CHAPTER 1

INTRODUCTION

1.1 Background

Noonan syndrome (NS; OMIM#163950) is a genetic disorder estimated between 1:1000—2.500 in worldwide population and mostly occurs on sporadic basis, due to *de novo* mutations, or inherited as autosomal dominant pattern [1].

The main characteristics of NS are distinctive facial feature, congenital heart defects, and short stature. The most striking facial features are in newborn and early-to-middle age, and then become less apparent in adulthood. The congenital heart defects are estimated between 50%-80% of NS patients. The most common defects are pulmonal valve stenosis, followed by hypertrophic obstructive cardiomyopathy, and other congenital heart defects. Short stature is more pronounce during prepuberty due to reduced or absent pubertal growth spurt and delayed puberty. However, growth catch up may present in late teen, thus some NS adult may have normal stature. Additional relatively frequent features are chest and skeletal deformity, cryptorchidism in males, delayed puberty, webbed neck, bleeding diathesis, and intellectual disability [1,2].

The molecular analysis to identify genes responsible for NS has evolved dramatically in the past decade. In 1994, linkage analysis of a large Dutch family with autosomal dominant NS was successfully identifying the location of NS gene on chromosome 12q [3]. Later, by sequencing method, *PTPN11* gene was identified as the first gene responsible for NS and accounts for approximately 50% of cases. *PTPN11* is located on 12q24.13 and encodes SHP-2 protein. The protein is involved in cell proliferation and differentiation, apoptosis and tissue development via RAS-MAPK signal transduction pathway [4]. Other

RAS-MAPK genes most commonly found in NS individuals without *PTPN11* mutation are *SOS1* (10%-15%), *RAF1* (5%-10%), and *KRAS* (2%). Small portion of NS cases have been reported to be mutated in *RIT1, NRAS,* and *BRAF* [2,5]. The involvement of *RRAS* and *A2ML1* is still unclear, whereas, the etiology of NS in individual with negative mutation in known genes is still remain to be identified [6–8].

Despite the phenotypic and genotypic heterogeneity, diagnosis of NS depends on clinical features, by observation of its cardinal signs. Several scoring systems have been develop to aid the diagnosis and the most recent and commonly used was develop in 1994 by Van der Burgt et al. Subsequently, NS clinical management guideline has been developed to promote proper diagnosis and management [1,2].

The differential diagnosis of NS includes syndromes related to dysregulation of RAS-MAPK pathway and Turner syndrome (TS), a well known sex chromosomal abnormality in females [1,2]. Indonesian population was estimated almost 250 million people in 2013 [9]. Therefore, between 100.000-250.000 NS cases should be present in Indonesia.

Automated ion semiconductor sequencing (ISS) was performed in order to screen the presence of mutation in four most common NS genes (*PTPN11, SOS1, RAF1,* and *KRAS*) in Indonesian patients suspected of having Turner syndrome phenotype with normal karyotype. As the first post-light sequencing, the process to determining the nucleotide order within human DNA using ISS become less time consuming and cost-effective [10].

1.2 Research Questions

What mutations can be found in *PTPN11, SOS1, RAF1,* and *KRAS* gene of Indonesian Turner phenotype patients with normal karyotype?

1.3 Research Objectives

1.3.1 General Research Objective

To identify and analyse genetic mutations in *PTPN11, SOS1, RAF1,* and *KRAS* of Indonesian Turner phenotype patients with normal karyotype.

1.3.2 Specific Research Objectives

1. To identify the presence of *PTPN11, SOS1, RAF1,* and *KRAS* mutations of Indonesian Turner phenotype patients with normal karyotype.
2. To analyse the potential pathogenic effects of amino acid changes in *PTPN11, SOS1, RAF1,* and *KRAS* genes of Indonesian Turner phenotype patients with normal karyotype**.**

1.4 Research Benefits

1. Knowing the causative mutation can confirm diagnosis and provide better management including genetic counselling.
2. Improving Indonesian public awareness of NS.
3. Encouragement of other studies of NS in Indonesian population.

1.5 Research Originality

NS studies have been done in different populations (Table 1). However, this is the first study to identify and analyses *PTPN11, SOS1, RAF1,* and *KRAS* mutations of Turner phenotype patients with normal karyotype in Indonesia by ion semiconductor sequencing.

Table 1. List of previous associated studies

No	Author	Title of publication	Method	Result
1.	Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al	Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome (Nature Genetics, 2001)	Sequencing analysis	Missense mutations in *PTPN11* were found in 11 out of 22 unrelated individuals with Noonan syndrome.
2.	Schubbert S, Zenker M, Rowe SL, Boll S, Klein C, Bollag G, et al	Germline *KRAS* mutations cause Noonan syndrome (Nature Genetics, 2006)	Sequencing analysis	Missense mutations in KRAS were found in individual with Noonan and CFC syndromes
3.	Roberts AE, Araki T, Swanson KD, Montgomery KT, Schiripo TA, Joshi VA, et al.	Germline gain-of-function mutations in *SOS1* cause Noonan syndrome (Nature Genetics, 2007)	Sequencing analysis	Missense mutations in *SOS1* were found in approximately 20% of cases of Noonan syndrome without *PTPN11* mutation.
4.	Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, et al.	Gain-of-function *RAF1* mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy (Nature Genetics, 2007)	Sequencing analysis	Missense mutations in *RAF1* were found in 18 of 231 individuals with Noonan syndrome without known mutations and two of six individuals with LEOPARD syndrome without *PTPN11* mutations.