fillmore et al., 2019). Along with its preclinical stages being asymptomatic, the test needed for confirmation of diagnosis requires bone marrow aspirate, which is an invasive and expensive procedure. This makes diagnosis in a resource-limiting setting difficult. Therefore, multiple myeloma patients may go years without diagnosis and may therefore present to clinics at much later stages and may affect the clinical outcomes of multiple myeloma patients. This may explain why the largest proportion of Ghanaian MM patients reported over 2 years after first occurrence of their symptoms.

Furthermore, the lack of any cytogenetic data, which may give an idea of the both the genesis of multiple myeloma and its pathogenesis in Ghanaians and Africans also diminishes the ability of clinicians to make comprehensive decisions. By comparing the presentation of Ghanaian African patients to patients with shared ancestry (CoMMpass African patients) and Caucasians (CoMMpass White patients), the study aimed to discover emerging patterns which may illuminate the differences multiple myeloma presentation among the patients.

Statistical analysis comparing the patients' clinical presentation at their first visit to the clinical facility yielded five main patterns. The five different patterns that emerged when comparing the patients are shown below:

In pattern 1, it may be said to be likely an environmental factor, as the two groups in the same locality (USA) have statistically identical figures, whilst the Ghanaian group has significantly different values than both the African American and White CoMMpass patients all showed pattern 1. The former three may all be linked, in that the M-component reflects the levels of

abnormal antibody components secreted by the plasma cells. These components are proteins and they are secreted by plasma cells. Therefore, the higher the number of abnormal plasma cells, the higher the number of abnormal antibodies they will secrete, which will manifest as a high M-component. This will lead to a high serum total protein. Lactate dehydrogenase, which is an intracellular enzyme is usually elevated when there is cellular damage (Kumar et al., 2018).

There exists a unique subset of multiple myeloma patients who are considered high risk, as their clinical course, median survival and genetics differ (Gertz et al., 2018). Such patients are considered to have plasma cell myeloma (Gertz et al., 2018). It was found that in those patients, high serum creatinine, LDH, and bone marrow plasma cells indicated a high international stage (Gertz et al., 2018). Therefore, it is likely that the average Ghanaian patient presents with plasma cell myeloma.

The comparison between creatinine levels showed only a significant difference between CoMMpass White and CoMMpass Black patients. Even though there was no significant difference for creatinine levels between the Ghanaian cohort and the other 2, it must be highlighted that the normal range for creatinine levels are 65.4 to 119.3 micromoles/L for adult men and 52.2 to 91.9 micromoles/L for adult women. The levels of creatinine are a reflection of the state of the kidney (Samra & Abcar, 2012). The major factors that affect creatinine level is the glomerular filtration rate (GFR) and creatinine production. In multiple myeloma, increased protein production may cause a decrease in kidney function causing a decreased GFR. Renal insufficiency is a hallmark of multiple myeloma and an eGFR > 25% has been associated with poor renal survival in multiple myeloma patient (Kastritis et al., 2018). In the Ghanaians, the median value of 96.5 micromoles/L may be considered high, but normal for

males, and abnormal for females. This suggest most of the patients presenting with multiple myeloma are on the verge of kidney damage but have normal renal function. This was corroborated in the current cohort, as less than 25% of the patients suspected of having multiple myeloma reported with kidney problems, as shown in Figure 4.4.6.

Serum albumin is key in staging multiple myeloma (Rajkumar et al., 2018). Albumin is a liver synthesised protein which is useful as an indicator of nutritional status as well as in carrying lipophilic substances. Albumin is also an acute phase protein, so elevation in the albumin levels may indicate the presence of an infection (Hoda & Hoda, 2020). The levels of albumin may indicate damage to the liver, which synthesises them, or the kidney, which when healthy, does not excrete albumin. The comparison of albumin levels showed pattern 2, which means there was no significant difference between Ghanaians and CoMMpass African Americans, but each of these groups have a significant difference between their median values and the CoMMpass White value. This may suggest there is a genetic component (due to the shared ancestory) associated with albumin levels, as the Africans and CoMMpass Africans had no significant difference between them. The normal range for albumin levels is 35.0 g/L to 50 g/L (Busher, 1990). Both CoMMpass White and CoMMpass African patients had a median value that fell into this range. However, the median value for the African patients was 33.3 g/L which is slightly below the normal range.

The comparison of haemoglobin levels showed pattern 5, where there was a significant difference between all the values. This is understandable as haemoglobin is affected by so many variables like age, sex and race. Environmental factors like nutritional status, altitude and medication can also affect the haemoglobin levels. Therefore, it is hard to draw a direct link between multiple myeloma and haemoglobin levels. However, anaemia is a hallmark of

multiple myeloma, so patients were expected to present with low levels of Hb. The levels of 5.585 g/dL, 6.758 g/dL and 6.076 g/dL firmly put all the patients in the anaemic range.

In the current cohort, majority of the patients are female, which is unexpected as being male is a risk factor. Males have also been shown to be twice as susceptible as females (S. K. Kumar & Rajkumar, 2018). The median age of 59.5 is expected, as a study done earlier in Ghana also reported a median age of 58 (Acquah et al., 2019). A significant environmental risk factor in the development of multiple myeloma is the exposure of patients to pesticides. As seen in Figure 4.4.2, majority (95%) of the patients have never lived near commercial farms, which can inadvertently cause them to be exposed to high doses of pesticides. The use of insecticides to get rid of insects, and especially in Ghana, mosquitos, can also expose patients to pesticides. However, half of the patients do not use insecticides. This means pesticide exposure is insufficient in explaining the early development of multiple myeloma in Ghanaian patients. Lastly, a large proportion of the patients reporting 2 years after symptoms begun corroborates the narrative that Ghanaian multiple myeloma patients present late (Acquah et al., 2019). This may explain the reason for their advanced stages at diagnosis.

