*Thesis N°: MM0232021*

**Mohammed V University**
**Faculty of Medicine and Pharmacy of Rabat**

Master's Thesis

**Master of Medical Biotechnology**
**Biomedical option**
***Promotion 2019-2021***

# Clinical and Molecular Study of the Kras gene in Noonan Syndrome: about a series of 54 cases

Defended By : EL MAKHZEN Nada

**Jury :**

| | | |
|---|---|---|
| **Pr. IBRAHIMI Azeddine** | FMPR | Committee president |
| **Pr. BOUGUENOUCH Laila** | FMPF, CHU of Fez | Supervisor |
| **Dr. ABBASSI Meriame** | CHU of Fez | Co-Supervisor |
| **Pr. OUADGHIRI Mouna** | FMPR | Examiner |
| **Pr. AANNIZ Tarik** | FMPR | Examiner |

# Abstract

Noonan syndrome (NS) is an autosomal dominant genetic disorder. However, most cases are sporadic. It affects 1/1000 - 1/2500 individuals. It is characterized by facial dysmorphia, cardiac malformation, small size, and mental retardation. The diagnosis is essentially clinical. The identified genetic mutations are involved in the RAS / MAPK signal transduction pathway and currently account for 60% of NS cases. The first gene described was *PTPN11*.

Our report highlighted a study of 54 Moroccan patients with Noonan syndrome and was tested negatively of the *PTPN11* gene at the Genetics and Oncogenetics Unit of the Hassan II University Hospital Center in Fez. 90% of our cohort, occurred sporadically and only in 10%, there was the first degree of consanguinity. The manifestations that led to the diagnosis were characteristic facies in 100% of cases, cardiopathy of pulmonary stenosis type in 61% of cases, cryptorchidism in 5%, short stature in 36%, and mental retardation in 20% of cases.

In the light of these clinical observations, our study focused on looking for mutations in exons 2 and 3 of the Kras gene by simplex PCR followed by sanger sequencing for our cohort. This study revealed the absence of mutations in exons 2 and 3 of the *Kras* gene in our cohort.

***Keywords:*** Noonan syndrome; Kras; pulmonary stenosis; short stature; mental retardation

# Résumé

Le syndrome de Noonan (SN) est une maladie génétique autosomique dominante. Cependant, la plupart des cas sont sporadiques. Il touche 1/1000 à 1/2500 individus. Il se caractérise par une dysmorphie faciale, une malformation cardiaque, une petite taille et un retard mental. Le diagnostic est essentiellement clinique. Les mutations génétiques identifiées sont impliquées dans la voie de transduction du signal RAS / MAPK et représentent actuellement 60% des cas de NS. Le premier gène décrit est le gène *PTPN11*.

Notre rapport met en évidence une étude de 54 patients marocains atteints du syndrome de Noonan et testés négativement du gène PTPN11 à l'Unité de Génétique et d'Oncogénétique du Centre Hospitalier Universitaire Hassan II de Fès. 90% de notre cohorte, est survenue de façon sporadique et seulement dans 10%, il y avait le premier degré de consanguinité. Les manifestations qui ont conduit au diagnostic sont un faciès caractéristique dans 100% des cas, une cardiopathie de type sténose pulmonaire dans 61% des cas, une cryptorchidie dans 5%, une petite taille dans 36% des cas et un retard mental dans 20% des cas.

À la lumière de ces observations cliniques, notre étude s'est attachée à rechercher des mutations dans les exons 2 et 3 du gène *Kras* par PCR simplex suivie d'un séquençage Sanger pour notre cohorte. Cette étude a révélé l'absence de mutations dans les exons 2 et 3 du gène Kras dans notre cohorte.

***Mots clés:*** Syndrome de Noonan ; Kras ; sténose pulmonaire ; petite taille ; retard mental

# Dedication

## *To my beloved parents*

You have always encouraged me, believed in me and prayed for my happiness and success.

All the words in the world cannot express the immense love and the gratitude and appreciation I feel for you, for the sacrifices you have made for my education and well-being. Please find in this project the expression of my deepest love and my most sincere affection.

May Allah bless you with good health, happiness and grant you the highest level of Jannah.

### *To my dear Brothers ; Yassine and Badreddine*

You have always been a great example of courage, patience, and perseverance. Thank you for encouraging me and for constantly believing in me.

Your structural and moral support will always be appreciated. Thank you for your love and care.

May Allah protect you and grant you a long life full of success.

### *To my dear friends and colleagues of Medical Biotechnology Master*

Thank you for reminding me that life is about balance.

Thank you for the good times we had together that I will never forget.

***To all my family***

***To all those who love me***

***To all those I love***

***I dedicate this humble work to you***

# Acknowledgement

I would like to thank the following people, without whom I would not have been able to complete this research, and without whom I would not have made it through my master's degree:

***Pr. IBRAHIMI Azeddine***

*Head of the Medical Biotechnology laboratory, Faculty of Medicine and Pharmacy of Rabat.*

I would like to express my sincere thanks to you for the effort you put into the Medical Biotechnology laboratory and for your interest in the improvement of our scientific knowledge. Not merely, I could not have imagined having a better professor for both my bachelor's and master's studies. Thank you, sir, for all the guidance and support you have given us.

*To my Supervisor*

***Pr. BOUGUENOUCH LAILA***

*Head of the Genetics and Oncogenetics Unit of the Hassan II University Hospital Center in Fez and professor at the Faculty of Medicine and Pharmacy of Fez.*

First of all, I would like to express my sincere thanks to you for giving me the opportunity to work on my master's thesis in the Genetics and Oncogenetics Unit of the Hassan II University Hospital. I am grateful for your consecutive support and your availability during the different stages of this project. The confidence and the autonomy that you granted me allowed me to work in the best conditions. I was greatly inspired by your open mindset, your qualities as "the Head of the Genetics Laboratory", and your enthusiastic and ambitious vision towards the future of human genetics in Morocco.

*To my Co-Supervisor*

***Dr. ABBASSI Meriame***

*Scientific doctor in the Genetics and Oncogenetics Unit of the Hassan II University Hospital Center in Fez*

Thank you so much for your support, the time, and the patience you gave me throughout the practical work of this thesis. Thank you for having guided my first steps in my research path. I would also like to thank you for your encouragement and accurate comments, which were critical during this project. Our cooperation was an inspiring experience. Please find here my respectful gratitude.

***Pr. OUADGHIRI Mouna & Pr. AANNIZ Tarik***

*Professors at the Faculty of Medicine and Pharmacy of Rabat*

I would like to express my gratitude for the honor you have given me for accepting being part of the review process. Your judgment will be of great value in the appreciation of this project. Thank you for the high quality of your coordination during my master's studies, your availability, and your dedication. Please accept, professors, the assurance of my respect and my gratitude.

Special thanks to the Genetics and Oncogenetics Laboratory staff for their great assistance and sympathy during the different aspects of this project. I had the pleasure to benefit from your experiences and explanations.

I also place my sense of; gratitude to one and all who directly or indirectly participated in this project.

# Table of contents

# List of abbreviation:

| | |
|---|---|
| **ASD** | Atrial septal defect |
| **BDT** | BigDyeTerminator |
| **BRAF** | V-Raf murine sarcoma viral oncogene homolog B1 |
| **CBL** | Casitas B-lineage lymphomas |
| **CCDS** | Consensus Coding Sequence |
| **CFC** | Cardio-Facio-Cutaneous Syndrome |
| **CHD** | Congenital heart defects |
| **CR1** | Converted region 1 |
| **CR2** | Converted region 2 |
| **CR3** | Converted region 3 |
| **CS** | Costello Syndrome |
| **DH** | Dbl homology |
| **ECG** | Electrocardiograms |
| **ERK** | Extracellular-regulated kinase |
| **EtBr** | Ethidium bromide |
| **ExoSAP** | Exonulease and Shrimp alkaline phosphatase |
| **FRET** | Resonant energy transfer |
| **GAB1** | Grb2 adaptor binder 1 |
| **GDP** | Guanosine Diphosphate |
| **GEF** | Guanosine exchange factors |
| **GH** | Growth hormone |
| **GRB2** | Growth factor receptor- bound protein 2 |
| **GTP** | Guanosine Triphosphate |
| **HCM** | Hypertrophic cardiomyopathy |
| **HD** | Histone domain |
| **JMML** | Juvenile myelomonocytic leukaemia |
| **KRAS** | kirsten rat sarcoma viral oncogene homolog |
| **LoF** | Loss-of-function |

| | |
|---|---|
| **LS** | LEOPARD syndrome |
| **MEK1** | Mitogen-activated protein kinase 1 |
| **MPD** | Myeloproliferative disorders |
| **NCFCS** | Neuro-cardio-facial-cutaneous syndromes |
| **NF1** | Neurofibromatosis Type 1 |
| **NRAS** | Neuroblastoma RAS viral oncogene homolog |
| **NS** | Noonan syndrome |
| **PCR** | Polymerase chain reaction |
| **PH** | Pleckstrin homology |
| **PTPN11** | Protein tyrosine phosphatase nonreceptor type 11 |
| **PTP** | Protein tyrosine phosphatases |
| **PS** | Pulmonic stenosis |
| **RAF1** | V-RAF-1 murine leukemia viral oncogene homolog 1 |
| **RAS** | Rat sarcoma viral oncogene homolog |
| **MAPK** | Mitogen activated protein kinase |
| **REM** | RAS exchanger motif |
| **SD** | Standard deviations |
| **SHOC2** | Leucine rich repeat scaffold protein |
| **SHP-2** | Src homology protein-tyrosine phosphatase-2 |
| **SH2** | Src-homology 2 |
| **SOS1** | Son of Sevenless homolog 1 |
| **TKR** | Tyrosine kinase receptors |

# List of figures :

# List of tables :

# Introduction

Noonan syndrome (NS) (OMIM 163950) is a common autosomal dominant genetic disorder with an estimated incidence of 1/ 1000–2500 live births. This syndrome is characterized by distinctive facial features, short stature, congenital heart defects (i.e., pulmonary valve stenosis and hypertrophic cardiomyopathy), mild-to-moderate developmental delay/learning disability, skeletal abnormalities (i.e., pectus, scoliosis), cryptorchidism, and predisposition to myeloproliferative disorders(Yart & Edouard, 2018). After Down's syndrome, NS is the most common syndromal cause of congenital heart disease(Rohrer, 2009). Many of the features of NS are similar to those seen in Turner syndrome, and this disorder has sometimes mistakenly been called "male Turner syndrome". This term is misleading, incorrect and should not be used. However, NS occurs in both males and females and the average age at diagnosis is 9 years (Judith E. Allanson & Roberts, 2021; Turner, 2014).

Moreover, several disorders have long been recognized to share clinical features with NS, including LEOPARD syndrome, cardiofacio- cutaneous syndrome, Noonan- like syndrome with loose anagen hair, and Costello syndrome. Recent research has shown that they are indeed biologically related disorders, all being because of germline mutations in genes involved in the RAS–MAPK (mitogen activated protein kinase) pathway. These disorders have been classified into a single family, the so-called neuro-cardio-facial-cutaneous syndromes (NCFCS), based on common pathogenetic mechanisms and clinical overlap(F. R. Lepri et al., 2014; Turner, 2014).

About 50% of NS patients have a missense mutation in the protein tyrosine phosphatase nonreceptor type 11 (*PTPN11*) gene encoding the Src homology protein-tyrosine phosphatase-2 (SHP-2). PTPN11 was the first gene identified with NS, and there are now more than eight known genes (*PTPN11, SOS1, RAF1, RIT1, KRAS, NRAS, BRAF, MAP2K1, RRAS, RASA2, A2ML1, SOS2, LZTR1*) that cause NS,(Kruszka et al., 2017; Yart & Edouard, 2018) with less common mutations notably in *SOS1*, *RAF1*, and *KRAS* genes, accounting for 10, 10, and less than 2%, respectively(Yart & Edouard, 2018).

In Morocco, congenital heart disease has not yet received the attention it deserves. On the one hand, the national incidence of congenital heart disease and its subtypes is unknown, and on the other hand, their etiology, particularly the genetic one, is very little studied.

Thus, we considered it interesting to study the molecular and bioinformatics characteristics of the main genetic factors involved in the manifestation or predisposition to congenital heart disease in the Moroccan population.

This work aimed to look for mutations only at exons 2 and 3 of the *Kras* gene causing NS since we couldn't receive exons 4, 5, and 6 primers of the Kras gene.

This study was performed on 54 Moroccan patients with NS and tested negatively of the *PTPN11* gene; at the Genetics and Oncogenetics Unit of the Hassan II University Hospital Center in Fez.

