CLASS III SKELETAL BASE IN MALAY POPULATION: CHARACTERISATION OF SKELETAL PHENOTYPES AND ASSOCIATION WITH FAMILIAL INHERITANCE

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CLASS III SKELETAL BASE IN MALAY POPULATION: CHARACTERISATION OF SKELETAL PHENOTYPES AND ASSOCIATION WITH FAMILIAL INHERITANCE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AMDI	Advanced Medical and Dental Institute
BSI	British Standard Institute
CA	Cluster Analysis
COVID	Corona Virus Disease
ICC	Intra-class Correlation
JPG	Joint Photographic Experts Group
PCA	Principal Component Analysis
PC	Principal Components
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
USA	United States of America
USM	Universiti Sains Malaysia
WHO	World Health Organisation

ASAS RANGKA KELAS III DALAM POPULASI MELAYU: PENCIRIAN FENOTIP-FENOTIP RANGKA DAN PERKAITAN DENGAN PEWARISAN KELUARGA

ABSTRAK

Masalah rangka Kelas III berpunca daripada pertumbuhan maksila dan mandibel yang tidak seimbang. Ciri-ciri rangka dentofasial adalah amat berlainan di antara etnik dan asas rangka Kelas III yang sukar dirawat, amat lazim di Malaysia, berbanding dengan kaum lain. Dalam populasi Melayu, pencirian fenotip rangka kelas III tidak pernah disiasat. Oleh itu, objektif am penyelidikan ini adalah untuk menyiasat dan mencirikan fenotip yang berbeza dalam populasi kaum Melayu dewasa yang mempunyai asas rangka Kelas III dan menentukan kaitannya dengan pewarisan keluarga. Kajian ini juga berusaha untuk mengetahui kadar prevalens asas rangka Kelas I, Kelas II, dan Kelas III dalam kalangan pesakit Melayu yang menghadiri klinik ortodontik Institut Perubatan dan Pergigian Termaju (IPPT), mencari prekursor untuk analisasi genetik dengan menggunakan kaedah statistik multivariate dan menyimpulkan perkadaran subjek asas rangka Kelas III yang mempunyai sejarah positif masalah yang sama dalam kalangan ahli keluarga. Kaedah penyelidikan ini merupakan kajian rentas keratan retrospektif dan dibahagikan kepada dua fasa. Dalam fasa pertama, prevalens asas rangka Kelas I, Kelas II, dan Kelas III diperolehi melalui analisasi 2182 radiograf cephalometrik pesakit Melayu yang menghadiri klinik orthodontic di IPPT. Dalam fasa kedua, analisasi kelompok digunakan pada 62 pembolehubah sefalometri untuk mendapatkan fenotip asas rangka Kelas III bagi 127 pesakit etnik Melayu. Fenotip yang diperolehi telah dicirikan dan dikaitkan dengan pewarisan keluarga menggunakan Ujian Pearson Chi Square. Ujian Principal

Component Analysis (PCA) juga dilaksanakan untuk mendapatkan pembolehubah sefalometrik yang paling penting dalam asas rangka Kelas III. 41.3%, 46.1%, dan 12.6% pesakit Melayu yang mengunjungi klinik ortodontik IPPT mempunyai asas rangka kelas I, II, dan III. Kajian ini mendapati perbezaan yang signifikan antara jantina tetapi tanpa perbezaaan antara kumpulan umur. Empat fenotip asas rangka Kelas III telah dikenalpasti dalam populasi Melayu tiga generasi. Kelompok-kelompok tersebut mempunyai ciri-ciri tertentu, dari segi saiz maksila dan mandibel, corak pertumbuhan, kepanjangan dan ketinggian rangka, serta kecenderungan gigi kacip. PCA daripada 62 pemboleubah sefalometrik mendapati lima komponen utama yang dapat menjelaskan 82.9% varians dalam data. Pembolehubah sefalometrik yang paling penting adalah ukuran menegak dan sagital, serta angulasi gigi kacip mandibel. Kebanyakan penemuan penyelidikan ini bersetuju dengan kajian terdahulu dan boleh mewakili fenotip dalam populasi Melayu dewasa. 21.3% subjek yang memiliki maloklusi asas rangka Kelas III didapati mempunyai sejarah positif masalah yang sama dalam kalangan ahli keluarga tetapi tidak terdapat perbezaan yang signifikan dalam taburan kelompok fenotip. Penyelidikan ini mengesahkan kewujudan pelbagai fenotip asas rangka Kelas III dalam populasi Malyu, dan mengaitkan asas rangka Kelas III kepada sejarah keluarga.

CLASS III SKELETAL BASE IN MALAY POPULATION: CHARACTERISATION OF SKELETAL PHENOTYPES AND ASSOCIATION WITH FAMILIAL INHERITANCE

ABSTRACT

Class III skeletal discrepancy results from the disproportionate growth of the maxilla and mandible. Dentofacial skeletal features vary significantly between different ethnicities and Class III malocclusions, which are difficult to treat, were highly prevalent in Malaysians compared to other races. In the Malay population, the skeletal phenotypes of Class III skeletal base have never been investigated. Therefore, the general objective of this study was to investigate and characterise the different Class III skeletal phenotypes in a racially homogenous adult Malay population and determine their association with familial inheritance. This study also sought to determine the prevalence of Class I, Class II and Class III skeletal base among Malay patients attending the Advanced Medical and Dental Institute (AMDI) orthodontic clinic, determine the precursor for genetic analysis using multivariate reduction method and ascertain the proportion of familial occurrence of Class III skeletal base. This was a retrospective cross-sectional observational study containing two phases. In this first phase, the prevalence of Class I, Class II, and Class III skeletal base were obtained by categorizing 2182 lateral cephalometric radiographs of Malay patients attending the orthodontic clinic in AMDI. In the second phase, Class III skeletal phenotypes of 127 Malay ethnicity patients were obtained using cluster analysis of 62 cephalometric variables. The obtained phenotypes were characterized and had their association with familial inheritance determined using Pearson's Chi-Square Test. Principal Component Analysis (PCA) was done to obtain the most highly correlated cephalometric variables in the Class III skeletal base. The proportion of Class I, Class II, and Class III skeletal base was 41.3%, 46.1% and 12.6% respectively with significant differences between the gender but not age groups. Four skeletal class III phenotypes were obtained in the homogenous Malay population. The clusters had certain characteristics, corresponding to the size of the maxilla and mandible, growth patterns, skeletal length and height, and incisor inclination. PCA of 62 cephalometric variables in the subjects found five principal components which covered 82.9% of the variance explained. The most heavily weighted cephalometric variables were vertical and sagittal measurements, as well as lower incisor angulation. 21.3% of subjects with Class III skeletal base had a positive history of family occurrence but there was no significant difference in the distribution within the phenotypes' clusters. This study confirmed the existence of variable skeletal class III phenotypes in the Malay population and linked Class III skeletal base to family history.