There was no significant difference in the urea levels, calcium level, $ß_2$ microglobulin level, platelet count, white blood cell count and lambda protein levels between the three groups. The range for normal urea levels is 2.1 to 8.5 mmol/L, and all three groups had medians that fell between this range. Calcium levels which reflect bone degradation in multiple myeloma patients were also similar in the three groups. The normal range for serum calcium is 2.2-2.6 mmol/L, which all 3 groups fell into. This may explain why majority (18/23) of the patients reported with no history of bone fractures. Nonetheless, this finding is not consistent with what has been described by Acquah *et al.,* where 96% of the patients presented with bone pains and

44% with bone fractures (Acquah et al., 2019). This may be because of the small sample size, and is evidenced by the atypical sampling group, where majority of the patients are female. Both the platelet count and white blood cell count fell in the normal ranges with no differences between the patient groups.

Cytogenetic Abnormalities

Out of the 9 patient samples which were sequenced, there were known cytogenetic abnormalities related to MM in 5 of them. This may be because either the patients were at different stages of development or the samples taken were of higher quality (Roy-Chowdhuri et al., 2015). The more advanced patients usually have a higher plasma cell percentage, which may be why it was easier to obtain abnormal plasma cells with the cytogenetic abnormalities (Al Saleh et al., 2020).

The predominant primary cytogenetic abnormality (seen in 4/5) of the patient was translocations. This was somewhat expected as translocations were found to be the dominant abnormalities in African Americans (Baker et al., 2013). Out of the 5 patients which had mm-related cytogenetic abnormalities, 3 had MYC-related aberrations. There were trisomies in 2 of the samples (patient 2 and patient 7), with patient 7, who had the highest tumour burden having trisomy of 6 out of 11 of the odd-numbered chromosomes. Dysregulation of the MYC gene was one of the pathways implicated in MM development (Corre et al., 2015).

The MYC signalling pathway is one of the three pathways that was shown to be responsible for tumour progression in 95% of newly diagnosed MM (Misund et al., 2020). MYC signalling has also been shown to be amplified in Black patients in head and neck squamous carcinoma and Triple-Negative Breast Cancer showing it may have a racial element (Shackelford et al.,

2022; Katsuta et. al., 2020). The increase in MYC signalling however is not synonymous with amplification of its gene (Katsuta et. al., 2020). Consequently, from our preliminary results, it is likely that Ghanaian MM patients have a disease mediated by *MYC* derangement.

Ongoing work

Sequencing of the DNA obtained from the bone marrow aspirates is still ongoing. Transformation of the competent cells is being repeated, after which the plasmids will be transfected into cell lines. The cells will be viewed under a fluorescent microscope and images will be taken. Also, qRT-PCR will be done, to determine the effects of the mutations on the expression of key genes that are associated with myoneurin and cancer hallmark genes

Conclusion

Africans do not have the typical presentation of multiple myeloma associated with Caucasians; they present with elevated: M-component, total protein, plasma cell percentage and lactate dehydrogenase levels partly owing to late presentation.

Recommendation

A control- long term study should be done on Ghanaian farmers who are constantly exposed to pesticides to investigate its effect on developing chromosomal abnormalities.

Limitation of the study

The quality of the samples needed were challenging to achieve. Lastly, there were challenges with respect to cloning the myoneurin gene. This stemmed from mistakes in the synthesized gene obtained.

References

Abdallah, N., Baughn, L. B., Vincent Rajkumar, S., Kapoor, P., Gertz, M. A., Dispenzieri, A., Lacy, M. Q., Hayman, S. R., Buadi, F. K., Dingli, D., Go, R. S., Hwa, Y. L., Fonder, A., Hobbs, M., Lin, Y., Leung, N., Kourelis, T., Warsame, R., Siddiqui, M., … Kumar, S. K. (2020). Implications of MYC rearrangements in newly diagnosed multiple myeloma. *Clinical Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-20-2283

Acquah, M. E., Hsing, A. W., McGuire, V., Wang, S., Birmann, B., & Dei-Adomakoh, Y. (2019a). Presentation and survival of multiple myeloma patients in Ghana: A review of 169 cases. *Ghana Medical Journal*, *53*(1), 52–58. https://doi.org/10.4314/gmj.v53i1.8

Acquah, M. E., Hsing, A. W., McGuire, V., Wang, S., Birmann, B., & Dei-Adomakoh, Y. (2019b). Presentation and survival of multiple myeloma patients in Ghana: A review of 169 cases. In *Ghana Medical Journal*. https://doi.org/10.4314/gmj.v53i1.8

Ahmad, K. F., Engel, C. K., & Privé, G. G. (1998). Crystal structure of the BTB domain from PLZF. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.95.21.12123

Al Saleh, A. S., Parmar, H. V., Visram, A., Muchtar, E., Buadi, F. K., Go, R. S., Dispenzieri, A., Kapoor, P., Warsame, R., Lacy, M. Q., Dingli, D., Leung, N., Gonsalves, W. I., Kourelis, T. V., Gertz, M. A., Kyle, R. A., Rajkumar, S. V., & Kumar, S. K. (2020). Increased Bone Marrow Plasma-Cell Percentage Predicts Outcomes in Newly Diagnosed Multiple Myeloma Patients. *Clinical Lymphoma, Myeloma and Leukemia*. https://doi.org/10.1016/j.clml.2020.03.012