Mutations research was done using different molecular biology tools, such as DNA extraction, polymerase chain reaction (PCR), and Sanger sequencing. To evaluate molecular biology results, we used Bioinformatics tools, including Blast sequence alignment.

The present work also aims to understand the physiopathology of the malformations studied and contributes to the characterization of the etiology of congenital cardiopathies in the Moroccan population. Moreover, this work allows; concerned families to benefit from genetic counseling and improve their life quality.

# LITERATURE REVIEW

# I. CLINICAL ASPECTS OF NOONAN SYNDROME

## 1. History of Noonan Syndrome

Noonan Syndrome (NS) has a long and complicated history. The first known publication of a case of NS was in 1883, in which Kobylinski described a 20-year-old male with clinical features compatible with NS (webbed neck and several other features). In 1930, Ullrich described several individuals, male and female, with short stature and webbed neck. some of whom represented Turner syndrome and, no doubt, some NS. Due to possible similarities to a mouse strain that Bonnevie had described, this entity was called the Bonnevie-Ullrich phenotype on the European continent and was used to describe children, some of whom would later be diagnosed with NS. In 1938, Turner described some patients with sexual infantilism who also had a webbed neck and short stature. Turner or Ullrich-Turner syndrome was later recognized as a sex chromosome abnormality with either the absence or an abnormality of one of the X chromosomes. However, Ullrich had described males with his disorder and that some female patients, who had features resembling the Ullrich-Turner phenotype, had two apparently normal X chromosomes. This led to the use of confusing terms such as “male Turner syndrome.”(Jacqueline A. Noonan, 1994; Marco Tartaglia & Gelb, 2005).

In 1963, NS was described by two pediatric cardiologists, Noonan & Ehmke in an oral presentation and later published by Noonan alone(Marco Tartaglia & Gelb, 2005). Nine patients, were reported (six males and three females) with remarkably similar facies with a previously unrecognized syndrome of valvular pulmonary stenosis and multiple extra cardiac anomalies. In 1968, Dr Noonan published a case series with these 9 plus an additional 10 patients (12 males and 7 females). All share a rather characteristic similar facies (hypertelorism, a relatively short neck, and apparently low-set ears. Micro¬ gnathia, a slight antimongoloid slant to the eyes etc.)(Jacqueline A. Noonan, 1968). However, in honor of Dr. Noonan, the eponym "Noonan syndrome" was created. Because she was the first to prove that this syndrome; existed in both genders, was linked with normal genes, contained congenital heart defects, and may be hereditary.(Romano et al., 2010).

## 2. Clinical features

### 2.1. Growth

Postnatal proportionate short stature, reported in up to 80% of patients, is one of the main features of NS and can lead to the diagnosis. Although, birth weight and length are usually normal(Yart & Edouard, 2018) and the average length at birth is 47 cm(J. E. Allanson, 1987). However, In some cases, marked weight loss occurs in the first week of life(Van Der Burgt, 2007).

Childhood growth follows conventional values. However**,** most are quite short. It exhibits retardation characterized by a prepubertal growth within the third centile, delayed onset of puberty with attenuated growth spurts, and an adult height approximately 2 (SD) below the norm(Carcavilla et al., 2020). In other words, pubertal growth was showed to be two years overdue in NS patients, and the mean height was in the third percentile, with female and male average heights of 151 cm and 161 cm respectively, and the average bone age delayed by two years(El Bouchikhi et al., 2016).

Treatment with Growth hormone (GH) can be used to accelerate growth during the first years of life. Initial reports on the long- term effects of this treatment show a beneficial effect. NS patients with a mutation in the PTPN11 gene respond less efficiently to GH than NS patients without a mutation in PTPN11(Van Der Burgt, 2007). However, this may be because patients with PTPN11 mutations presented with more severe short stature and, therefore reached a lower adult height(Yart & Edouard, 2018).

### 2.2. Craniofacial features

Facial appearance is the key to the diagnosis of NS. However, it is most characteristic in infancy and early-to-middle childhood and becomes more subtle in adulthood(Romano et al., 2010; Turner, 2014). In the newborn infant with NS, the main features are hypertelorism with downward slanting palpebral fissures (95%), low set, posteriorly rotated ears with a thick helix (90%), deeply grooved philtrum with high, wide peaks of the vermilion border of the upper lip (95%), high arched palate (45%), micrognathia (25%), and excess nuchal skin with low posterior hairline (55%). The head appears large with a small face tucked beneath a large

cranium, a tall forehead, and narrowing at the temples. The eyes are wide-spaced and prominent with epicanthal folds, ptosis, hypertelorism, and thick hooded eyelids. The nose is short and broad with a depressed root and full tip. In childhood, facial appearance often lacks expression and resembles the face of an individual with a myopathy(J. E. Allanson, 1987; Romano et al., 2010).

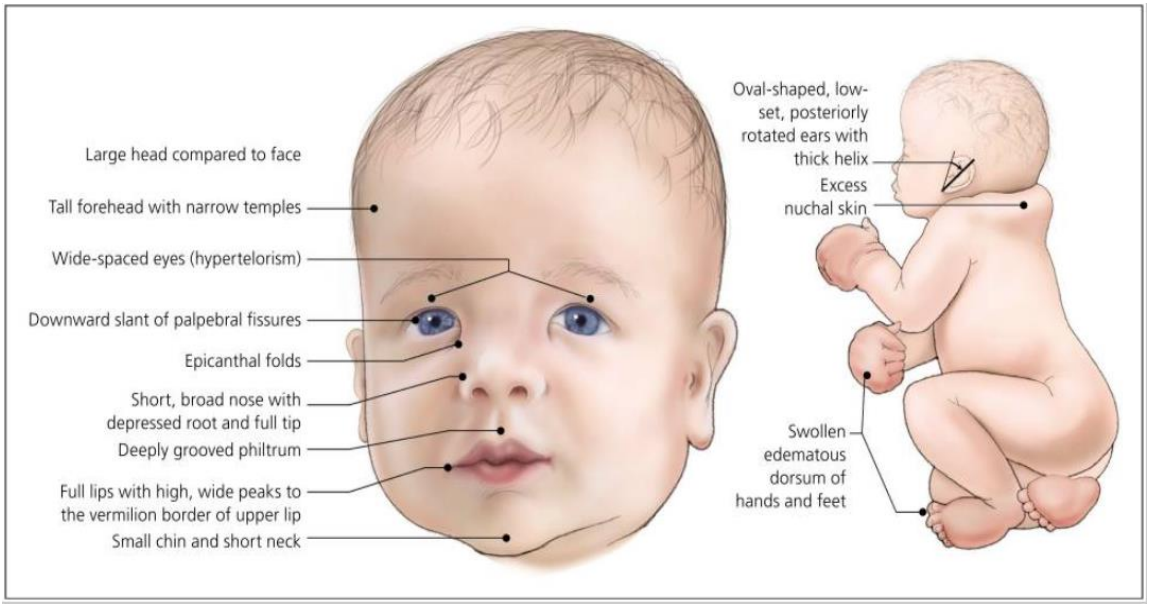


**Figure 1** : Newborn with Noonan syndrome.("Noonan Syndrome," n.d.)

In the adolescent and young adult, face shape becomes more triangular. Eyes are less prominent. Features are sharper. There is a narrow nasal root with a thin bridge. The neck is longer, accentuating skin webbing or prominence of the trapezius muscle. The adult face may be quite unremarkable. Some adults, however, have prominent nasolabial folds, a high anterior hairline, and transparent, wrinkled skin. Hair may be wispy in the toddler, whereas it is often curly or woolly in the older child and adolescent. Regardless of age, Features that are often present are pale blue or blue-green eyes, diamond-shaped, arched eyebrows, and low set, posteriorly rotated ears with a thick helix (J. E. Allanson, 1987; Romano et al., 2010).

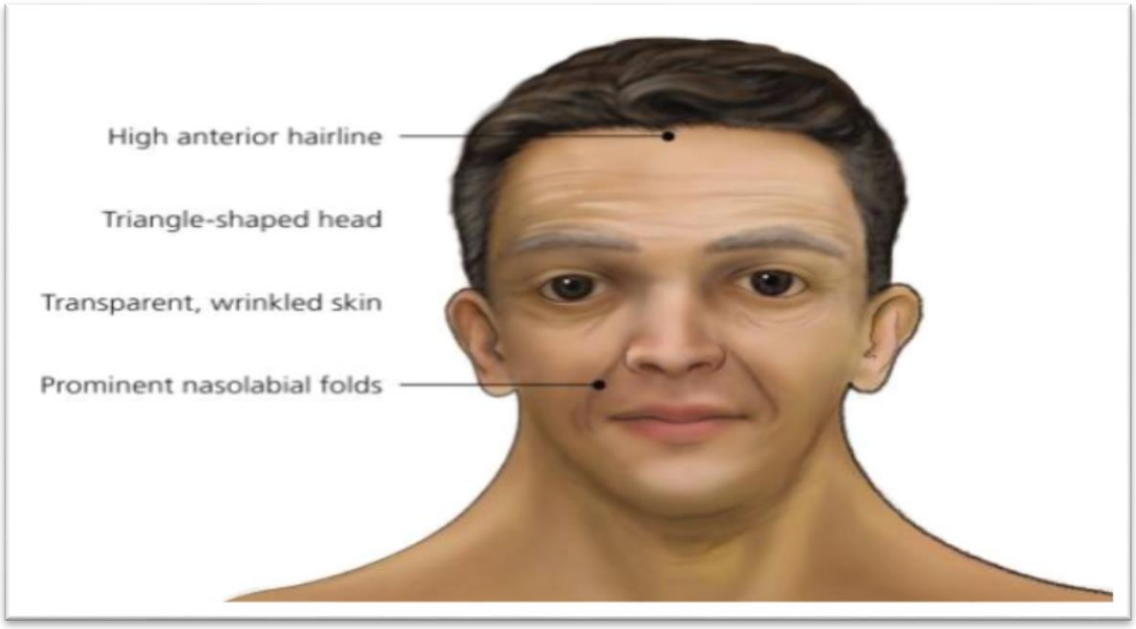


**Figure 2** : Adult with Noonan syndrome.(“Noonan Syndrome,” n.d.)

### 2.3. Cardiovascular features

Congenital heart defects (CHD) are one of the main clinical features of NS. The prevalence of cardiovascular abnormalities is estimated at 70%-80% of cases(Carcavilla et al., 2020). Common anomalies include pulmonic stenosis (PS) (50-60%), hypertrophic cardiomyopathy (HCM) (20%), and atrial septal defect (ASD) (6-10%). The other CHDs, such as ventricular septal defect, atrioventricular canal defect, and aortic coarctation, are observed less frequently(Carcavilla et al., 2020; El Bouchikhi et al., 2016).

Electrocardiograms (ECG) from NS patients also described electrocardiographic abnormalities (50%). Wide-ranging QRS intervals with a mainly negative pattern in the left precordial leads and left axis deviation with giant Q waves are often involved.(Carcavilla et al., 2020; Van Der Burgt, 2007).

## 2.4. Genitourinary and renal manifestations

Puberty is usually delayed in Noonan syndrome patients of both sexes. The average age of puberty onset in males is 14.5 years and 14 years in females. The majority of females are fertile(J. E. Allanson, 2009). In NS males gonadal dysfunction with impaired spermatogenesis has been reported; this may explain the sex ratio distortion/predominant transmission by the mother in familial cases. Cryptorchidism, which affects 60-80% of NS males, can be related to decreased fertility(Yart & Edouard, 2018).

Renal abnormalities (duplex collecting system, solitary kidney, pyeloureteral stenosis and renal pelvis dilation) are present in 10% of cases(Carcavilla et al., 2020).

## 2.5. Development and Behaviour

Children with NS demonstrate mild motor delay, with the average age of sitting at 10 months, the first unsupported walking occurring at 21 months, and basic two-word sentences occurring at 31 months. Mental retardation is present in 15%–35% of cases and is generally mild; moreover, most children can do better in a typical school environment, although 10–40% will need extra assistance(J. E. Allanson, 2009; Van Der Burgt, 2007). It is characterized by specific visual-constructional problems, and verbal performance discrepancy (15%). Mild hearing loss (12%), or articulation abnormalities (72%) are frequent(J. E. Allanson, 1987). The average full-scale intelligence quotient (IQ) is 85, but there is a wide range in the level of intelligence.Clumsiness, feeding problems, fidgety or stub-born spells, echolalia, and irritability are common behavioral issues. Social issues and attention loss have also been identified(Van Der Burgt, 2007).