CHAPTER 1

INTRODUCTION

1.1 Background of Study: Class III Skeletal Discrepancy and Genetics

Classically, the Class III skeletal base describes the anteroposterior relationship between the maxilla and mandible. It occurs when there is an overgrowth of the mandible with or without undergrowth of the maxilla, which makes the mandible looks more prominent than the maxilla and results in a concave facial profile and reverse overjet in the anterior teeth. There are three conditions in which this could happen. First, a hypoplastic/retrognathic maxilla with a normal mandible. Second, a normal maxilla with a prognathic mandible, and third, a hypoplastic maxilla together with a prognathic mandible can present as Class III skeletal discrepancy.

Class III skeletal discrepancy is caused by factors like genetic inheritance, ethnic aggregation, and environmental influence. Genetic components play an important role in its aetiology. Genotype refers to the individual's collection of genes and phenotype is the characteristics or traits seen in the individual due to these particular genes. In addition, there can be specific genotypes that cause Class III skeletal discrepancy. Previous genetic research done in Class III skeletal base attempted to identify and shed light on the link of genetics with the occurrence of the prognathic mandible. However, since Class III skeletal discrepancy is a complex disorder, multidimensional, and has poorly defined subtypes, one of the limitations of previous genetic works was the lack of phenotypes definition cannot express the complexity of the gene involved in the disorder (Moreno Uribe *et al.*, 2014). Therefore, to identify the genes involved, researchers should first identify and characterise in detail the phenotypes of Class III skeletal discrepancy to establish distinct subtypes or subclasses among the broad manifestation of the disorder, which can be linked to the expression of the genotype.

Then, with abundant demographic, familial history, and proper phenotypes data at hand, further genetic research can eventually identify the aetiology or genotype that was prone to Class III skeletal base.

Other than that, it is well-known that skeletal features vary between different ethnicities (Alam *et al.*, 2016a). Cases of malocclusions especially Class III are highly prevalent in oriental and southeast Asians, including Malaysians, compared to other races or regions (Alhammadi *et al.*, 2018; Hardy *et al.*, 2012; Zere *et al.*, 2018). The integration of environmental factors and factors like race can influence the manifestation of phenotypes in the Class III skeletal base. Therefore, phenotype characterisation done only in specific races and populations can reduce genetic heterogeneity and obtain more targeted results (Li *et al.*, 2016). Furthermore, the convergence of phenotypes in such focused studies can accurately represent the genotype in that particular race and is generalisable to the population of the same race.

The foundation of this study was based on previous studies which had successfully characterised Class III skeletal base phenotypes in other races based on shape analysis and cephalometric analysis (Bui *et al.*, 2006; de Frutos-Valle *et al.*, 2020; Li *et al.*, 2016; Mackay *et al.*, 1992; Moreno Uribe *et al.*, 2014). However, studies of malocclusion on specific populations or races in the country can better serve their purpose to establish a norm or baseline data for more accurate future genetic research and community-targeted treatment planning and resource allocation.

1.2 Problem Statement

Many previous epidemiological studies in Malaysia used Angle's classification of malocclusion to determine the prevalence of malocclusion in the Malay population (Elfseyie *et al.*, 2020; Mohd Azlan Sunil and Dhanraj, 2019; Mulimani *et al.*, 2017).

There was evidence that only approximately 60% correlation exists between the anteroposterior relationship between the dental arches (using Angle's classification of malocclusion) and the skeletal base pattern (using angular measurement ANB on lateral cephalogram) (Al-Jabaa and Aldrees, 2014; Zhou *et al.*, 2008). In other words, a person presenting with Class I malocclusion based on Angle's Classification may have a Class II or Class III skeletal base relationship and vice versa. Sometimes the dental and soft tissue positions can mask underlying skeletal discrepancies. The results were therefore not comparable and did not represent the prevalence of true Class III skeletal base in the Malay population.

Fortunately, there were few studies on the cephalometric norm in the Malay and Chinese populations in the eastern and central region of Malaysia and in these studies the prevalence of skeletal base was determined using cephalometry, which was similar to the present study (Al-Jaf *et al.*, 2020; Alam *et al.*, 2016b; Ahmad Razin *et al.*, 2019). There is a need to determine the proportion of Class I, Class II, and Class III skeletal base in the Malay population in the northern region of Malaysia to complete the comparison.

The underlying genetic mechanism of Class III has also never been investigated in the Malay population. However, before such genome-wide linkage studies can be carried out, a previous study recommended that a skeletal phenotype characterisation be identified to provide precursor information for future genetic studies (Bui *et al.*, 2006). In other words, the establishment of Class III skeletal phenotypes in a specific population can serve as a good bedrock for future genotype studies and increase the strength of future genetic studies.