Alliel, P. M., Seddiqi, N., Goudou, D., Cifuentes-Diaz, C., Romero, N., Velasco, E., Rieger, F., & Périn, J. P. (2000). Myoneurin, a novel member of the BTB/POZ-zinc finger family highly expressed in human muscle. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1006/bbrc.2000.2862

Baffour-Awuah, S., Annan, A. A., Maiga-Ascofare, O., Dieudonné, S. D., Adjei-Kusi, P., Owusu-Dabo, E., & Obiri-Danso, K. (2016). Insecticide resistance in malaria vectors in Kumasi, Ghana. *Parasites and Vectors*. https://doi.org/10.1186/s13071-016-1923-5

Baker, A., Braggio, E., Jacobus, S., Jung, S., Larson, D., Therneau, T., Dispenzieri, A., Van Wier, S. A., Ahmann, G., Levy, J., Perkins, L., Kim, S., Henderson, K., Vesole, D., Vincent Rajkumar, S., Jelinek, D. F., Carpten, J., & Fonseca, R. (2013). Uncovering the biology of multiple myeloma among African Americans: A comprehensive genomics approach. *Blood*. https://doi.org/10.1182/blood-2012-07-443606

Baughn, L. B., Pearce, K., Larson, D., Polley, M. Y., Elhaik, E., Baird, M., Colby, C., Benson, J., Li, Z., Asmann, Y., Therneau, T., Cerhan, J. R., Vachon, C. M., Stewart, A. K., Bergsagel, P. L., Dispenzieri, A., Kumar, S., & Rajkumar, S. V. (2018a). Differences in genomic abnormalities among African individuals with monoclonal gammopathies using calculated ancestry. *Blood Cancer Journal*. https://doi.org/10.1038/s41408-018-0132-1

Baughn, L. B., Pearce, K., Larson, D., Polley, M. Y., Elhaik, E., Baird, M., Colby, C., Benson, J., Li, Z., Asmann, Y., Therneau, T., Cerhan, J. R., Vachon, C. M., Stewart, A. K., Bergsagel, P. L., Dispenzieri, A., Kumar, S., & Rajkumar, S. V. (2018b). Differences in genomic abnormalities among African individuals with monoclonal gammopathies using calculated ancestry. *Blood Cancer Journal*. https://doi.org/10.1038/s41408-018-0132-1

Bolli, N., Avet-Loiseau, H., Wedge, D. C., Van Loo, P., Alexandrov, L. B., Martincorena, I., Dawson, K. J., Iorio, F., Nik-Zainal, S., Bignell, G. R., Hinton, J. W., Li, Y., Tubio, J. M. C., McLaren, S., O'Meara, S., Butler, A. P., Teague, J. W., Mudie, L., Anderson, E., … Munshi, N. C. (2014). Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nature Communications*. https://doi.org/10.1038/ncomms3997

Busher, J. T. (1990). Serum Albumin and Globulin. In *Clinical Methods: The History, Physical, and Laboratory Examinations*.

Chen, Y. (2012). Calcium Phosphate Transfection of Eukaryotic Cells. *BIO-PROTOCOL*. https://doi.org/10.21769/bioprotoc.86

Corre, J., Munshi, N., & Avet-Loiseau, H. (2015). Genetics of multiple myeloma: Another heterogeneity level? In *Blood*. https://doi.org/10.1182/blood-2014-10-567370

Cyster, J. G., & Allen, C. D. C. (2019). B Cell Responses: Cell Interaction Dynamics and Decisions. In *Cell*. https://doi.org/10.1016/j.cell.2019.03.016

Du, Z., Weinhold, N., Song, G. C., Rand, K. A., Van Den Berg, D. J., Hwang, A. E., Sheng, X., Hom, V., Ailawadhi, S., Nooka, A. K., Singhal, S., Pawlish, K., Peters, E. S., Bock, C., Mohrbacher, A., Stram, A., Berndt, S. I., Blot, W. J., Casey, G., … Cozen, W. (2020). A meta-analysis of genome-wide association studies of multiple myeloma among men and women of African ancestry. *Blood Advances*. https://doi.org/10.1182/bloodadvances.2019000491

Fedotova, A. A., Bonchuk, A. N., Mogila, V. A., & Georgiev, P. G. (2017). C2H2 zinc finger proteins: The largest but poorly explored family of higher eukaryotic transcription factors. In *Acta Naturae*. https://doi.org/10.32607/20758251-2017-9-2-47-58

Fillmore, N. R., Yellapragada, S. V., Ifeorah, C., Mehta, A., Cirstea, D., White, P. S., Rivero, G., Zimolzak, A., Pyarajan, S., Do, N., Brophy, M., & Munshi, N. C. (2019). With equal access, African American patients have superior survival compared to white patients with multiple myeloma: A VA study. In *Blood*. https://doi.org/10.1182/blood.2019000406

Forthal, D. N. (2014). Functions of Antibodies. *Microbiology Spectrum*. https://doi.org/10.1128/microbiolspec.aid-0019-2014

Gaitatzes A, Johnson SH, Smadbeck JB, Vasmatzis G. Genome U-Plot: a whole genome

visualization. Bioinformatics. 2018;34(10):1629-34.