## 2.6. Ophthalmological features

Ophthalmic abnormalities are frequent: strabismus (48%–63%), refractive errors (61%), and amblyopia (33%). Anterior segment changes (63%). Fundal abnormalities and nystagmus may be present in (20%) and (10%) of NS patients respectively(Carcavilla et al., 2020; Van Der Burgt, 2007).

## 2.7. Musculoskeletal abnormalities

Chest deformities are seen with pectus carinatum superiorly and pectus excavatum inferiorly. These sternal abnormalities are present in 70%–95% of cases(J. E. Allanson, 1987; Carcavilla et al., 2020; Van Der Burgt, 2007). The thorax is broad with wide-spaced nipples. Shoulders are often rounded, and the upper chest appears long with low-set nipples and axillary webbing(J. E. Allanson, 2009). Common orthopedic features include cubitus valgus (50%), hand anomalies including brachydactyly and blunt fingertips (30%), radioulnar synostosis (2%), joint hyperextensibility (50%), and talipes equinovarus (12%)(J. E. Allanson, 1987; Van Der Burgt, 2007).

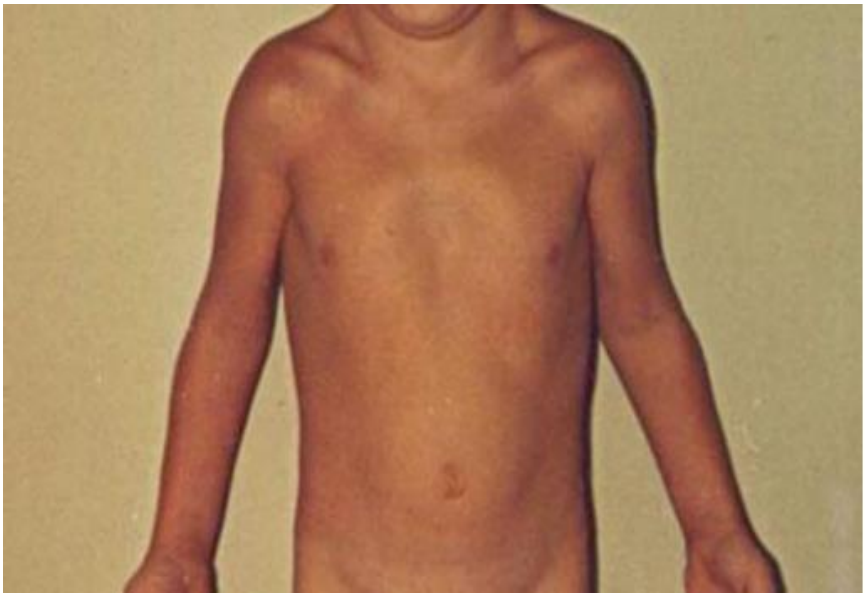

**Figure3** : The chest phenotype showing wide-spaced and low-set nipples, pectus deformity with pectus carina- tum superiorly and pectus excavatum inferiorly, and rounded shoulders.(J. E. Allanson, 2009).

## 2.8. Blood disorders and cancer

Bleeding disorders have been identified in 30% to 65% of patients with NS(Romano et al., 2010). It includes factor XI deficiency, factor XII deficiency, factor VIII deficiency, von Willebrand disease (vWD), and platelet dysfunction, which may be associated with trimethylaminuria or a cyclooxygenase deficiency(J. E. Allanson, 2009). In many instances, there is no correlation between coagulation test results and bleeding diathesis.

As for all RASopathies, NS presents an elevated incidence of blood cancers and solid tumors that is estimated to be 8.1 times higher than the general population(Carcavilla et al., 2020). However, several patients have been diagnosed with acute leukemia and myeloproliferative disorders (MPD). In rare cases, individuals with NS can develop fatal MPD, typically juvenile myelomonocytic leukemia (JMML)(Van Der Burgt, 2007). One specific *PTPN11* mutation, The73Ile, is present in almost half of children with NS and MPD but is rare in individuals with NS but without MPD. However, somatic mutations in PTPN11 are a common cause of MPD that is unrelated to NS(J. E. Allanson, 2009). Recent research showed the highest neonatal mortality in NS due to severe JMML, suggesting that JMML may go undetected in this age group due to the fatal outcome of these cases(Carcavilla et al., 2020).

## 2.9. Other features

Other features have also been identified, including Lymphatic vessel dysplasia, hypoplasia, or aplasia (20%), pyeloureteral stenosis and/or hydronephrosis (10%), Hepatosplenomegaly often present in infancy (26%–51%), and abnormalities of pigmentation including pigmented naevi (25%), cafe-au-lait spots (10%) and lentigines (3%)(Van Der Burgt, 2007).

## 3. Diagnostic Criteria

Despite advances in molecular testing that can confirm 70% of cases, NS diagnosis is based on clinical features(Carcavilla et al., 2020; "Noonan Syndrome," n.d.). Van der Burgt created the diagnostic criteria that are now universally accepted in 1994 and revised in 2007, which take into account the facial features, growth pattern, chest deformity, and cardiac defects(Carcavilla et al., 2020; Jorge, Malaquias, Arnhold, & Mendonca, 2009). In van der Burgt's scoring system, patients are firstly classified as having typical or suggestive NS characteristics based on their facial features. The diagnosis of NS is confirmed by a typical face and any other major sign or two minor signs, whereas patients with suggestive NS face features require two major or three minor clinical characteristics to confirm the NS diagnosis(Jorge et al., 2009).

**Table 1 :** Noonan syndrome diagnostic criteria (adapted from van der Burgt et al.)(Jorge et al., 2009)

| Clinical characteristics | Major | Minor |
|---|---|---|
| 1 Facial | typical face | suggestive face |
| 2 Cardiac | pulmonary valve stenosis and/or typical ECG | other defects |
| 3 Height | <3rd centile | <10th centile |
| 4 Chest wall | pectus carinatum/excavatum | broad thorax |
| 5 Family history | first-degree relative with definite diagnosis | first-degree relative with suggestive diagnosis |
| 6 Other:<br>Mental retardation<br>Cryptorchidism<br>Lymphatic dysplasia | all 3 | any of the 3 |

Definite NS: typical face + one major or two minor clinical characteristics or suggestive face + two major or three minor clinical characteristics.

Once a child is diagnosed, genetic counseling should be enrolled for parents to provide accurate recurrence risks. When a mutation in one of the NS genes is detected, a search for NS mutations in parents can be performed. However, in the absence of a known NS mutation in a family, parental screening should involve a comprehensive physical examination to look for distinguishing features(Judith E. Allanson & Roberts, 2021).

## 4. Differential Diagnosis

The differential diagnosis must include other RASopathies as well as other syndromes unrelated to the RAS-MAPK pathway, and that is characterized by facial dysmorphology, short stature, and cardiac defects such as Aarskog syndrome, Baraitser-Winter syndrome, Fetal alcohol syndrome, and Williams syndrome(Carcavilla et al., 2020; Romano et al., 2010; Van Der Burgt, 2007).

**Table 2 :** Differential Diagnosis: Similarities and Differences Between NS and Other related facial dysmorphology disorders (Romano et al., 2010).

| Syndrome | Similarities with NS | Differences from NS |
|---|---|---|
| Aarskog syndrome | The similarities between NS and Aarskog syndrome are largely facial and skeletal (hypertelorism, down-slanting palpebral fissures, and low stature). | There are no cardiovascular malformations of Aarskog syndrome, but there is a shawl scrotum; this is an X-linked recessive condition. |
| Baraitser-Winter syndrome | Hypertelorism, eyelid ptosis, a short jaw, short stature, and developmental delays are all characteristics shared with both NS and Baraitser-Winter syndrome. | With Baraitser-Winter syndrome, there can be iris coloboma, pachygyria, lissencephaly, bicuspid aortic valve stenosis, and aortic stenosis |
| Fetal alcohol syndrome | Growth and developmental delays, hypertelorism, epicanthal folds, PVS, and ASD are all common characteristics of both NS and fetal alcohol syndrome. | With fetal alcohol syndrome, born small for gestational age, microcephaly, cleft palate, small palpebral fissures, smooth philtrum, thin-lip vermilion, and a wider range of cardiac defects including ventricular septal defect, endocardial cushion defect, conotruncal abnormalities, and coarctation of the aorta |

The differential diagnosis of other RASopathies include (Judith E. Allanson & Roberts, 2021; J. A. Noonan, 2009; Roberts, Allanson, Tartaglia, & Gelb, 2013; Marco Tartaglia, Gelb, & Zenker, 2011) :

LEOPARD syndrome (LS) (OMIM 151100) is a rare autosomal dominant disorder that overlaps phenotypically with NS. It is also allelic with NS caused by different missense mutations in the PTPN11 gene. About 90% of LS patients have a PTPN11 mutation. Moreover, LS has been causally linked to mutations in RAF1 for the remaining 10%. In addition to NS, LS is characterized by multiple lentigines and café-au-lait spots as well as deafness. Although NS is characterized by PS and, less often, HCM, LS is characterized by HCM and a lower prevalence of PS.

Cardio-Facio-Cutaneous Syndrome (CFC) (OMIM 115150) is a rare, sporadic, multiple anomaly syndrome with significant mental retardation. The face in CFC syndrome shares many facial features with NS, such as ptosis, a short nose with a relatively broad base, a well-grooved philtrum with cupids-bow lip, and a small chin. However, there is usually significant cutaneous involvement consisting of dry hyperkeratotic scaly skin, sparse or absent eyebrows, and sparse or absent eyebrows, and sparse slow-growing curly hair. The majority of affected individuals have heart problems, which most usually include PS, HCM, and septal defects. BRAF appears to be the most often mutated gene in CFC, followed by MEK1 and MEK2, and occasional patients with a KRAS mutation. Whereas individuals with PTPN11 and SOS1 mutations have not been identified with clear-cut CFC syndrome.

Costello Syndrome (CS) (OMIM 218040) is a rare disorder characterized by high birthweight and delayed growth, developmental delay, coarse facial features, wide nasal bridge, loose and soft skin, increased pigmentation over time, deep palmar and plantar creases, facial or perianal papillomata, premature aging, and hair loss, moderate intellectual disability, flexion or ulnar deviation of the wrist and fingers, and cardiac abnormalities (PS in about 40% and HCM in another 40%). Children and young adults with CS are reported to have certain malignancies (rhabdomyosarcoma, most commonly), which have been estimated to occur in approximately 15% of affected individuals. CS is caused by germline missense mutations in the HRAS gene that cause roughly 85% of cases.

Neurofibromatosis Type 1 (NF1) (OMIM 162200) is an autosomal dominant disease. It is characterized by subcutaneous and, or plexiform neurofibromas, café-au-lait spots, axillary and, or inguinal freckling, Lisch nodules in the iris, skeletal deformities, vascular defects, learning disabilities, and behavioral problems, short stature, macrocephaly, and a predisposition for developing benign and malignant neoplasia. NF1 is caused by mutations or deletions in the neurofibromin-1 gene (NF1). It acts as a negative regulator of the RAS-mediated signal transduction pathway. However, the etiology of NF-NS is indistinct. A report of a patient who had appearance features of the two syndromes was found to have two mutations, a PTPN11 mutation which was inherited from the dad, and a de novo NF1 mutation. This is the only report so far of molecular occurrence of the two diseases in a similar patient(Hüffmeier, Zenker, Hoyer, Fahsold, & Rauch, 2006).

**Table3 :** Genetic causes and summary of major features of other RASopathies.(Jorge et al., 2009).

| Disorder | Causative gene | Phenotype |
|---|---|---|
| Noonan syndrome | *PTPN11, SOS1, RAF1, MEK1, KRAS* | |
| Neurofibromatosis type 1 | *NF1* | Familial cancer syndrome; hallmark features include hyperpigmented skin lesions and benign neurofibromas; learning disabilities are common |
| Leopard syndrome | *PTPN11, RAF1* | Multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, growth retardation and deafness |
| Costello syndrome | *HRAS, KRAS, BRAF, MEK1* | Mental retardation, high birth weight, neonatal feeding problems, curly hair, coarse face, thick lips, nasal papillomata, diffuse skin hyperpigmentation, and nail dystrophy |
| Cardio-facio-cutaneous syndrome | *KRAS, BRAF, MEK1, MEK2* | Coarse face, congenital heart defects, ectodermal anomalies (follicular and palmar hyperkeratosis), short stature, variable degrees of mental retardation (moderate to severe) and facial features reminiscent of NS and Costello syndrome |

# II. MOLECULAR ASPECTS OF NOONAN SYNDROME

## 1. the Ras/MAPK (mitogen-activated protein kinase) signal transduction pathway

The RAS/MAPK pathway is an important signal transduction pathway through which extracellular ligands stimulate cell proliferation, differentiation, survival, and metabolism(Zadeh, 2019). This pathway has been studied widely with regards to oncogenesis since its dysregulation is one of the essential drivers of cancer, with Ras, discovered to be somatically mutated in roughly 20% of malignancies(Tidyman & Rauen, 2009). Germline mutations in the pathway can result in distinctive RASopathies; a group of syndromes characterized by mutations in the RAS -MAPK pathway, including NS, CS, and NF1(Jafry & Sidbury, 2020).