Although multiple previous studies established the phenotypes of Class III skeletal base and described the characterisation, one of the most important research questions was formed due to the generalisability of previous research to other races or populations. It was confirmed that craniofacial morphology differed significantly in different ethnicities and nationalities should be considered when dentofacial research was being carried out (Loster *et al.*, 2015). Therefore, there were gaps and limitations in this research area because no previous studies had investigated the phenotypes in Class III skeletal discrepancy among Southeast Asian or Malay populations and whether they are influenced by familial inheritance, which was important because this population was repeatedly reported to have one of the highest prevalence of Class III malocclusion.

1.3 Objectives

1.3.1 General Objective

To investigate the phenotypes of Class III skeletal base in the Malay population and its association with familial inheritance.

1.3.2 Specific Objectives

1) To determine the prevalence of Class I, Class II, and Class III skeletal base amongst Malay patients attending the Advanced Medical and Dental Institute (AMDI) orthodontic clinic and its association with gender and age group.

2) To investigate and characterise the skeletal phenotypes of Class III skeletal base in Malay patients using digital cephalometric analyses.

3) To determine the precursors for genetic analysis using principal component analysis (PCA).

4) To determine the proportion and association between Class III skeletal phenotypes and familial occurrence.

1.4 Research Questions

a) What is the prevalence of Class I, Class II, and Class III skeletal base among Malay patients attending AMDI orthodontic clinic?

b) How many clusters exist and what are the characteristics of different phenotypes of Class III skeletal base in Malay population?

c) What are the most significant cephalometric variables in representing Class III skeletal base using Principal Component Analysis?

d) What is the proportion of familial occurrence in patients with Class III skeletal base?

1.5 Research Hypotheses

a) There is no significant association between the prevalence of Class I, Class II, and Class III skeletal base to gender.

b) There is no significant association between the prevalence of Class I, Class II, and Class III skeletal base to age group.

c) There is no significant association between Class III skeletal phenotypes with familial occurrence.

1.6 Justification of the study

Skeletal Class III development emerges gradually at a young age and accelerates during puberty. Cases of Class III skeletal discrepancy may worsen with age if left untreated. Therefore, early detection and treatment of developing Class III malocclusion in children and adolescents are important, as timely interception might prevent further damage to oral tissues and reduce the severity of future orthodontic treatment (Zere *et* *al.*, 2018). Besides, treatment of Class III skeletal base is also often more costly, complex and difficult, involving a combination of orthognathic surgery and orthodontic treatment (Stellzig-Eisenhauer *et al.*, 2002). Hence, the effort to identify children and adolescents who have developing Class III tendencies and remain interested in their condition can be beneficial to the patients in the long term and helpful to the department's treatment planning. Research to investigate the reasons behind possible genetic predisposition and family study of Class III skeletal base is vital for the region in which the majority population was reported to have a higher prevalence rate of the problem than other countries.

1.7 Significance of the study

This study investigated and characterised the different phenotypes in the Class III skeletal base in racially homogenous adult Malay populations using the multivariate reduction method. Replication studies using a similar method in a different population with more careful selection criteria are indispensable to determine the rationality of this method for phenotypic characterisation by evaluating if the findings could be at least partially reproduced.

This study also provided useful additions to the variation of established Class III skeletal phenotypes that were generalisable to the Malay population and can be useful clinically or in future genetic studies. Therefore, this study added to the literature and strengthened the knowledge of the existence of different subclasses of Class III skeletal base. Furthermore, the organization of Class III malocclusion into subtypes based on their morphological features can also assist clinicians in diagnosis, communication and decision-making in treatment planning.

This study is also the first research investigating the existence of different phenotypes in the Malay population and whether they are influenced by familial inheritance. The greatest strength of this study was an additional step in obtaining family history data from the participants to establish the link of Class III phenotypes to familial inheritance, which was proposed but not done before in previous studies.

The results from this study, in which there exists a high prevalence of skeletal base discrepancy problems in the Malay population compared to other regions of the world, especially of Class III skeletal base, can also act as the baseline data to raise the concern of the country's major stakeholders to tackle the problems by increasing orthodontic speciality training and facilities and promoting education of the public.

CHAPTER 2

LITERATURE REVIEW

2.1 Malocclusion

Occlusion is the way the maxillary and mandibular teeth contact when they are closed or in function. According to a widely cited paper, the ideal occlusion contains six keys which are: (1) correct molar relationship, (2) correct crown angulation, (3) correct crown inclination, (4) no rotations, (5) no spaces, and (6) flat occlusal planes (Andrews, 1972). Any appreciable deviations from the ideal occlusion constitute malocclusion.

Malocclusion is one of the most common dental disorders in the oral cavity, alongside dental caries and periodontal diseases. A person can suffer from a malocclusion originating from either dental or skeletal aetiology or both. They are closely interrelated yet different entities in aetiology, treatment planning, and prognosis. For example, Class III skeletal base often requires orthognathic surgery, while Class III dental malocclusion can often be treated with fixed appliance therapy (Mageet, 2016).

Well-proportionate upper and lower jaws demonstrate a normal and harmonious skeletal relationship known as a Class I skeletal base. Skeletal base discrepancy happens when the maxilla and the mandible grow disproportionately, resulting in a larger-than-normal discrepancy (Jaradat, 2018). When the maxilla is relatively hyperplastic or the mandible is relatively hypoplastic, it is classified as a Class II skeletal base. When the maxilla is relatively hypoplastic and the mandible is relatively hyperplastic, it is classified as a Class III skeletal base (Figure 2.1).