Gertz, M. A., Rosinol, L., & Bladé, J. (2018). Plasma Cell Leukemia and Extramedullary Plasmacytoma. In *Hematologic Malignancies*. https://doi.org/10.1007/978-3-319-25586-6_9

Hoda, S. A., & Hoda, R. S. (2020). Robbins and Cotran Pathologic Basis of Disease. *American Journal of Clinical Pathology*. https://doi.org/10.1093/ajcp/aqaa163

Horsak, R. D., Bedient, P. B., Hamilton, M. C., & Thomas, F. Ben. (1964). Pesticides. In *Environmental Forensics: Contaminant Specific Guide*. https://doi.org/10.1016/B978-012507751-4/50030-6

Jian, Y., Chen, X., Zhou, H., Zhu, W., Liu, N., Geng, C., & Chen, W. (2016). Prognostic impact of cytogenetic abnormalities in multiple myeloma: A retrospective analysis of 229 patients. *Medicine (United States)*. https://doi.org/10.1097/MD.0000000000003521

Karimzadeh M, Ernst C, Kundaje A, Hoffman MM. Umap and Bismap: quantifying genome and methylome mappability. Nucleic Acids Res. 2018;46(20):e120.

Kastritis, E., Wechalekar, A., & Merlini, G. (2018). Primary Systemic Amyloidosis. In *Hematologic Malignancies*. https://doi.org/10.1007/978-3-319-25586-6_12

Katsuta, E., Yan, L., Takeshita, T., McDonald, K. A., Dasgupta, S., Opyrchal, M., & Takabe, K. (2019). High MYC mRNA Expression Is More Clinically Relevant than MYC DNA Amplification in Triple-Negative Breast Cancer. *International journal of molecular sciences*, *21*(1), 217. https://doi.org/10.3390/ijms21010217

Kristinsson, S. Y., Landgren, O., Dickman, P. W., Derolf, Å. R., & Björkholm, M. (2007). Patterns of survival in multiple myeloma: A population-based study of patients diagnosed in Sweden from 1973 to 2003. *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.2006.09.0100

Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of cell viability by the lactate

dehydrogenase assay. *Cold Spring Harbor Protocols*. https://doi.org/10.1101/pdb.prot095497

Kumar, S. K., & Rajkumar, S. V. (2018). The multiple myelomas - Current concepts in cytogenetic classification and therapy. In *Nature Reviews Clinical Oncology*. https://doi.org/10.1038/s41571-018-0018-y

Landgren, O., Kyle, R. A., Hoppin, J. A., Beane Freeman, L. E., Cerhan, J. R., Katzmann, J. A., Rajkumar, S. V., & Alavanja, M. C. (2009). Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood*. https://doi.org/10.1182/blood-2009-02-203471

Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM: arXiv; 2013 [Available from: https://arxiv.org/abs/1303.3997.

Lutz, J., Heideman, M. R., Roth, E., Van Den Berk, P., Müller, W., Raman, C., Wabl, M., Jacobs, H., & Jäck, H. M. (2011). Pro-B cells sense productive immunoglobulin heavy chain rearrangement irrespective of polypeptide production. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1019224108

Misund, K., Keane, N., Stein, C. K., Asmann, Y. W., Day, G., Welsh, S., Van Wier, S. A., Riggs, D. L., Ahmann, G., Chesi, M., Viswanatha, D. S., Kumar, S. K., Dispenzieri, A., Gonzalez-Calle, V., Kyle, R. A., O'Dwyer, M., Rajkumar, S. V., Kortüm, K. M., Keats, J. J., … Bergsagel, P. L. (2020). MYC dysregulation in the progression of multiple myeloma. In *Leukemia*. https://doi.org/10.1038/s41375-019-0543-4

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. In *Frontiers in Public Health*. https://doi.org/10.3389/fpubh.2016.00148

Onwona Kwakye, M., Mengistie, B., Ofosu-Anim, J., Nuer, A. T. K., & Van den Brink, P. J.

(2019). Pesticide registration, distribution and use practices in Ghana. *Environment, Development and Sustainability*. https://doi.org/10.1007/s10668-018-0154-7

Packard, E., Shahid, Z., Groff, A., Patel, R., & Jain, R. (2019). Multiple Myeloma in an Agricultural Worker Exposed to Pesticides. *Cureus*. https://doi.org/10.7759/cureus.4762

Pahwa, P., Karunanayake, C. P., Dosman, J. A., Spinelli, J. J., McDuffie, H. H., & McLaughlin, J. R. (2012). Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study. *Journal of Agromedicine*. https://doi.org/10.1080/1059924X.2012.632339

Palumbo, A., & Anderson, K. (2011). Medical Progress Multiple Myeloma. *N Engl J Med*.