RAS proteins are small guanosine-binding proteins that act as signal switch molecules that integrate activated cell membrane receptors, mainly tyrosine kinase receptors (TKR) and downstream effectors. Ras genes are part of a larger gene family that comprises HRAS, NRAS, and KRAS. Tyrosine residues in the TKR's intracytoplasmic domain are phosphorylated after activation by hormones. In other words, this activation occurs when a growth factor binds to RTK, inducing autophosphorylation and interaction with the adaptor protein GRB2. GRB2 is bound to SOS and then recruited to the plasma membrane. SOS proteins are guanosine nucleotide exchange factors (GEF) that enable RAS proteins to convert from an inactive GDP-bound to an active GTP-bound conformation. Activated RAS can positively activate the RAF-MEK-ERK kinase cascade, which results in the transcription of target genes, encouraging cell proliferation and differentiation while decreasing apoptosis. The MEK-ERK kinase cascade is activated by three RAF serine/threonine kinases (ARAF, BRAF, and RAF1). Raf phosphorylates and activates MEK1 and, or MEK2 (MAPK kinase), which activates and phosphorylates ERK1 and, or ERK2. ERK1/2 can phosphorylate both cytosolic and nuclear substrates, which include transcription factors that control the cell cycle(Jorge et al., 2009; Malaquias & Jorge, 2021; Tidyman & Rauen, 2009).

As previously mentioned, NS and other RASopathies are distinguished molecularly by increased signal transduction through the RAS/MAPK cascade. Gain-of-function mutations in the genes encoding tyrosine phosphatase (PTPN11/SHP2), GNEFs (SOS1, SOS2), RAS superfamily members (HRAS, KRAS, MRAS, NRAS, RIT1, RRAS, RRAS2), MAPK pathway components (BRAF, RAF1, MAP2K1/MEK1, MAP2K2/MEK2, and MAPK1), or proteins that interact with RAS to activate the MAPK cascade (SHOC2, PPP1CB). Besides, Loss-of-function (LoF) mutations in genes that encode GTPase-activating proteins (NF1, RASA2), inhibitors of RAS/MAPK pathway activation (LZTR1, SPRED1), or proteins that promote TKR degradation can also trigger RASopathies (CBL)(Malaquias & Jorge, 2021).

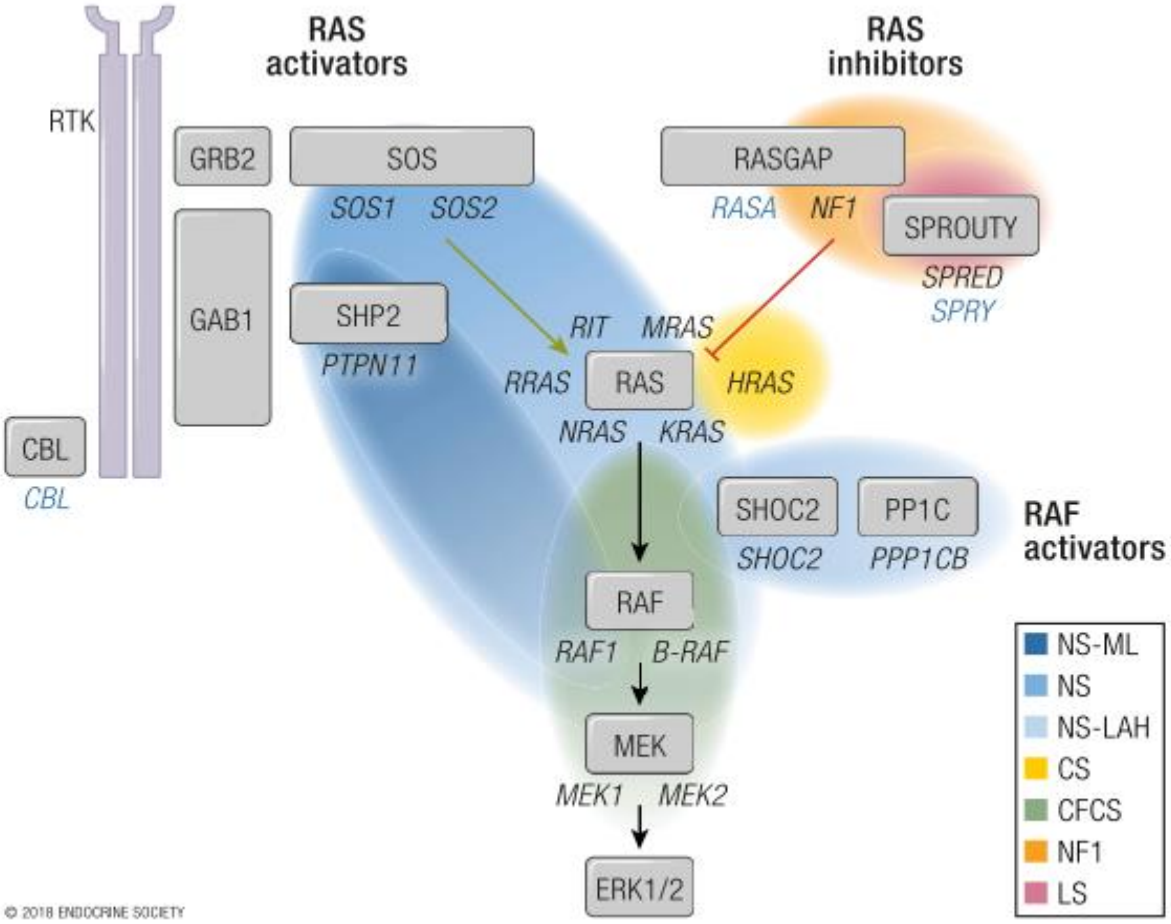


**Figure 4 :** The RAS/MAPK pathway and the RASopathies disease genes.(Tajan, Paccoud, Branka, Edouard, & Yart, 2018)

A simplified diagram of the canonical RAS/MAPK signaling pathway activated by tyrosine kinase receptors (RTK). Proteins are represented by gray rectangles (with corresponding genes italicized). SHP2 participates in RAS activation by dephosphorylating many inhibitory phosphotyrosines, including the docking site for p120RASGAP carried by GAB1 and the tyrosine on RAS that mediates its interaction with p120RASGAP. SPROUTY proteins negatively regulate the RAS/MAPK pathway by sequestering RAS activators, and CBL drives RTK ubiquitination, which deactivates signals. Colored ovals represent major disease genes for each RASopathy and show function/phenotype relationships: In blue, RAS/RAF activator mutations are the primary cause of NS and NS-like syndromes (NS-ML, NS-LAH). NF1 and LS are linked with RAS inhibitors in orange/pink. Mutations that cause CS and CFCS affect the RAS/MAPK backbone in yellow and green. Some NS-associated genes are not hidden by the blue oval but are written in blue for improved readability, and disease genes unrelated to the RAS/MAPK pathway are not shown. CBL, Casitas B-lineage lymphomas; ERK, extracellular signal-regulated kinase; GAB1, Grb2 adaptor binder 1; GDP, guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; GTP, guanosine triphosphate; MEK, MAPK/ERK kinase; PP, protein phosphatase; RAF, rapidly accelerated fibrosarcoma; SHOC2, Soc-2 homolog; SOS, son of sevenless.

## 2. Historical approach to the Genes involved

### 2.1. *PTPN11* (protein-tyrosine phosphatase, nonreceptor-type 11)

In 1994, a large Dutch kindred was studied by Jamieson and co-workers transmitting the trait to perform a genome-wide scan and observed linkage with several markers at chromosome 12q22-qt, which they named NS1. Using novel STRs, the NS1 locus was refined to a region of around 7.5 cm. In addition, Legius and co-workers studied, in turn, a four-generation Belgian family transmitting the trait, achieving independent linkage to NS1, and narrowing the critical interval further to approximately 5 cm. A few years later, Tartaglia and co-workers established *PTPN11* (OMIM 176876) which encodes the non–receptor-type protein tyrosine phosphatase SHP-2 (src homology region 2-domain phosphatase–2) as the NS1 disease gene(M. Tartaglia & Gelb, 2009). In a population of 22 unrelated subjects with familial or sporadic NS, missense mutations in *PTPN11* accounted for 50% of the cases(Marco Tartaglia et al., 2002). Based on these statistics, many studies have attempted to establish genotype-phenotype correlations of *PTPN11* NS cases. In some studies, PS and short stature were more prevalent in the NS group with *PTPN11* gene mutations. On the other hand, HCM was less often present in the NS group with *PTPN11* gene mutations(Ko, Kim, Kim, & Yoo, 2008; M. Tartaglia & Gelb, 2009).

The *PTPN11* gene spans more than 90 kb, comprising 16 exons with an open reading frame of 1,779 bases(M. Tartaglia & Gelb, 2009). It encodes SHP, a member of a small subfamily of cytosolic protein tyrosine phosphatases (PTPs) that includes SHP-1 (encoded by PTPN6) and the Drosophila SHP2–homolog corkscrew (csw). However, SHP-2 is widely expressed in both embryonic and adult tissues and is required during development. SHP-2 structure is composed of two tandemly arranged amino-terminal src-homology 2 (SH2) domains (N-SH2 and C-SH2), a PTP, and a carboxy-terminal tail(M. Tartaglia & Gelb, 2009; Marco Tartaglia & Gelb, 2005; Marco Tartaglia et al., 2002). SHP-2, in contrast to SHP-1, is ubiquitously expressed and is included in mesodermal patterning, limb development, hematopoietic cell differentiation, and semilunar vasculogenesis. Moreover, SHP-2 plays an important role in the cellular response to growth factors, hormones, cytokines, and cell adhesion molecules(Marco Tartaglia et al., 2002).

It is required for the Ras/mitogen-activated protein (MAP) kinase cascade activation, induced by epidermal, fibroblast, and hepatocyte growth factors(Marco Tartaglia et al., 2002). Depending upon its binding partner and interactions with downstream signaling networks, SHP-2 can either positively or negatively modulate the signal flow(M. Tartaglia & Gelb, 2009; Marco Tartaglia et al., 2002).

Activation of SHP-2 results from binding of both N-SH2 and C-SH2 domains to short amino acid motifs containing a phosphotyrosyine residue and promote SHP- 2's association with cell surface receptors, cell adhesion molecules, and scaffolding adapters. While both SH2 domains positively modulate SHP2 phosphatase activity, the N-SH2 domain plays a crucial role in controlling its activation. Crystallographic data indicate that the N-SH2 domain also interacts with the PTP domain in the inactive conformation, blocking the catalytic site. The binding of the N-SH2 phosphopeptide-binding site to a phosphotyrosyine ligand causes a conformational change in the domain that reduces the intermolecular interaction between the N-SH2 and PTP domains makes the catalytic site available to substrate(M. Tartaglia & Gelb, 2009; Marco Tartaglia et al., 2002).