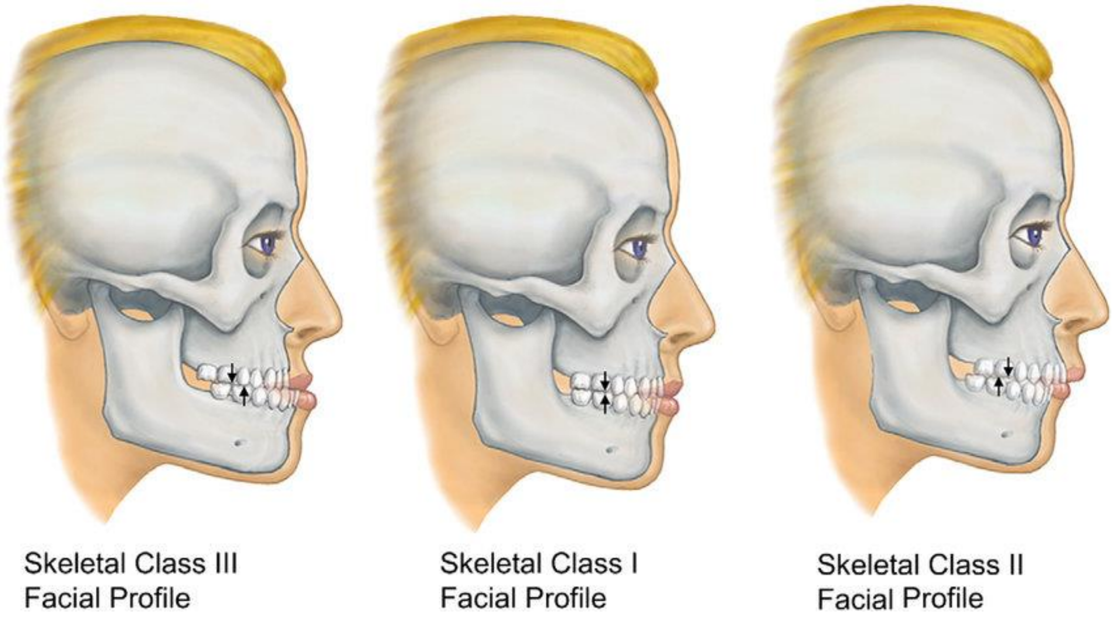

Figure 2.1 An illustration of Class I, Class II, and Class III Skeletal Base (Morcos, S.S. and Patel, P.K., 2007. The Vocabulary of Dentofacial Deformities. *Clinics in Plastic Surgery*, 34(3), Figure 13, Page 598)

On the other hand, dental malocclusion depends on the relationship between the teeth in the maxillary and mandibular jaws, which can happen due to many local factors (Petrović *et al.*, 2013). Therefore, one of the most used dental malocclusion classifications is Angle's molar relationship classification. Angle's Class I is defined as the mesiobuccal cusp of the maxillary first molar occluding in line with the buccal groove of the mandibular first molar. Class II is when the mesiobuccal cusp of the maxillary first molar is occluding anterior to the buccal groove of the mandibular first molar, and Class III is when the mesiobuccal cusp of the maxillary first molar is occluding posterior with the buccal groove of the mandibular first molar (Figure 2.2).

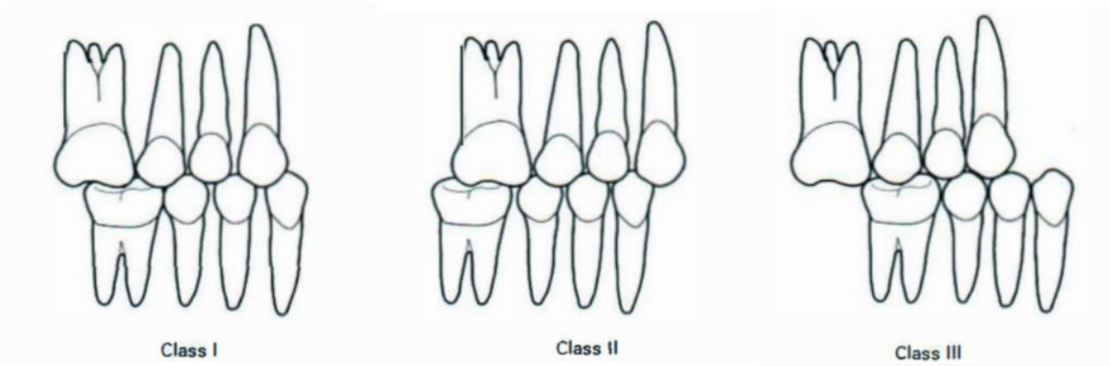

Figure 2.2 Angle's Classification of Malocclusion according to First Molar Relationship (Mitchell, L., 2007. The aetiology and classification of malocclusion. *An Introduction To Orthodontics,* Third Edition. Oxford University Press Inc, New York, Figure 2.1, Page 9)

Another popular classification of dental malocclusion would be The British Standard Institute (BSI) Incisor Classification (1983). In this classification, Class I is defined by the mandibular incisor edges lie or are immediately below the cingulum of the maxillary incisors, Class II is when mandibular incisor edges lie more posteriorly, and Class III is when mandibular incisor edges lie more anteriorly to the cingulum of maxillary central incisors (Figure 2.3). Apart from that, the canine relationship is classified as Class I when the tip of the maxillary canine lies on the embrasure between the mandibular premolar and mandibular canine, Class II when the tip of the maxillary canine lies anteriorly, and Class III when it lies posteriorly.