Polat, F., Yilmaz, M., & Diler, S. B. (2019). The association of MYNN and TERC gene polymorphisms and bladder cancer in a Turkish population. *Urology Journal*. https://doi.org/10.22037/uj.v0i0.4083

Presutti, R., Harris, S. A., Kachuri, L., Spinelli, J. J., Pahwa, M., Blair, A., Zahm, S. H., Cantor, K. P., Weisenburger, D. D., Pahwa, P., McLaughlin, J. R., Dosman, J. A., & Freeman, L. B. (2016). Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project. *International Journal of Cancer*. https://doi.org/10.1002/ijc.30218

Prideaux, S. M., Conway O'Brien, E., & Chevassut, T. J. (2014). The genetic architecture of multiple myeloma. In *Advances in Hematology*. https://doi.org/10.1155/2014/864058

Rajan, A. M., & Rajkumar, S. V. (2015). Interpretation of cytogenetic results in multiple myeloma for clinical practice. In *Blood Cancer Journal*. https://doi.org/10.1038/bcj.2015.92

Rajkumar, S. V., Fonseca, R., & San Miguel, J. F. (2018). Diagnosis and Staging of Multiple Myeloma and Related Disorders. In *Hematologic Malignancies*. https://doi.org/10.1007/978-3-319-25586-6_2

Roy-Chowdhuri, S., Goswami, R. S., Chen, H., Patel, K. P., Routbort, M. J., Singh, R. R., Broaddus, R. R., Barkoh, B. A., Manekia, J., Yao, H., Medeiros, L. J., Staerkel, G., Luthra, R., & Stewart, J. (2015). Factors affecting the success of next-generation sequencing in cytology specimens. *Cancer Cytopathology*. https://doi.org/10.1002/cncy.21597

Samra, M., & Abcar, A. C. (2012). False estimates of elevated creatinine. *The Permanente Journal*. https://doi.org/10.7812/TPP/11-121

Seyfert, V. L., Allman, D., He, Y., & Staudt, L. M. (1996). Transcriptional repression by the proto-oncogene BCL-6. *Oncogene*.

Shackelford, A., Momen-Heravi, F., Dubey, S., Mezghani, M. N., Taylor, A., Yao, M. A., ... & Philipone, E. (2022). The Impact of Race on C-MYC Expression and Resulting Prognostic Implications in Head and Neck Squamous Cell Carcinoma. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *133*(5), e126.

Soediono, B. (1989). Alberts - Molecular Biology Of The Cell 4th Ed. *Journal of Chemical Information and Modeling*. https://doi.org/10.1017/CBO9781107415324.004

Sonneveld, P., Einsele, H., Brioli, A. M., & Cavo, M. (2018). Treatment of Transplant Eligible Patients with Multiple Myeloma. In *Hematologic Malignancies*. https://doi.org/10.1007/978-3-319-25586-6_3

Vasquez, J. F., Diaz, E., & Poquioma, E. (2020). Assessment of 1112 Newly Diagnosed Multiple Myeloma Patients According to Age and Year of Diagnosis at a Referral Cancer Center. *Blood*. https://doi.org/10.1182/blood-2020-142066

Went, M., Sud, A., Försti, A., Halvarsson, B. M., Weinhold, N., Kimber, S., van Duin, M., Thorleifsson, G., Holroyd, A., Johnson, D. C., Li, N., Orlando, G., Law, P. J., Ali, M., Chen, B., Mitchell, J. S., Gudbjartsson, D. F., Kuiper, R., Stephens, O. W., … Thibodeau, S. N. (2018). Identification of multiple risk loci and regulatory mechanisms

influencing susceptibility to multiple myeloma. *Nature Communications*. https://doi.org/10.1038/s41467-018-04989-w

World Bank. (2020). World Development Indicators: Ghana. Retrieved March 7, 2023, from https://databank.worldbank.org/data/source/world-development-indicators