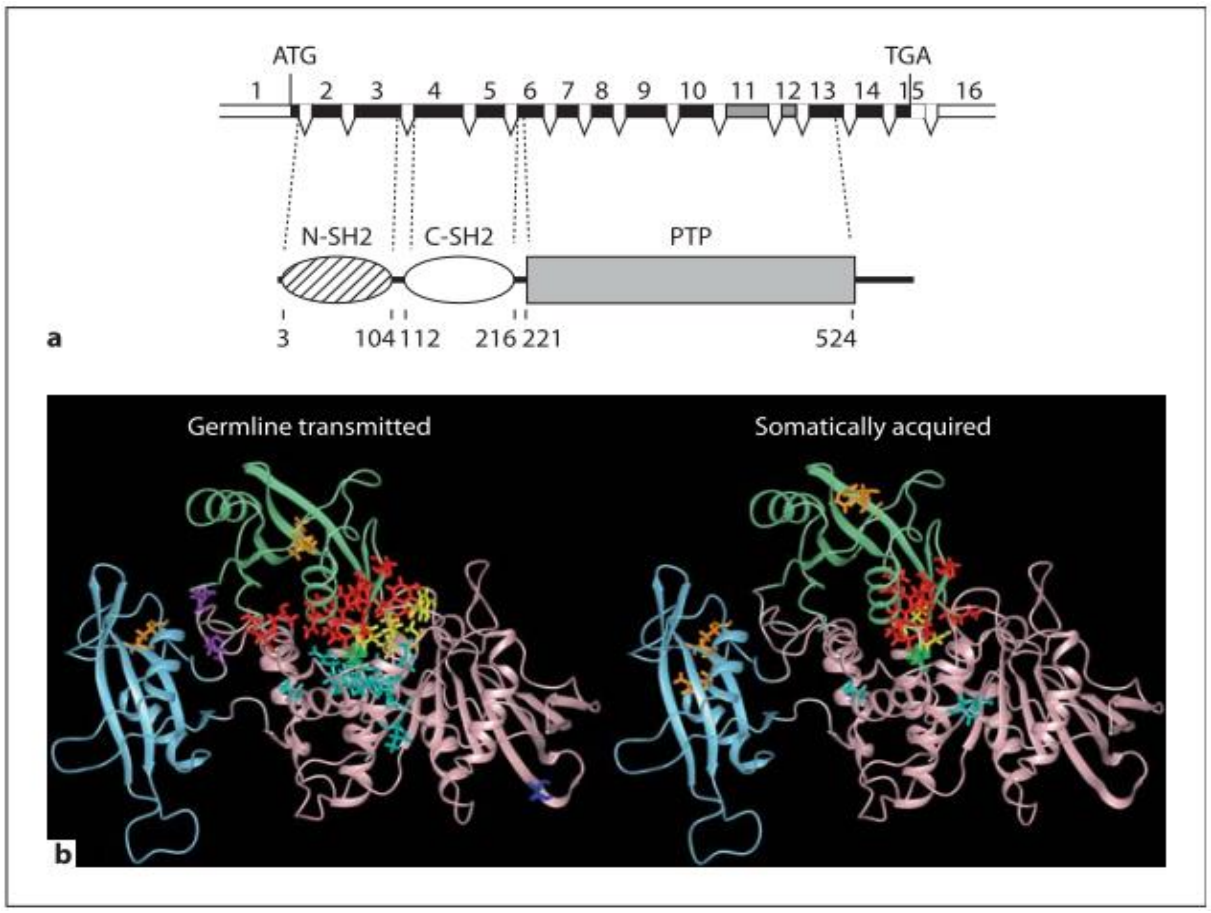


**Figure 5 :** *PTPN11* gene organization, SHP-2 domain structure and location of affected residues in human disease.(M. Tartaglia & Gelb, 2009)

(a) The *PTPN11* gene and its encoded protein: The coding exons are shown by the numbered, filled boxes at the top, as are the locations of the ATG and TGA codons. The functional domains of the SHP-2 protein are shown below, and they consist of two tandemly arranged SH2 domains at the N-terminus (N-SH2 and C-SH2) followed by a protein tyrosine phosphatase (PTP) domain. The numbers underneath the cartoon represent the amino acid boundaries of those domains. (b) Mutated residues location in the three-dimensional configuration of SHP-2 in its catalytically inactive conformation (green, N-SH2 domain; cyan, C-SH2 domain; pink, PTP domain). The lateral chains of residues affected by germline (left) or somatically acquired (right) mutations are colored according to the classification suggested by Tartaglia et al. (2006) (red, group I; yellow, group II; green, group III; cyan, group IV; orange, group V; violet, group VI; blue, unclassified).(M. Tartaglia & Gelb, 2009)

## 2.2. *SOS1* (Son of Sevenless homolog 1)

Recent studies confirmed *SOS1* (OMIM 182530) as the second major gene for NS(F. Lepri et al., 2011; Zenker et al., 2007). According to Roberts et al., *SOS1* is responsible for nearly 20% of NS patients in the absence of *PTPN11* mutations(El Bouchikhi et al., 2016).

The *SOS1* gene, located in chromosome band 2p22.1(Jongmans et al., 2010), encompasses 23 exons and encodes a 150-kD multidomain protein(Baban et al., 2019). It is a RAS-specific guanine nucleotide exchange factor (GEF) ; that catalyzes GDP/GTP exchange in response to growth factors and cytokines, which is necessary for RAS activation(Jongmans et al., 2010). *SOS1* is a large multidomain protein characterized by an N-terminal portion of the protein that contains a histone domain (HD; ≈200 residues characterized by two tandemly arranged histone folds and is followed by a Dbl homology (DH) domain (≈200 residues) and a pleckstrin homology (PH) domain (≈150 residues), which are involved in the activation of RAC, a small GTPase of the RHO/CDC42 family. And a C-terminal which, makes up half of the protein contains the RAS exchanger motif (REM) domain (≈200 residues) and the Cdc25 domain (≈300 residues), which are required for the RAS-specific nucleotide exchange activity of *SOS1*. Finally, the C-terminus region includes SH3 domain recognition sites and mediates *SOS1* interaction with SH3 domain-containing adaptor proteins that carry *SOS1* to the membrane upon receptor activation(M. Tartaglia & Gelb, 2009). On the other hand, the majority of NS mutations ; are located in the DH, PH, and REM domains, which are all thought to contribute to auto-inhibition of the Cdc25 domain(Jongmans et al., 2010).

According to the reported data, *SOS1* mutation carriers have a distinct NS phenotype with a high prevalence of ptosis and ectodermal symptoms, a normal intelligence, and a lower frequency of short stature as compared to individuals with a *PTPN11* mutation(Jongmans et al., 2010; F. Lepri et al., 2011; Zenker et al., 2007). However, The prevalence of PS was higher in patients with SOS1 mutations (83.3%) than those with *PTPN11* mutations(El Bouchikhi et al., 2016).

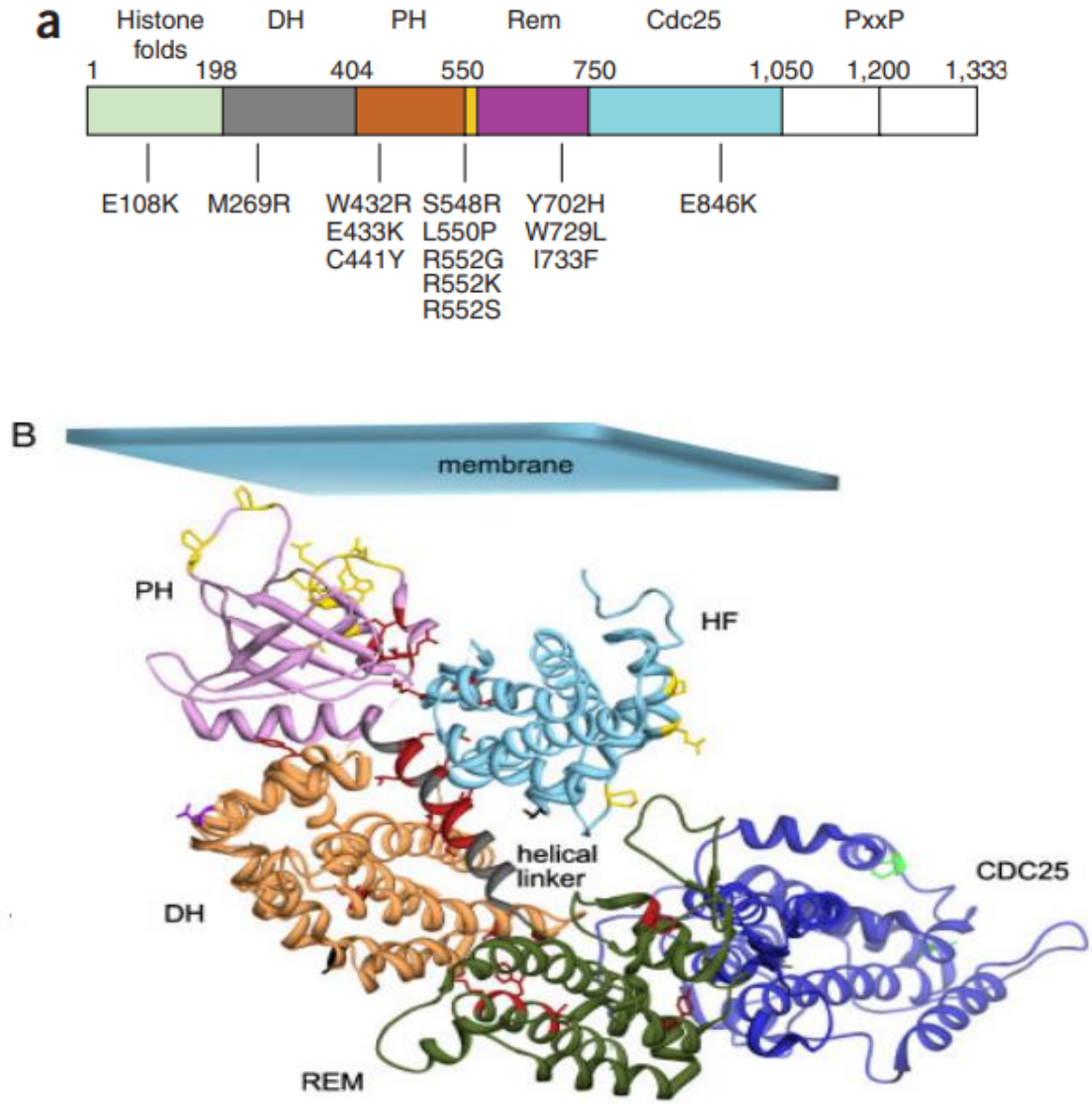


**Figure 6 :** *SOS1* domain structure and location of residues altered in Noonan syndrome.(F. Lepri et al., 2011; Marco Tartaglia et al., 2007).

**(a) :** *SOS1* cartoon with predicted amino acid substitutions from 14 *SOS1* missense mutations and functional domains above. DH, Dbl homology domain; PH, pleckstrin homology domain; Rem, RAS exchanger motif(Marco Tartaglia et al., 2007). **(B):** According to the crystal structure of the protein truncated at the C-terminus (residues 1–1049) (PDB ID: 3KSY), the location of affected residues in *SOS1* is represented in its inactive conformation. [Guerasko et al., 2010]. Ca ribbon trace of the HF (sky blue), DH (sandy brown), PH (plum), REM (dark green), and CDC25 (blue) domains, and the helical linker connecting the PH and REM domains (gray)(F. Lepri et al., 2011).

## 2.3. *KRAS* (kirsten rat sarcoma viral oncogene homolog)

*KRAS* (OMIM 190070), another RAS-MAPK gene is located on chromosome 12p12 and consists of 6 exons that code for the P loop, as well as a switch I and switch II domains(El Bouchikhi et al., 2016). Ras isoforms, which are encoded by the three genes *KRAS*, *HRAS*, and *NRAS*, act as molecular switches by cycling between an active GTP-bound and an inactive GDP-bound state. In their active form, they relay growth signals to several effector proteins and thus control fundamental cellular pathways(Md Abdur Razzaque et al., 2012; Marco Tartaglia et al., 2007).

Through alternative splicing, the *KRAS* gene generates two transcripts, *KRAS*A and *KRAS*B. *KRAS*A is expressed in a tissue- and temporally-restricted way, whereas KRASB is expressed everywhere. Moreover, The C-terminal tails of *KRAS*A and *KRAS*B are different. This region in *KRAS*A contains a CAAX motif and is palmitoylated at cysteine residues with effects on membrane localization of the protein. In *KRAS*B, the CAAX domain near the C-terminus is substituted with a polylysine stretch. This differential processing has significant practical consequences, resulting in alternate trafficking pathways to the plasma membrane and distinct membrane localization(Gelb & Tartaglia, 2006).

Mutations in *KRAS*, account for about 3% of NS cases and 7% of all RASopathies, including CFC and few individuals with CS(Addissie et al., 2015). However, according to Schubbert et al, *KRAS* was the second reported gene for NS and its mutation was first reported in a minority (five patients, ~4%) of 124 individuals with NS without *PTPN11* mutations(Amanda Salem Brasil,1 Alexandre C. Pereira,2 Luciana Turolla Wanderley,1 Chong Ae Kim,1 Alexsandra C. Malaquias,3 Alexander A.L. Jorge,3 Jose´ Eduardo Krieger & Noonan, 1981; Marco Tartaglia et al., 2007).