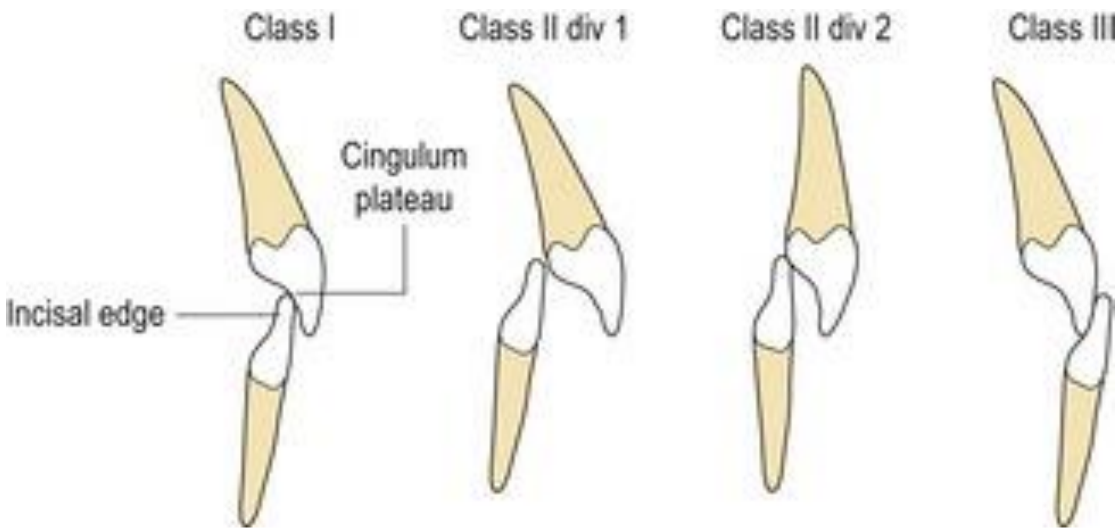

Figure 2.3 British Standards Institute Incisor Classification (Mrzezo 2015, Occlusion and malocclusion, blog, viewed 31 March 2022, <https://pocketdentistry.com/occlusion-and-malocclusion/>. Figure 1.3)

2.1.1 Effect of Class III Malocclusion on Health and Function

It was well-established that malocclusion, especially if severe, can be associated with other medical problems (Joshi, 2014) and affect the quality of life (Masood *et al.*, 2013). In addition, Class III malocclusion can adversely affect speech (Lathrop-Marshall *et al.*, 2021) and masticatory efficacy (Picinato-Pirola *et al.*, 2012). These functional problems may subsequently result in digestive disorders and compromised nutritional status. Nevertheless, the most critical concerns for the patients are still their self-image and confidence in their appearance, which often become the main reason and motivation for patients with an unpleasant, concave Class III facial profile to seek orthodontic or even orthognathic surgical treatment.

2.1.2 Management of Class III Malocclusion

The treatment of Class III malocclusion requires accurate and critical diagnosis and planning. Beyond the incisors, canines and molar relationship, Class III malocclusion is often complicated by underlying Class III skeletal base. Many factors should be considered while doing treatment planning for Class III malocclusions, such as the severity of the skeletal pattern, potential of future growth, timing of treatment, presence of functional displacement, amount of overbite and dentoalveolar compensation present.

Depending on the presentation and age of the patient, various treatment modalities can be considered. Children in early mixed dentition presenting with simple anterior crossbite or reverse overjet with functional shift can be treated effectively with removable or fixed appliances known to some as 2 by 4 or 2 by 6 (Wiedel and Bondemark, 2014). Chin cup therapy and reverse-pull headgear/facemask are used depending on the type of skeletal discrepancy present, such as retrognathic maxilla or prognathic mandible. The success rate of reverse-pull headgear was reported to decrease after age 10 (Wells *et al.*, 2006), indicating the significance of early detection and treatment of Class III skeletal discrepancy. Functional appliances have also been used to treat Class III malocclusion with varying success as they are reported to contribute mainly to the dentoalveolar effect (Zere *et al.*, 2018).

Meanwhile, for adult patients, camouflage treatment by fixed appliances is the usual treatment of choice for mild to moderate Class III malocclusion. This can be done in combination with orthognathic surgery for fully grown patients with more severe skeletal discrepancy (Mitchell, 2007). It has been suggested that patients with ANB value $< -4^{o}$ and lower incisor to mandibular plane angle $< 83^{o}$ will almost certainly require surgical correction and this can be used as a guideline in treatment planning decisions (Kerr *et al.*, 1992).

2.2 Worldwide Prevalence of Class I, Class II, and Class III Malocclusion

A systematic review of the worldwide prevalence of malocclusion reported that approximately 74.7% of the population was Class I (range: 31%-97%), 19.56% was Class II (range: 2%-63%), and 5.93% was Class III (range: 1%-20%), the large differences in percentage was most likely due to different sampling method of the studies (Alhammadi *et al.*, 2018). In another systematic review with a meta-analysis of the prevalence of Class III malocclusion globally, the prevalence ranged from 0% to 26.7% and was at an average of 7.04% (Hardy *et al.*, 2012). It was reported in that review that a higher prevalence was found in China and Malaysia compared to other racial groups (Hardy *et al.*, 2012). The author concluded that populations with notably high prevalence require more attention from policymakers and clinicians to address their issues. However, all these studies used Angle's classification of malocclusion to classify occlusion, which means that the prevalence represented more of the dental origin than the underlying skeletal base discrepancy.

On the other hand, some other studies used cephalometric measurements, including ANB angle, to determine the prevalence of skeletal bases in their populations (Aldrees, 2012; Almasri, 2014; Boeck *et al.*, 2011). One of the studies found the prevalence of different skeletal bases to be Class I: 51.7%, Class II: 40.2%, and Class III: 8.1%, with no significant difference between gender. They concluded that most countries and prevalence studies agreed with this result (Aldrees, 2012).

2.2.1 Prevalence of Class I, Class II, and Class III Malocclusion in Malaysia

A few epidemiological studies done in Malaysia determined the prevalence of types of occlusion of all three ethnicities, and also specifically in the Malay population in Shah Alam, the central region of Malaysia. Based on the literature reviews, in the Malay population, the prevalence of Class I occlusion was reported to be in the range of 33.3% to 39.3%, Class II: 12% to 30%, Class III: 12.2% to 48.7% (Elfseyie *et al.*, 2020; Mohd Azlan Sunil and Dhanraj, 2019; Mulimani *et al.*, 2017). The large variation between different studies was most likely due to different sampling methods. For example, the study conducted by Mulimani et al. (2017) was done only on orthodontic patients of the clinic. Hence, there was a higher proportion of Class II and Class III malocclusion, as they were more likely to seek orthodontic treatment than patients with Class I malocclusion, which was considered normal. Besides that, Elseyie et al. (2020) reported that a significant difference in the occlusal status was found between the gender in the Malay population, in which Class III malocclusion was more prevalent in the male than the female group of their study. One previous study found a significant association between gender and age towards the types of malocclusion (Ismail *et al.*, 2017) in the general Malaysian population, dominated by Malay ethnicity. However, it is essential to note that the mentioned studies used Angle's Classification of Malocclusion or British Standard Institute Incisor Classification to determine the proportion of malocclusion, so it was a different categorization method and type of malocclusion reported when compared to this study.