Appendix

MYNN gene sequence with restriction sites added

AGA TCT CGA GCT CAA GCT TCG AAT GCA GTA TTC GCA CCA CTG TGA GCA CCT TTT AGA GAG ACT GAA CAA ACA GCG GGA AGC AGG TTT TCT CTG TGA CTG TAC CAT AGT GAT TGG GGA ATT CCA GTT TAA AGC TCA TAG GAA TGT GCT GGC CTC CTT TAG TGA GTA TTT TGG TGC GAT CTA CAG AAG CAC TTC TGA GAA CAA TGT CTT TCT TGA TCA GAG TCA GGT GAA GGC TGA TGG ATT TCA GAA ACT GTT GGA GTT TAT ATA CAC AGG AAC TTT AAA TCT TGA CAG TTG GAA TGT TAA AGA AAT TCA TCA GGC TGC TGA CTA TCT CAA AGT GGA AGA GGT GGT CAC TAA ATG CAA AAT AAA GAT GGA AGA TTT TGC TTT TAT TGC TAA TCC TTC TTC TAC AGA GAT ATC TAG TAT TAC TGG AAA CAT TGA ATT GAA TCA ACA GAC TTG TCT TCT TAC TCT GCG AGA TTA TAA TAA TCG AGA GAA ATC AGA AGT ATC TAC AGA TTT GAT TCA GGC AAA TCC TAA ACA AGG CGC GTT AGC GAA AAA GTC ATC TCA AAC GAA AAA GAA GAA GAA GGC TTT CAA CTC CCC GAA AAC AGG GCA GAA TAA AAC AGT GCA ATA TCC CAG TGA CAT CTT AGA GAA TGC ATC TGT TGA ATT ATT CCT AGA TGC AAA TAA ACT GCC CAC ACC TGT AGT AGA ACA AGT TGC ACA AAT AAA TGA TAA TTC AGA ACT CGA GTT GAC ATC AGT TGT GGA AAA TAC TTT TCC AGC ACA AGA TAT TGT GCA CAC TGT TAC AGT GAA ACG GAA ACG TGG AAA ATC ACA GCC AAA CTG TGC TCT GAA AGA ACA CTC TAT GTC TAA TAT AGC CAG CGT CAA GAG TCC TTA TGA GGC GGA GAA CTC CGG GGA AGA GCT GGA TCA GAG GTA TTC CAA GGC CAA GCC AAT GTG TAA CAC ATG TGG GAA AGT GTT TTC AGA AGC CAG CAG TTT GAG AAG GCA CAT GAG AAT ACA TAA AGG AGT CAA ACC TTA CGT CTG CCA CTT ATG TGG AAA GGC ATT TAC CCA ATG TAA CCA GCT GAA AAC GCA TGT AAG AAC TCA TAC AGG TGA GAA GCC ATA CAA ATG TGA ATT GTG TGA TAA AGG ATT TGC TCA GAA ATG TCA GCT AGT CTT CCA TAG TCG CAT GCA TCA TGG TGA AGA AAA ACC CTA TAA ATG TGA TGT ATG CAA CTT ACA GTT TGC AAC TTC TAG CAA TCT CAA GAT TCA TGC AAG GAA GCA TAG TGG AGA GAA GCC ATA TGT CTG TGA TAG GTG TGG ACA GAG ATT TGC TCA AGC CAG CAC ACT GAC CTA TCA TGT CCG TAG GCA TAC TGG AGA AAA GCC TTA TGT ATG TGA TAC CTG TGG GAA GGC ATT TGC TGT CTC TAG TTC TCT TAT CAC TCA TTC TCG AAA ACA TAC AGG TGA AAA ACC ATA CAT ATG TGG TAT TTG TGG GAA AAG TTT TAT TTC CTC AGG AGA GCT CAA CAA ACA CTT TCG GTC CCA TAC AGG AGA AAG ACC ATT TAT CTG CGA ATT ATG TGG AAA TTC TTA CAC AGA TAT TAA AAA TTT AAA GAA GCA CAA AAC AAA AGT CCA TTC TGG TGC AGA TAA AAC TCT AGA CTC CAG TGC AGA GGA TCA TAC TTT GAG TGA ACA GGA TTC CAT ACA AAA AAG TCC TTT ATC AGA AAC TAT GGA TGT GAA GCC TTC TGA TAT GAC TTT ACC ATT AGC TCT TCC ACT TGG GAC TGA GGA CCA TCA CAT GCT TCT GCC TGT CAC GGA TAC TCA GTC TCC TAC ATC AGA TAC ATT GTT GAG GTC AAC TGT GAA TGG GTA TTC AGA ACC ACA GTT GAT TTT TTT ACA ACA ATT ATA CAA AGA GGT ACC GA

Table showing the primers used for site-directed mutagenesis

	Forward Primer	Reverse Primer
MYNN	CAA GCT TCG AAT GCA GTA TTC GCA CCA **C**TG	TCG GTA CCT CTT TGT ATA ATT GTT G
MYNN- mutation 1	CAA GCT TCG AAT GCA GTA TTC GCA CCA **G**T	TCG GTA CCT CTT TGT ATA ATT GTT G
MYNN- mutation 2	CAA GCT TCG AAT GCA GTA TTC GCA CCA **T**T	TCG GTA CCT CTT TGT ATA ATT GTT G

Shown in bold is the base that has been mutated

PCR protocol for Site-directed mutagenesis- 25 uL reaction

Component	Volume (uL)
Master mix	12.5
Forward Primer (10 uM)	0.5
Reverse Primer (10 uM)	0.5
Template gene (MYNN gene)	5
Nuclease free water	6.5

PCR cycling conditions for site-directed mutagenesis

Step	Temperature (°C)	Time (seconds)
Initial denaturation	94	30
Denaturation	94	15
Extension	51	15
Annealing	68	105
Final Hold	68	300

Restriction digest components

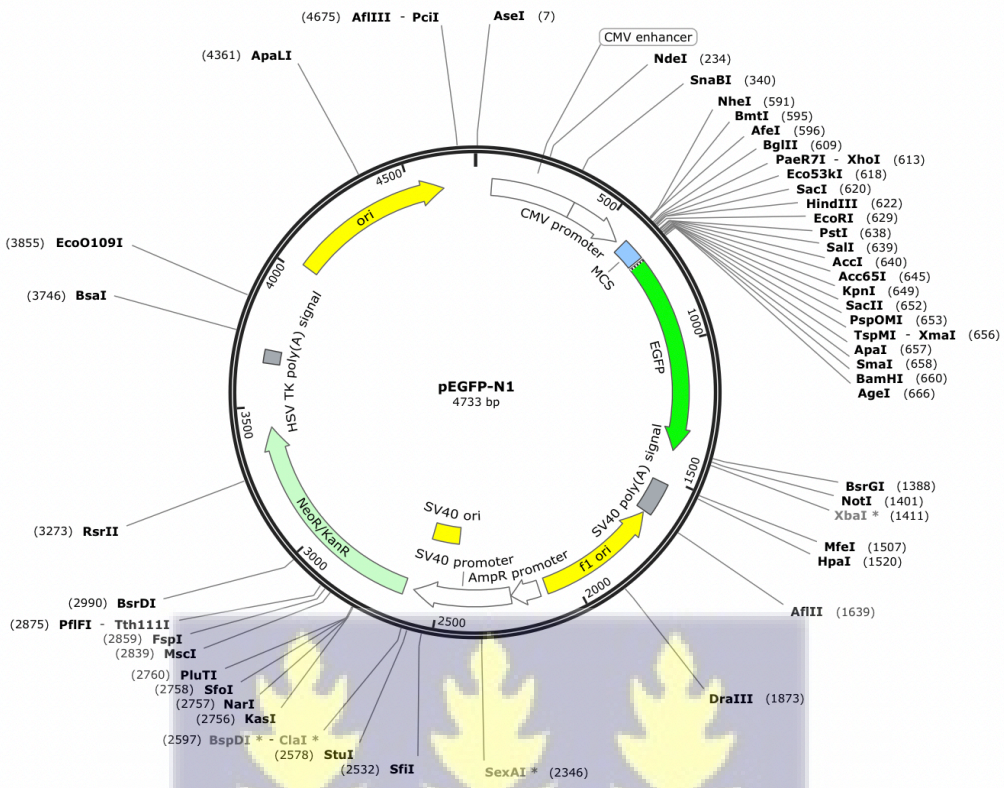
Component	Volume (uL)
DNA/plasmid	10
10x buffer	2
KpnI	0.5
HindIII	0.5
Nuclease free water	7