NS patients with *KRAS* mutations were reported to have severe phenotypes with mental retardation(El Bouchikhi et al., 2016; Ko et al., 2008). Somatic *KRAS* mutations are found in myeloid malignancies and other cancers, including approximately 25% of JMMLs(Schubbert et al., 2006). It has been suggested that germline mutations in the *KRAS* gene are causally involved in craniosynostosis; a developmental defect that disrupts the cranial morphogenetic program, leading to variable dysmorphic craniofacial features and associated functional abnormalities, supporting the role of the RAS-MAPK pathway as a mediator of aberrant bone growth in cranial sutures(Addissie et al., 2015; Brasil et al., 2012; Kratz et al., 2009).

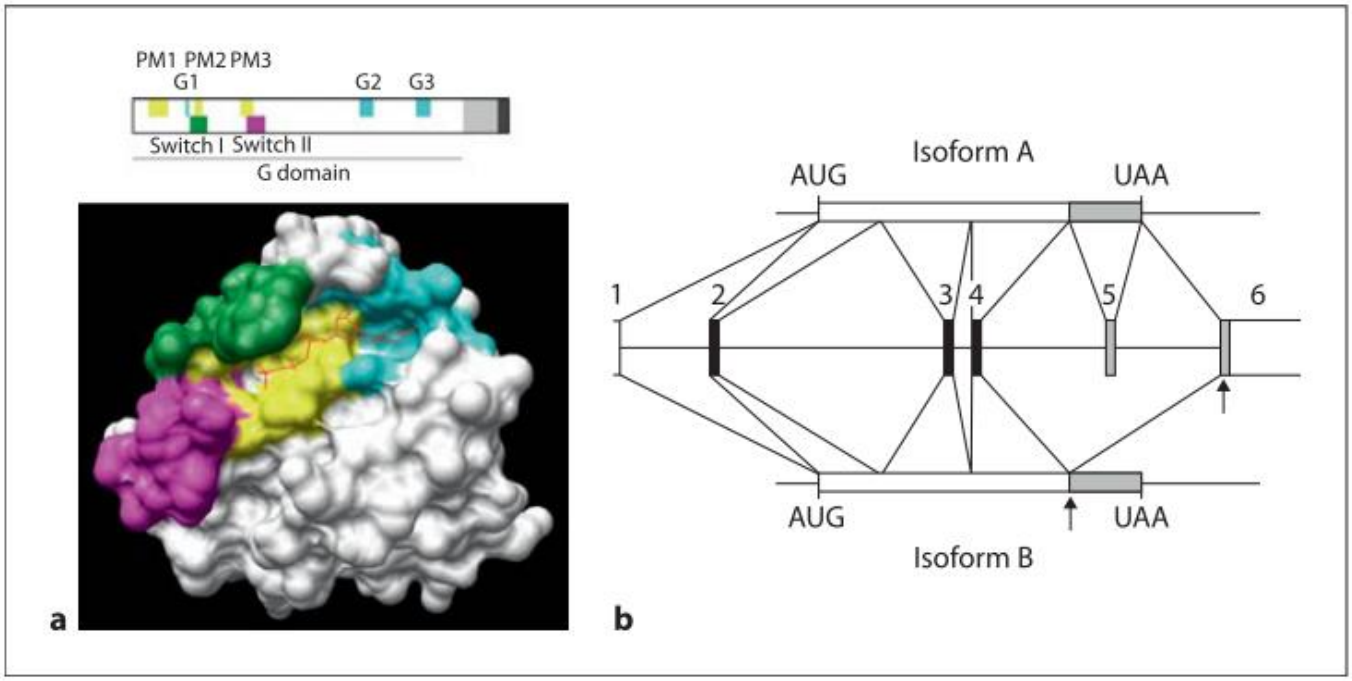


**Figure 7 :** *KRAS* gene organization and protein domain structure(M. Tartaglia & Gelb, 2009).

**(a)** The structural and functional domains identified within RAS proteins are represented schematically and in three dimensions. The conserved domain (G domain) is shown, along with the motifs needed for signaling function (PM1 to PM3 indicate residues involved in binding to the phosphate groups, while G1 to G3 are those involved in binding to the guanine base). The hypervariable region, as well as the C-terminal motifs that direct post-translational processing and plasma membrane anchoring are represented in grey and dark grey respectively. According to the GTP-bound RAS conformation, the GTP/GDP binding pocket is shown in cyan (guanine ring binding surface) and yellow (triphosphate group binding surface), along with the Switch I (green) and Switch II (magenta) domains. **(b)** *KRAS* gene organization and transcript processing result in the alternate KRAS isoforms A and B. The numbered black and grey boxes represent invariant coding exons and alternative splicing exons, respectively. Exon 5 skipping results in *KRAS*B mRNA. Exon 6 of *KRAS*A mRNA encodes the 3′-UTR.(M. Tartaglia & Gelb, 2009)

## 2.4. Other Genes

*RAF1* (v-RAF-1 murine leukemia viral oncogene homolog 1) (OMIM 164760) is a member of the RAF serine-threonine kinase family that transmits RAS signaling upstream to downstream MEK and ERK. It is a member of the proto-oncogene family, and it shares with *ARAF* and *BRAF* three conserved regions, CR1, CR2, and CR3(Kobayashi et al., 2010; M. Abdur Razzaque et al., 2007). Nevertheless, reported *RAF1* mutations in NS were located in the CR2 domain and some in the CR3 domain. Mutations in *RAF1* cause 3–17% of NS cases(Kobayashi et al., 2010). Recent data have suggested that there might be an association between *RAF1* gene mutation and HCM(Ko et al., 2008; Li et al., 2019; Pandit et al., 2007; M. Abdur Razzaque et al., 2007).

*BRAF* (V-Raf murine sarcoma viral oncogene homolog B1) (OMIM 164757), a member of the RAF family, was shown to play a role in Noonan syndrome pathogenesis by enhancing ERK activation(El Bouchikhi et al., 2016). Mutations in the *BRAF* gene appear to underlie only a small fraction of NS (<2%). However, molecular screening of the *BRAF* gene would be worthwhile in individuals with clinical features fitting NS with moderate to severe cognitive deficits(Lee et al., 2011; Sarkozy et al., 2009).

*MEK1* (MAP2K1, mitogen-activated protein kinase 1) (OMIM 176872) is located in the 15q22 region and comprises 11 exons. It is essential for embryonic development, especially cell migration and placental development(El Bouchikhi et al., 2016). For the first time, Nava et al, have shown mutations in *MEK1* related to NS patients(Nava et al., 2007).

*NRAS* (neuroblastoma RAS viral oncogene homolog) (OMIM 164790) is mapped to 1p13.2 region. It contains 7 exons("OMIM Entry - * 164790 - NRAS PROTOONCOGENE, GTPase; NRAS," n.d.). Recent data have demonstrated *NRAS* gene mutations in a few individuals with a clinical diagnosis of NS(Cirstea et al., 2010; Denayer et al., 2012; Runtuwene et al., 2011).

*SHOC2* (leucine-rich repeat scaffold protein) (OMIM 602775) gene is mapped to chromosome 10q25(Selfors, Schutzman, Borland, & Stern, 1998). It is a commonly expressed protein that is almost entirely composed of leucine-rich repeats (LRR) with a lysine-rich sequence at the N terminus. It has been demonstrated that mutations in the *SHOC2* gene revealed a consistent phenotype, previously known as Noonan-like syndrome with loose anagen hair(Cordeddu et al., 2009).

# MATERIALS & METHODS

# I. Samples

Our cohort consists of 54 patients referred to the Medical Genetics and Oncogenetics Unit of the CHU Hassan II of Fez for suspicion of Noonan syndrome. For this study, our analysis focused on negative cases after molecular analysis of the PTPN11 gene.

Following a genetic consultation in which the patient is examined, the results of the additional examinations already carried out are recorded and, a family tree is drawn up and, after informed consent is obtained, we collect a blood sample from the patient on two EDTA tubes (2 to 4ml) for DNA extraction. Extracted DNA can be stored at +4°C for use within 72 hours or -20°C for post-run extraction.

# II. DNA Extraction

DNA extraction is the process of isolating DNA from cells or tissues. The DNA thus extracted is then used for molecular biology research, such as PCR. There are several protocols to extract DNA, but the basic concept is nearly always the same:

- ✓ Cell lysis, which means that a detergent is used to break cell and nuclear membranes.
- ✓ Protein elimination.
- ✓ Elimination of other nucleic acids (RNA ...).
- ✓ DNA precipitation.

## 1. Genomic DNA Extraction by the Pure Link™ Genomic DNA Kit

### 1.1. Overview

The PureLink Genomic DNA Kits are based on the selective binding of DNA to the silica-based membrane in the presence of chaotropic salts. The lysate is prepared from the blood. The cells are digested with proteinase K at 55°C using an optimized digestion buffer formulation that facilitates the denaturing of proteins and reinforces the activity of proteinase K. Lysate is mixed with ethanol and PureLink Genomic Binding Buffer that allows strong DNA binding to the rotation column.

DNA binds to the silica-based membrane in the column, and impurities are removed by deep washing with Wash Buffers. The genomic DNA is then eluted into the low-salt elution buffer.

This technique is simple, faster, and advantageous when there is a small volume of blood.

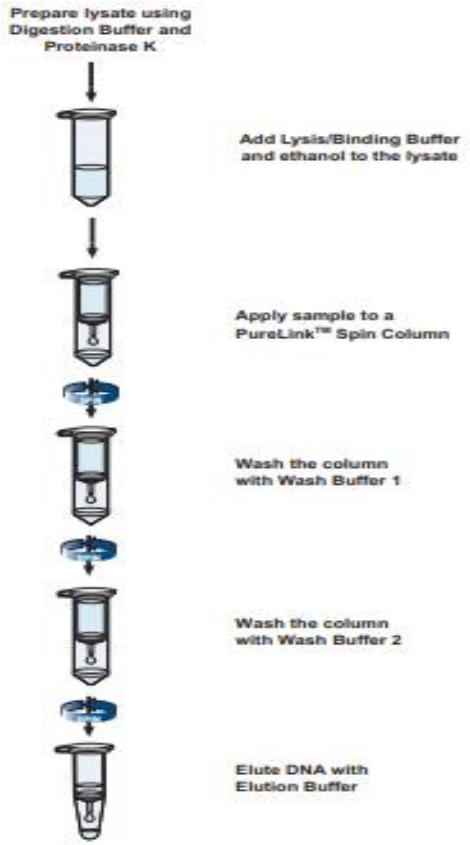


**Figure 8 :** An overview of DNA extraction using The PureLink Genomic DNA Mini Kit. ("PureLink® Genomic DNA Kits," n.d.)

## 1.2. Reagents

Kit Pure Link™ Genomic DNA Kits containing the following solutions :

- 50 ml PureLink™ Genomic Lysis/Binding Buffer
- 45 ml PureLink™ Genomic Digestion Buffer
- 50 ml PureLink™ Genomic Wash Buffer 1
- 37.5 ml PureLink™ Genomic Wash Buffer 2
- 50 ml PureLink™ Genomic Elution Buffer

• 5 ml RNase A (20 mg/ml)
• 5 ml Proteinase K (20 mg/ml)
• 5 × 50 each PureLink™ Spin Columns with Collection Tubes
• 5 × 100 PureLink™ Collection Tubes (2.0 ml)

### 1.3. Protocol

In a 1.5 ml Eppendorf tube, 200µl of blood is mixed with 20µl of Proteinase k and 200µl of lysis solution; vortexed and then incubated for 10min at 60°C. A series of washes are performed using 200µl of absolute ethanol at first. After that, the mixture is returned to a PureLink® Spin Column and centrifuged for 1min at 8000rpm. The collection tube supplied in the kit must be discarded each time. A second wash is done with 400µl of Wash Buffer1, the mixture is centrifuged for 1min at 8000rpm. The last wash is done with 400µl of Wash Buffer2 and centrifuged for 3min at 14000rpm.

The column is placed in a 1.5ml Eppendorf tube, and 70µl of elution buffer is added. Incubation is performed for 1 min at room temperature. Final centrifugation of 1min at 8000rpm is performed to recover purified genomic DNA.

## III. DNA quantification

The concentration of extracted DNA is determined by spectrophotometry; by measuring the optical density at 260 nm (DO260nm), the maximum wavelength at which purine and pyrimidine bases absorb, whereas proteins absorb at 280 nm. One DO unit at 260 nm =50 µg/ml of DNA. The absorbance value (or optical density DO) at 280 nm will determine any protein contamination. Thus, the DO260/DO280 ratio is used to estimate DNA purity. An extracted DNA is considered pure when it has a DO260/DO280 ratio between 1.8 and 2.