However, few studies found in the literature were comparable to this study as ANB angle in the lateral cephalometric radiographs was used to classify skeletal base in Malaysian's Malay population. The prevalence of Class I skeletal base was reported to be 32.3-41.87%, Class II: 31.3-33.74%, and Class III: 24.39-36.4% respectively (Al-Jaf *et al.*, 2020; Alam *et al.*, 2016b).

Nonetheless, it was noted that many previous studies reported the Malay population was one of the ethnic groups to have a higher prevalence of Class III malocclusion compared to other races (Hardy *et al.*, 2012; Mohd Azlan Sunil and Dhanraj, 2019; Mulimani *et al.*, 2017; Zere *et al.*, 2018).

2.3 The Malay Population

Malaysia is a unique country in which three major ethnicities consisting of Malay, Chinese, and Indian, make up the population of the country (Noor and Leong, 2013). With globalisation and cross-cultural marriage becoming more common, the concept of pure racial genetics is diminishing. Nevertheless, for this study, the Malay population has to be defined. As discussed earlier, phenotypic characterisation in a specific race facilitates subsequent genetic research as noise and genetic heterogeneity are reduced.

Malay is an ethnic group native to Southeast Asia and is spread mainly in Malaysia, Indonesia, Borneo, Singapore, and some parts of Thailand (Figure 2.4). They are concentrated in Malaysia and Indonesia, making up the majority of the population in the world. Today, the word "Malay" has been largely extended to other ethnic groups and includes descendants of Acehnese, Buginese, Minangkabau and Javanese people that live within the Malay world (Milner, 2010). Based on Article 160(2) Federal Constitution of Malaysia 1957, "Malay" in Malaysia means a person who professes the religion of Islam, habitually speaks the Malay language and conforms to Malay custom. It is interesting to note that the ethnicity information can be obtained from the microchip-embedded MyKad (National Identification Card) carried by all Malaysian citizens by using appropriate card-readers. This is considered the official documentation of a person's ethnic group by the National Registration Department, as defined by the Constitution of Malaysia (Nagaraj *et al.*, 2009). Furthermore, there should be no existence of any inter racial marriage with other races or people from another region other than with Malay in the Malay World within the generation of their parents and grandparents for them to be considered as unmixed or true Malay ancestry (Mohammad *et al.*, 2011).

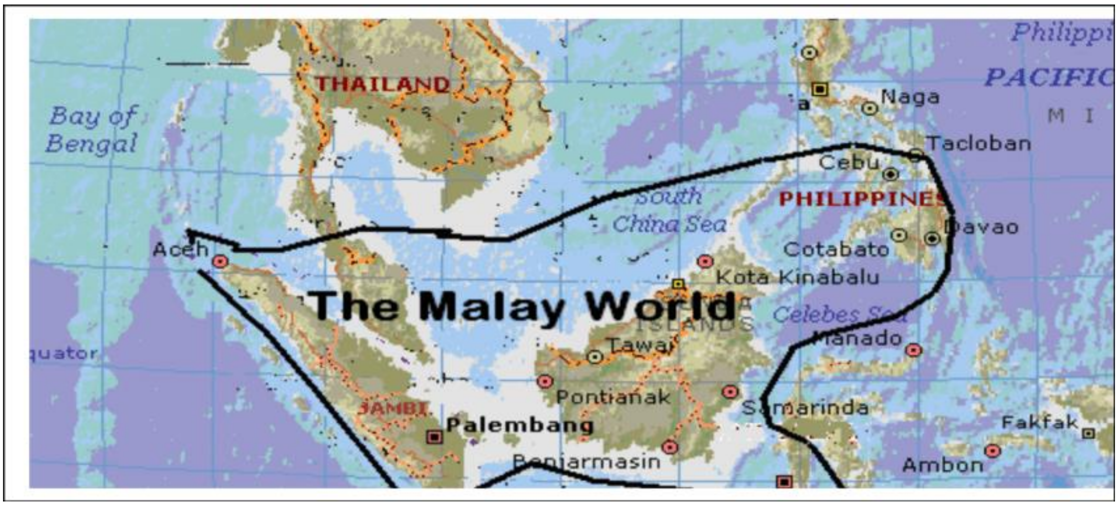

Figure 2.4 The Malay World (Omar Din, M.A. and Mohamad, M., 2016. Nusantara and the Malay World: The Southeast Asian Indigenous Maritime Borders. *Proceeding of Workshop on High Impact Journal Writing and Publishing 2016,* ISBN 978-967-0899-42-8, Figure 3, Page 106)

2.4 Lateral Cephalometric Radiograph

A lateral cephalometric radiograph is a standardized, reproducible radiograph that is done to capture the skull from the sagittal plane. Cephalometric radiographs provide an abundance of information on the morphological features of the skeletal base underneath the face, and it is primarily used by orthodontists in diagnosis and treatment planning. Using cephalometry radiographs for Class III skeletal base phenotype research has several advantages because they are relatively cheap, quick, and convenient to capture. It is also easily stored and retrieved when it has been digitized. Most orthodontic centres would have most of their patients' cephalometric radiographs taken before the treatment and the files stored in the system. This makes conducting research and studies based on lateral cephalograms convenient and accessible. In addition, a moderate to large sample size can also be obtained easily.