Components of ligation reaction

Component	Volume
Nuclease free water	1
Ligase buffer 1x	1
Vector (plasmid)	2
Oligos (cut gene)	5
T4 DNA ligase	1

E. coli transformation protocol

1. Place 10 ng/uL DNA sample in 1.5 ml tube
2. Fill ice bucket and prechill each tube
3. Thaw competent cells on ice
4. Pipette 30 uL of competent cells in each tube
5. Pipette 3 uL of 10 ng/uL resuspended DNA in each tube
6. Close tubes and incubate for 30 mins on ice
7. Heat shock at 42ºC for 30 seconds
8. Place tube back on ice
9. Pipette 120 uL of LB broth into each transformant
10. Incubate at 37 ºC for 1 hour at 180 rpm
11. Plate on 25 uL kanamycin and incubate for 18 hours at 37 ºC



pEGFP-N1 plasmid sequence and key features

Sample size calculation

Based on the estimated prevalence by Globocan, 2018 (International Agency for Research, 2019), for an incidence of 117 cases in Ghana in 2018, 100 samples will be needed to 99% CI and 5% margin of error. But with an incidence rate of 4.1/100,000 in Caucasians and a study finding the rate in Black people to be 9.1/100,000 people (Lewis et al., 1994), the cases are believed to be underreported. Using the incidence rate in Blacks, and a Ghanaian population estimated by the world bank to be 29.77 million (in 2018), there would have been about 2,709 cases in Ghana. For a confidence level of 99% with 5% margin of error, 535 samples will be needed. Therefore, the sample size of cases will be 600 samples. On average, five case samples per study site per month are expected.

$$\text{Sample size} = \frac{\frac{z^2 \times p\,(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p\,(1-p)}{e^2 N}\right)}$$

N= population size
e= margin of error
Z= z score

Questionnaire:

Characterisation and Karyotyping Multiple Myeloma in patients in Ghana

Participant Questionnaire form

Name of Hospital: .....

ID: .....

A. Participant General History

Age_____

Age:

- ☐ < 30 years
- ☐ 30 – 34 years
- ☐ 35 – 39 years
- ☐ 40 – 44 years
- ☐ 45 – 49 years
- ☐ 50 – 54 years
- ☐ 55 – 59 years
- ☐ 60 – 64 years
- ☐ 65 + years

Gender:

- ☐ Male
- ☐ Female
- ☐ Prefer not to say

Race:

- ☐ Black or African
- ☐ African American
- ☐ Caucasian or White
- ☐ Asian
- ☐ American Indian
- ☐ Hispanic or Latino
- ☐ Mixed Race or Others (please state)

.....

First Occurrence of symptoms: .....

Date of diagnosis: .....

Occupation: .....

Residence (Location): .....

Ethnic group (tribe): .....

Highest education achieved.....

B. Participant Medical History

1. Have you ever been diagnosed with plasmacytoma?
   Yes No

2. Have you ever been diagnosed with lymphoma?
   Yes No

3. Have you ever been diagnosed with any other blood cancer?
   Yes No

4. Have you ever been diagnosed with kidney disease?
   Yes No

5. Do you have any relatives that have been diagnosed with a blood cancer?
   Yes No

If yes, what cancer was it?
.....

6. Have you received blood transfusion in the past 3 months?
   Yes No

7. Have you ever had a bone marrow transplant?
   Yes No

8. Have you ever had a bone fracture?
   Yes No
   If yes, how recent was it?.....

9. What symptoms made you go to the hospital?

- ☐ Bone pains
- ☐ Bone fractures
- ☐ Fatigue
- ☐ Recurring Infections
- ☐ Shortness of breath
- ☐ Numbness in legs
- ☐ Weight loss
- ☐ Other (please state)
  .....
- ☐ Nausea
- ☐ Constipation
- ☐ Increased thirst and urination
- ☐ Anaemia
- ☐ Kidney problems
- ☐ Confusion

10. Have you ever lived near a commercial farm?
    Yes No
    If yes, for how long?.....

11. Are you usually exposed to insecticides/pesticides (do you spray your room)?
    - ☐ Everyday
    - ☐ Once a week
    - ☐ A few times a week
    - ☐ Never
    - ☐ Other (please state)
        - ○ .....

    If yes, which ones?
    - ☐ Insecticide sprays
    - ☐ Mosquito coils
    - ☐ Rat poison
    - ☐ Other (please state)
        - ○ .....