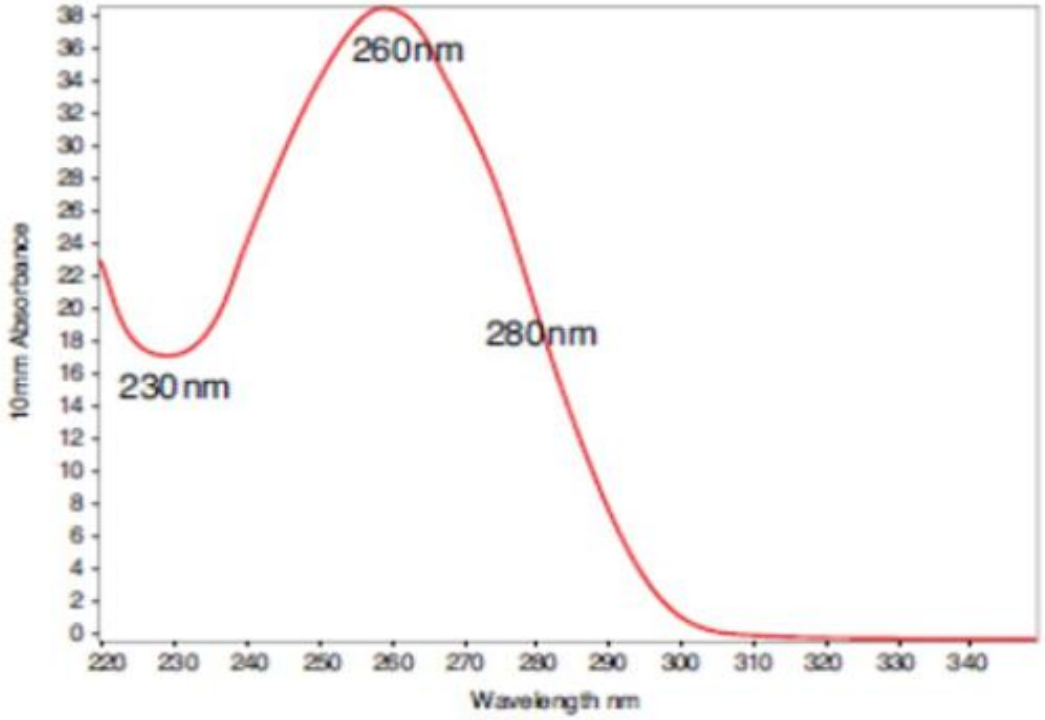


**Figure 9 :** Absorption spectrum of pure DNA.

- If the ratio is >2 : There is RNA contamination.
- If the ratio is <1.8: There is protein contamination.

In the Medical Genetics Laboratory, the NanoVue Plus Spectrophotometer is the instrument used to measure DNA concentration. It is enough to pipette DNA samples of 1 to 2 µl directly onto the sample plate to measure the DNA concentration.

**Figure 10 :** Nanovue spectrophotometer used in Medical Genetics Laboratory.

## IV. Polymerase chain reaction (PCR)

### 1. Overview

Polymerase chain reaction (PCR) is a technique developed in 1983 by Kary Mullis, used for fast in vitro enzymatic amplification of specific DNA sequences(Bartlett & Stirling, 2003). It provides a sufficient and detectable amount of a selected DNA sequence from specific primers; containing sequences complementary to the target region along with a heat-stable DNA polymerase, such as Taq polymerase, are key components to enable selective and repeated amplification. The method relies on thermal cycling, consisting of a succession of many cycles. Each PCR cycle consists of three steps, performed at different temperatures, allowing the control of the enzymatic activity:

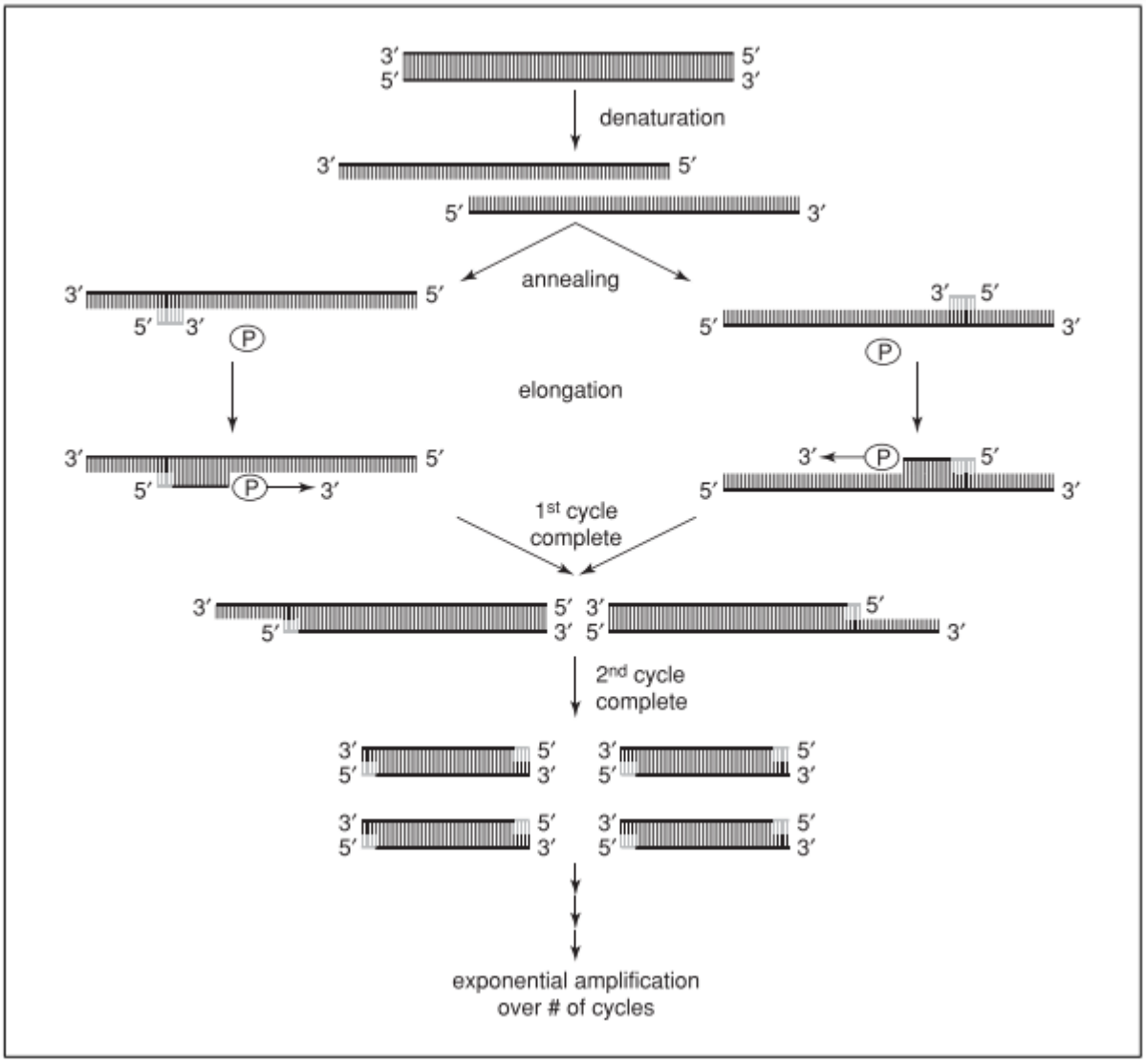


**Figure 11 :** PCR process of the in vitro amplification of DNA(Kuslich, Chui, & Yamashiro, 2019).

• **Denaturation** step is performed at approximately 95°C for complete dissociation of two strands of DNA. Indeed, DNA loses its characteristic structure in a double helix because the hydrogen bonds connecting the bases of each strand of DNA are unstable to this temperature. Double-stranded (2-strand) DNA is denatured into single-strand DNA.

• **Annealing** step is done at a temperature between 50°C and 60°C. This temperature depends on the deoxyribonucleotide composition (dATP, dTTP, dGTP, and dCTP) primers. Primers recognize and attach to their complementary sequences by reformatting hydrogen bonds.

• **Elongation** step is done at approximately 72°C, the optimal functioning temperature of the Taq polymerase. During this step, the strands complementary to the matrix DNA are synthesized from the free 3'OH ends hybridized primers due to free dNTPs present in the reaction medium.

## 2. Protocol

For this study, we are interested in the research of mutations in exon 2 and 3 of the *Kras* gene for our cohort. The primers used to amplify these regions were derived from the work of (Carta et al., 2006).

**Table 4 :** Primer Pairs and Annealing Temperatures (*T*ann) Used to Amplify the *KRAS* gene and the Size of PCR Products.

| Exon | Primer F | Primer R | *T*ann (°C) | Product Length (bp) |
|---|---|---|---|---|
| **2** | GATACACGTCTGCAGTCAACTG | GGTCCTGCACCAGTAATATGC | 58 | 340 |
| **3** | GGTGCACTGTAATAATCCAGACT | CATGGCATTAGCAAAGACTCA | 56 | 300 |

This PCR reaction is carried out using the Kit «DreamTaq Green PCR Master Mix» which is a ready-to-use solution containing: DreamTaq DNA Polymerase, DreamTaq Green buffer optimized, MgCl2, dNTP, and Water (Water nuclease-free).

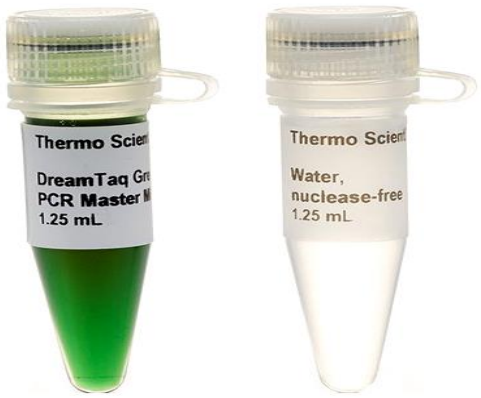


**Figure 12 :** Reagents of the Kit «DreamTaq Green PCR Master Mix».

For each sample, a reaction medium is prepared in an Eppendorf tube. The composition of each Eppendorf tube is detailed in the table below (Table 5 ) :

**Table 5 :** Composition of the reaction medium for the detection of mutations in the kras gene.

| PCR Mix | volume for a reaction (µl) |
|---|---|
| Dream Taq Green PCR Master Mix | 10 |
| Water nuclease-free | 12 |
| Primer F | 1 |
| Primer R | 1 |
| DNA | 1 |

Each tube prepared has thus a final volume of 25 µl.

For each primer, two tubes were prepared, one containing the DNA and the other one includes all PCR reagents except DNA and is called "Negative control"; It ensures the absence of contamination.

**Table 6 :** PCR Tubes.

| Tube 1 | Tube 2 |
|---|---|
| Negative control without DNA | DNA |

The tubes are then centrifuged for 1 min, then deposited in a thermocycler (Applied Biosystem); amplification is carried out in 35 temperature cycles, preceded by an initial denaturation step of 3 min at 95°C, and completed with a final 9 min at 72°C elongation step.

**Table 7 :** Cycle program T° for the amplification of exon 2 and 3 of the Kras gene in the thermocycler.

| Step | | T° | Duration | Number of cycles |
|---|---|---|---|---|
| **Initial Dénaturation** | | 95°C | 3 min | X 1 |
| **Dénaturation** | | 95°C | 30 secs | X 35 |
| **Annealing** | Exon 2 | 58 °C | 30 secs | |
| | Exon 3 | 56°C | | |
| **Elongation** | | 72°C | 1 min | |
| **Final Elongation** | | 72°C | 9 min | X 1 |

## V. Visualization of PCR Product by Agarose Gel Electrophoresis

### 1. Overview

By agarose gel electrophoresis, DNA fragments (e.g., PCR products) are separated according to size. When applying an electrical field across the agarose gel, the DNA; which is negatively charged, will migrate towards the anode. The larger fragments migrate slowly because of their progress; is encounter obstruction from the gel matrix. Indeed, this method is used in molecular biology to confirm; the success of the gene amplification and verify the size of the amplified DNA fragment.