Besides that, cephalograms have been the mainstay of orthodontic practice for almost a century since Broadbent used them in the 1920s (Hans *et al.*, 2015). Then over the next few decades, numerous orthodontists described and published their analysis of the skull and craniofacial skeleton based on cephalograms, which became the cephalometric analyses that are now widely used. Some of the more popular cephalometric analyses are Steiner analysis, Downs analysis, Tweed analysis, Jarabak analysis, Ricketts analysis and McNamara analysis. These analyses use almost the same landmarks on the lateral cephalometric radiographs of the skull (with minor differences in some of the landmark definitions) to derive different measurements and angles that describe the skull's morphology. There may be overlap measurements in some of the analyses, but most are well established and developed for different purposes, primarily for research, skeletal or soft tissue clinical analysis (Kula and Ghoneima, 2018).

Using the measurement values obtained in these analyses, one can describe the morphological features, dimensions, and relationships between different craniofacial structures. Hence, the values obtained from the analysis represent the skull's morphology from the sagittal plane in a two-dimensional view. Furthermore, the convergence of these values obtained from the Class III skeletal base subjects into separate groups represents the different morphological features in different groups. These differences in morphological features between Class III skeletal base subjects and their characterisation form the basis of phenotype classification in this study. Therefore, with its numerous advantages, cephalometric analysis was still the best way to investigate phenotypes in the Class III skeletal base. Nevertheless, as technology advances, three-dimensional morphological capture of the skull and advanced analysis methods will surely gain traction and become more important in the future.

2.5 Association between Class III Skeletal Base and Genetics

The presence of mandibular prognathism in portraits of European Royalty and their descendants known as the Hapsburg Jaw had caught interest for decades and suggested the role of genetics in Class III skeletal base (Xue *et al.*, 2010).

As a result, genetic research was done but the evidence of inheritance patterns varies. The inheritance patterns range from autosomal dominant in Brazilian, Chinese, and Mediterranean populations (Cruz *et al.*, 2008; Genno *et al.*, 2019; Guan *et al.*, 2015) to dominant inheritance with incomplete penetrance in Chinese Han and Libyan populations (El-Gheriani *et al.*, 2003; Li *et al.*, 2010), or a polygenic threshold model in Korean population (Ko *et al.*, 2013), and autosomal-recessive inheritance could not be rejected as well (El-Gheriani *et al.*, 2003). This ethnic variation in genetic findings indicates that the causative gene of Class III skeletal base may be caused by a variety of genes not unique to any population and may differ in different races.

2.6 Cluster Analysis (CA) and Principal Component Analysis (PCA)

Previous studies had used multivariate methods like cluster analysis (CA) and principal component analysis (PCA) to ascertain specific phenotypes for Class III skeletal base. Multiple cephalometric variables or their derivations obtained from the cephalograms of Class III skeletal base subjects can undergo multivariate statistical analysis to translate into fewer but more important components and allow researchers to understand better how they relate to each other.

Principal component analysis is a powerful statistical method that reduces complex multivariate data containing many components and organises them into fewer components on a best-suited linear axis model without much data loss. When PCA was used on the cephalometric variables of the Class III skeletal base subjects, it can find the most highly correlated cephalometric variables in the dataset that could explain the maximum amount of variance in the subjects. PCA reduces the dimension of multiple variables into fewer variables (components) and ranks them in order of importance that contributes to the data.

Cluster analysis is another statistical method that can group the sets of data so that subjects in the same group are more homogenous than those in the other groups/clusters. For example, when cluster analysis was done on the cephalometric values of Class III skeletal base subjects, one can find subjects of specific morphological features grouped into separate clusters and hence form a subclass or phenotypes that was distinctly different from the other groups. These homogenous groups can represent a phenotype with reduced genetic heterogeneity, and similar methods have been used to identify subtypes of other diseases (Arif *et al.*, 2014; Deliu *et al.*, 2016). Once the phenotypes are obtained, the clusters can be tested with familial cases to identify any significant difference in the distribution or if there were any convergence of familial inheritance in specific phenotypes. For example, suppose there was a high concentration of cases of positive family history for mandibular prognathism in specific phenotypes or clusters. In that case, it will prove invaluable to the future genetic and etiologic understanding of the diseases by forming the basis for genetic linkage studies.

2.6.1 Phenotypic Characterisation of Class III Skeletal Base

Mackay et al. (1992) were among the earliest to identify Class III skeletal base phenotypes. However, they employed the centroid method instead of cephalometric values to perform cluster analysis. They identified five clusters of Class III phenotypes in 50 adult subjects from the English population who needed surgical correction. From these five clusters: (1) 14% exhibited a combination of short, retrognathic maxilla, slightly prognathic mandible and retroclined lower incisors, (2) 42% exhibited a combination of normal maxilla with a slightly prognathic mandible, reduced maxillo-mandibular plane angle and decreased lower face height percentage, (3) 4% exhibited a combination of normal maxilla with very prognathic mandible, and markedly retroclined lower incisors, (4) 22% exhibited a combination of normal maxilla with a slightly prognathic mandible, average maxillo-mandibular plane angle and increased lower face height percentage and larger gonion-menton length, and (5) 18% exhibited normal maxilla with a moderately prognathic mandible, moderately increased gonion-menton length and markedly proclined upper incisors. However, as the sample population were only those who required surgical correction, milder phenotypes manifestation of Class III skeletal base may be missed by the study.

Hong and Yi (2001) identified seven clusters of Class III phenotypes in 106 subjects of the Korean population who were referred to a clinic for surgical-orthodontic correction. They analysed the cephalograms using modified Delaire’s analysis for Korean and the measurements obtained were put through cluster analysis for grouping. Seven clusters were identified. They reported that Class III skeletal phenotypes were not only influenced by the facial bones and dentition but also involved the cranial base, cranial vault, and cervical spine. The posterior part of the cranial vault and craniocervical junction is closely related to the direction of mandibular rotation. This population difference detected in the phenotypes is believed to have occurred because of different causative genes in each population. Although this study used a different analysis that included other features in the cephalometric radiographs aside from craniofacial structures, they also confirmed the existence of subtypes among subjects of Class III skeletal base.