12. Multiple Myeloma status:
    a. Already Diagnosed
    b. Newly Diagnosed
    c. Suspected
    d. Archived

    If already diagnosed, what treatment were you recommended/given?
    - ☐ Corticosteroids (e.g. Dexamethasone)
    - ☐ Chemotherapeutic agents (e.g. doxorubicines)
    - ☐ Proteasome inhibitors (e.g. bortezomib)
    - ☐ Immunomodulatory drugs (e.g. thalidomide, lenalidomide)
    - ☐ Bone Marrow transplant
    - ☐ Other (please state)
        - ○ .....

13. Are there any drugs/supplements you take for your symptoms?
    Yes No
    If yes, what is it.....

C. Relative History

Do you have a relative with Multiple Myeloma?

☐ Yes ☐ No

1.Relationship	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin
2. Age at Diagnosis (if unknown, enter DK)			
3. Deceased/ Alive			

Would you like any of your relatives/neighbours to be checked for Multiple Myeloma?

☐ Yes

☐ No

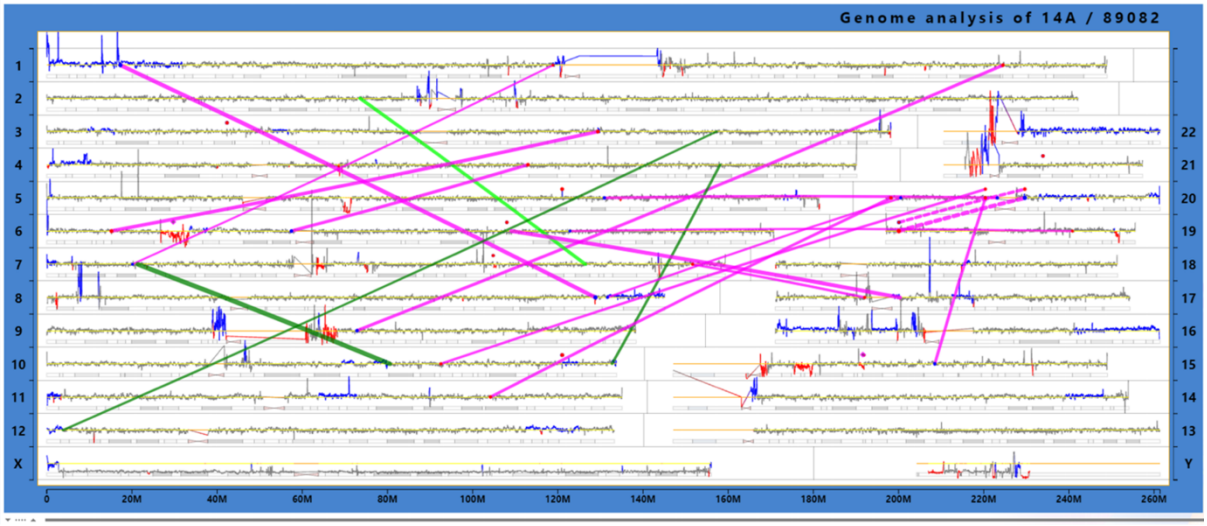
1.Relationship	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin ☐ Neighbour	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin ☐ Neighbour	☐ Great grandmother ☐ Great grandfather ☐ Grandmother ☐ Grandfather ☐ Father ☐ Mother ☐ Sister ☐ Brother ☐ Husband ☐ Daughter ☐ Son ☐ Auntie ☐ Uncle ☐ Cousin ☐ Neighbour

For official use only

Participant ID: Relative ID:

Cytogenetics Results

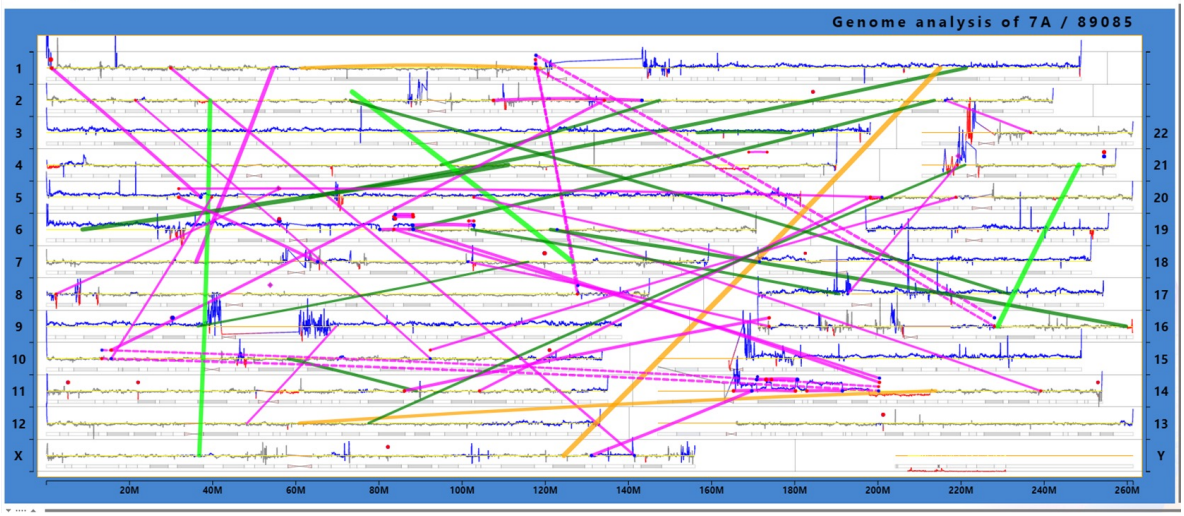
Patient 14



Patient 8

Patient 12

Patient 7



Patient 2