Abu Alhaija and Richardson (2003) detected three clusters of Class III phenotypes using hierarchical cluster analysis which was the same as this study in 115 subjects of the Caucasian population referred to the orthodontic department. Their results correspond to severe horizontal discrepancy cases, an intermediate group and long face types. In the first cluster described as horizontal discrepancy, the mandible length was increased, and the Y-axis angle reduced, indicating a horizontal growth tendency. In the third cluster described as long face, the maxillary/mandibular plane angle and Y-axis angle were both increased, and the total facial height was markedly increased, indicating a long face type. Cluster two was described as an intermediate between clusters one and three. However, the study did not provide stringent sample selection criteria, so there may be a mixture of syndromic or post-trauma subjects in the sample, which reduced genetic homogeneity.

A study with a larger sample was done in North Carolina by Bui et al. (2006) They revealed 5 clusters in a multi-racial sample of 356 subjects who attended the orthodontic clinic and were clinically diagnosed as skeletal Class III. Using normalised cephalometric values in cluster analysis, they reported the 5 clusters as (1) Mandibular prognathic, long face, (2) Maxillary deficient, low angle, (3) Maxillary deficient, high angle, (4) Mildly mandibular prognathic, normal, (5) Combination, normal. However, this study involved multiple populations in different proportions and also included children as young as six years old, where the phenotypes may not be fully expressed yet. Therefore, there was uncertainty within the results regarding generalisability and the homogeneity of the sample population.

Another study in Iowa which involved 292 adult Caucasians had clearly defined inclusion and exclusion criteria. Their sample was more homogenous and could represent the true Class III skeletal base. They also reported five distinct subphenotypes for Class III skeletal base, which consisted of (1) borderline Class III with mild maxillary retrognathism and mandibular prognathism, flat mandibular plane, and retroclined lower incisors, (2) borderline Class III with moderate maxillary retrognathism and mild mandibular prognathism, normal mandibular plane, normal lower incisors, (3) Vertical Class III with an increased anterior facial height, and proclined lower incisors, (4) severe mandibular prognathism and normal maxilla, with retroclined lower incisors, (5) normal mandible and severely retrognathic maxilla, and proclined lower incisors (Moreno Uribe *et al.*, 2014). Li et al. (2016) examined 144 Han Chinese individuals with the clinical diagnosis of Class III malocclusion and who were being treated orthodontically. Their work revealed four subtypes: (1) mild mandibular prognathism with a steep mandibular plane, (2) prognathic mandibular and retrusive maxilla with a flat or normal mandibular plane, (3) severe mandibular prognathism and normal mandibular plane, (4) mild retrognathic maxilla and severe mandibular prognathism with lowest mandibular plane angle. They also had rigorous sampling criteria regarding race and age and included cases from mild to severe Class III skeletal base. In these two studies, their method for obtaining different phenotypes was slightly different from this study as they used the first six principal components obtained from PCA as the basis of their cluster formation instead of using normalised cephalometric values.

The most recent study on Class III skeletal base phenotypes was done by de Frutos-Valle et al. (2020) in a population in southern Europe. They also had a careful selection of the sample population and retrieved six clusters: (1) the most prognathic mandible, increased anterior facial height, and enlarged posterior cranial base, (2) vertical component with an increased mandibular plane, low posterior facial height to anterior facial height proportion, and bimaxillary retrusion (3) slight class III skeletal base, reduced upper facial height and only slight mandible prognathism (4) severe class III malocclusion with maxillary hypoplasia, reduced anterior and posterior cranial base (5) increased maxillary and mandibular dimension (6) moderate class III malocclusion with a short face, reduced anterior facial height. In addition, the authors also found significant convergence of phenotypes in gender in clusters 1, 3 and 5. However, there was a slight difference in their statistical method from other studies as this paper had used ten main models from PCA to determine the clusters.

2.6.2 Principal Component Analysis of Cephalometric Variables

When PCA was done on cephalometric variables obtained from Class III skeletal base subjects, Bui et al. (2006) found that cephalometric variables representing the anteroposterior and vertical dimensions were cephalometric variables more significant than cephalometric measurements of particular craniofacial structures. The first five principal components explained 67% of the variation. Hence, it meant that the subtypes were differentiated by dimensions rather than the size of specific structures. They also found that commonly used clinical parameters such as overjet and Wits analysis were not as important as other variables in the first few components. The author concluded that these findings should drive researchers to challenge the use of conventional cephalometric variables to define Class III malocclusion.

Moreno Uribe et al. (2014) captured an even higher percentage of variance explained using the first five principal components (PC) derived from cephalometric analysis of multiple variables. There was an additional 7% at 74% compared to 67% in the previous study. The authors concluded that the difference was most likely due to the larger sample size, the more homogenous population in terms of race and age, and the more rigorous selection criteria for the sample. Despite the differences, they had almost similar results to PCA and duplicated of most significant cephalometric variables. The results strengthened the plausibility of using such phenotypic methods in providing phenotypes and contributing to future genetic studies.

Li et al. (2016) obtained only 68.7% of variance explained when the first five principal components were considered but increased to 73.6% when all six were included. They had similar findings: PC 1 and PC 2 contained mostly vertical and sagittal variables, and PC 3 had lower incisor parameters. One notable difference was that the ANB angle was not present in the first few important components in their study when compared to previous studies. They postulated that it was due to the inclusion of mild cases of Class III skeletal base. Hence the difference was observed.

On the other hand, de Frutos-Valle et al. (2020) captured 73.6% of variance explained with five principal components but extended their study to include ten components that explained 92.9%. The most highly correlated variables were also sagittal and vertical measurements, as they were ranked in the top three components. This study concurred with all previous studies that performed PCA on cephalometric measurements to find the most highly correlated variables, further confirming the importance of vertical dimensions.