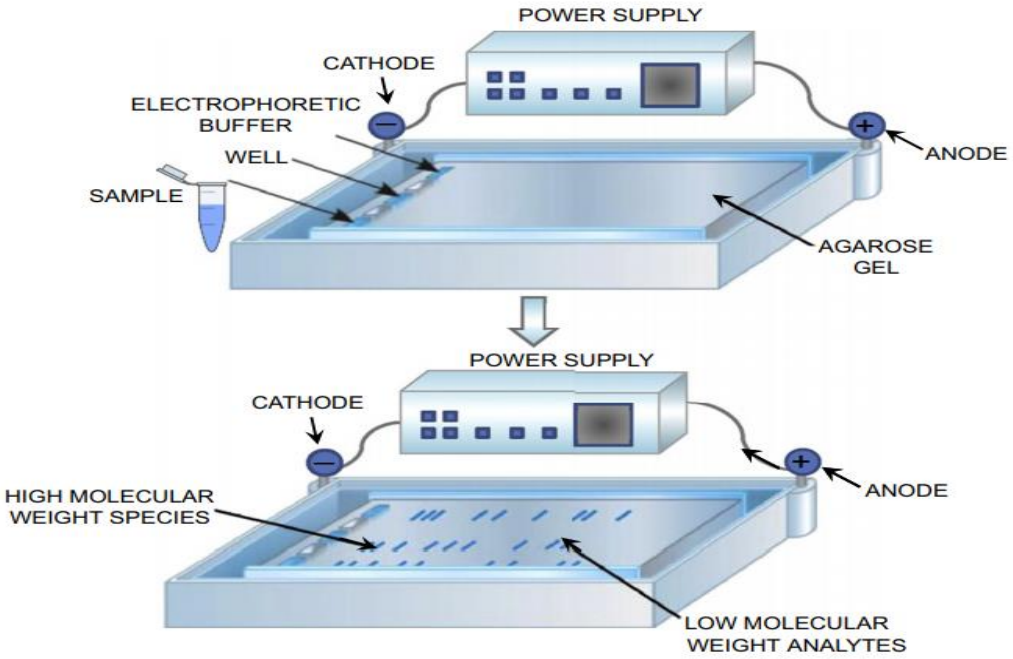


**Figure 13:** Agarose electrophoresis system(Drabik, Bodzoń-Kułakowska, & Silberring, 2016).

## 2. Protocol

Preparation of 2% agarose gel: 2g of agarose gel is weighed and mixed with 100ml of the gel running buffer TBE (45 mM Tris-borate; 1 mM EDTA). The agarose/buffer mixture is melted by heating in a microwave until boiling and obtaining a transparent liquid. 4µl of ethidium bromide (EtBr) is added. The mixture is poured then into a gel casting tray with a comb. The wells formed within the gel are used to deposit the samples to be tested (PCR products). After cooling (about 30min), the gel is then placed in the gel box filled with the migration buffer.

Setting up of Gel Apparatus and Separation of DNA Fragments : 6 µl of the PCR product is deposited directly in the bottom of the gel well. The leads of the gel box are attached to the power supply, with the cathode (black leads) closer to the wells than the anode (red leads).

Visualization of PCR products by ultraviolet light: After PCR products have migrated, the gel is removed from the gel casting tray and exposed to UV light. DNA bands should show up as orange fluorescent bands.

If the PCR results are successful, sequencing is performed to detect genetic mutations.

# VI. Sequencing

## 1. Purification of the PCR product by ExoSAP

Before sequencing the DNA, the amplification product to be sequenced must be purified. For our study, we used the ExoSAP kit to purify our previously amplified DNA.

ExoSAP (Exonulease and Shrimp alkaline phosphatase) uses two hydrolytic enzymes, exonuclease I (Exo) and shrimp alkaline phosphatase (SAP). Exonuclease I removes primers single strand residual and all single strand foreign DNAs produced in the PCR. The Alkaline phosphatase removes the remaining dNTPs in the PCR mixture. This method is designed to require a minimum of manipulation time.

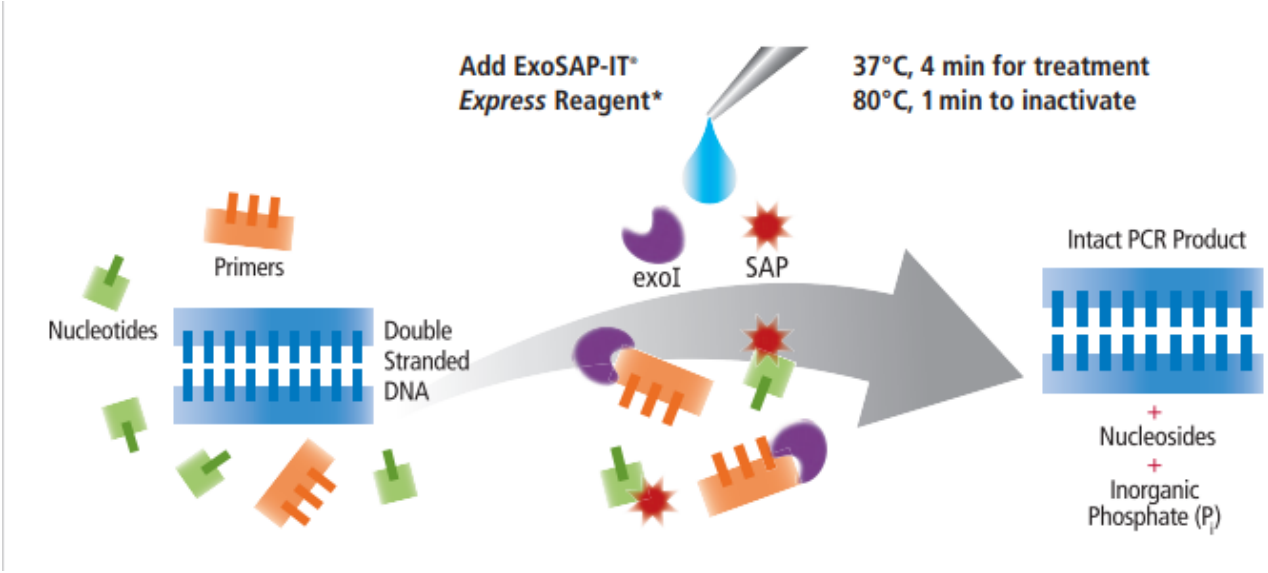


**Figure 14:** Summary of ExoSap-IT PCR product treatment(*ExoSAP-IT ® PCR Cleanup reagent.*, n.d.).

For the purification of the PCR products of this study, 2.5 µl of exoSAP was added directly to 5.5 µl of PCR product and then centrifuged for 1 minute at 8000 rpm and incubated at 37°C for 5 min. The ExoSAP reagent was then inactivated by a simple 80°C heater for 1 min.

## 2. BigDye® Terminator V3.1 Sequencing Reaction

DNA sequencing involves determining the sequence of nucleotides (A, C, G, and T) that make up a given DNA molecule. Currently, it has become a routine technique for molecular biology laboratories. In our study, we based on the SANGER method for sequencing.

The principle of SANGER sequencing is to perform a specific PCR reaction (sequence reaction) containing, in addition to the usual reagents, four types of didésoxyribonucleotide (ddATP, ddCTP, ddGTP et ddTTP) marked by different fluorochromes that block the PCR reaction and lead to the release of fragments of different sizes which all contain one of the ddNTPs at the end of their respective sequences. The fluorescence emitted by the different ddNTP will be captured and transformed into a peak of different colors corresponding to the sequence of bases relative to each patient.

The BigDyeTerminator (BDT) technology uses a resonant energy transfer (FRET) system between two fluorochromes attached to the same ddNTP and linked together by a linker. The first is fluorescein (6 carboxyfluorescein) called donor fluorochrome, common to the four ddNTP. The second is DichloroRhodamines (dRhodamine) which acts as an acceptor fluorochrome. In addition, the emission spectrum of dichloroRhodamine is different for each type of ddNTP which makes the spectrum of the fluorescence emitted, specific for each type of ddNTP.

The composition of the reaction mixture of the sequenced PCR as well as the programming conditions are summarized in Tables 8 and 9.

**Table 8:** The composition of the reaction mixture of the sequenced PCR.

| Reagents | Volume (µl) |
|---|---|
| **Big Dye terminator V3.1** | 1 µl |
| **Primers (F or R)** | 1 µl |
| **Sterile water** | 4 µl |
| **Purified PCR product** | 4 µl |

**Table 9:** Cycles and program T° for PCR product sequencing in the thermocycler.

| Temperature and cycles | | Duration |
|---|---|---|
| 96°C | | 1 min |
| 95°C | | 10 sec |
| 50°C | × 25 | 5 sec |
| 60°C | | 5 min |
| 8°C | | ∞ |

## 3. Purification of sequencing products and determination of the sequence

Once the sequence reaction is complete, it is necessary to purify the sequenced products. For this purification, the BigDye® Xterminator™ kit was used. This kit consists of two reagents: one containing the “SAM Solution" (store at room temperature or 4°C) and The other is the "XTerminator Solution" (store at 4°C). This kit allows the capture of dyes not incorporated in the sequence reaction, salts, and other charged molecules that can interfere with the detection of bases by capillary electrophoresis.

For this study, 45µl of the SAM solution and 10µl of the XTerminator solution was added to the sequence reaction products. This mixture is vortexed for 30 min at 1800rpm and then centrifuged for 3 min at 100000rpm. After that, 10 µl of the supernatant of each tube are pipetted and placed on a plate ( MicroAmp™ Optical 96-Well Reaction Plate).

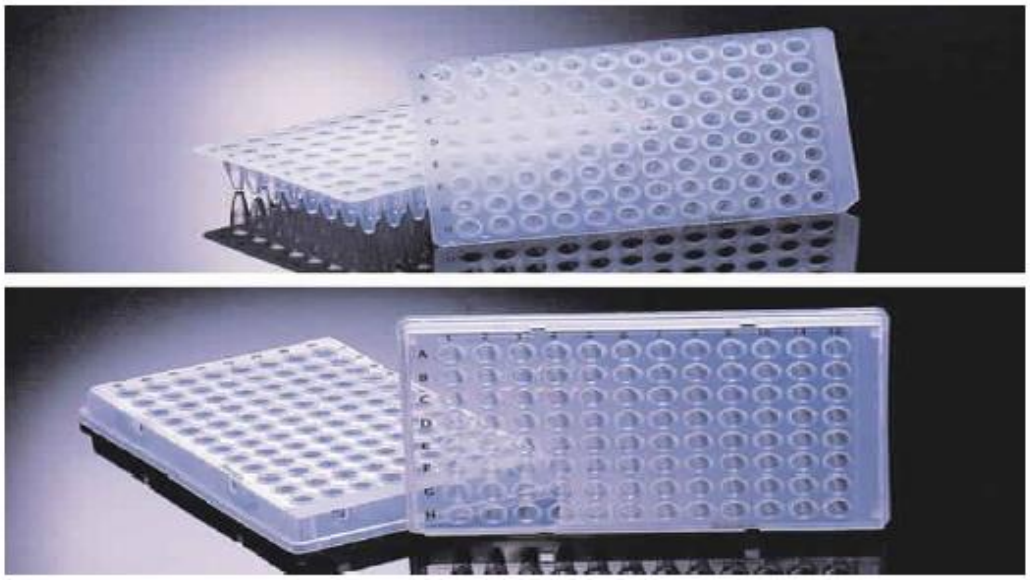

**Figure 15:** MicroAmp™ Optical 96-Well Reaction Plate.

This reaction plate is placed in the Applied Biosystems DNA analyzer available in the laboratory (Figure 16). The electropherograms are then analyzed using Sequencing Analyzing software and compared to databases.

**Figure 16:** The automatic sequencer (Genetic Analyzer 3500 Dx) available in the Genetic laboratory ( CHU fez).

## VII. Bioinformatics tool

Bioinformatics is becoming an indispensable tool for data processing in molecular biology laboratories. However, The results of molecular biology are always highlighted by bioinformatics tools.

In this study, we used the following sites and databases:

- **BLAST (http://blast.ncbi.nlm.nih.gov)**: The sequences of the different patients were aligned with those in the NCBI database via the BLAST tool. In short, the principle of this tool is to identify homologous sequences by searching one or more databases usually hosted by NCBI on the query sequence of interest. After this analysis, BLAST presents the information of the most homologous sequence with the corresponding percentage of identity. It also displays the detailed alignment of homologous sequences.

- **NCBI- CCDS database (https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi):** The Consensus Coding Sequence (CCDS) displays the consensus coding sequence of the searched gene, starting with the ATG start codon; and ending with the stop codon. It also displays the protein sequence of the searched gene, which allows the localization of the mutation at the level of the coding part of the studied gene and eventually to know the amino acid affected by the mutation on the corresponding protein sequence.

# Results & Discussion

# I. Results

## 1. Epidemiological data

### 1.1. Distribution by gender

NS affects both sexes equally but may be more recognizable in boys as they may have cryptorchidism. In this study, we noted a predominance of males with a sex ratio of 2 (36M/18F).

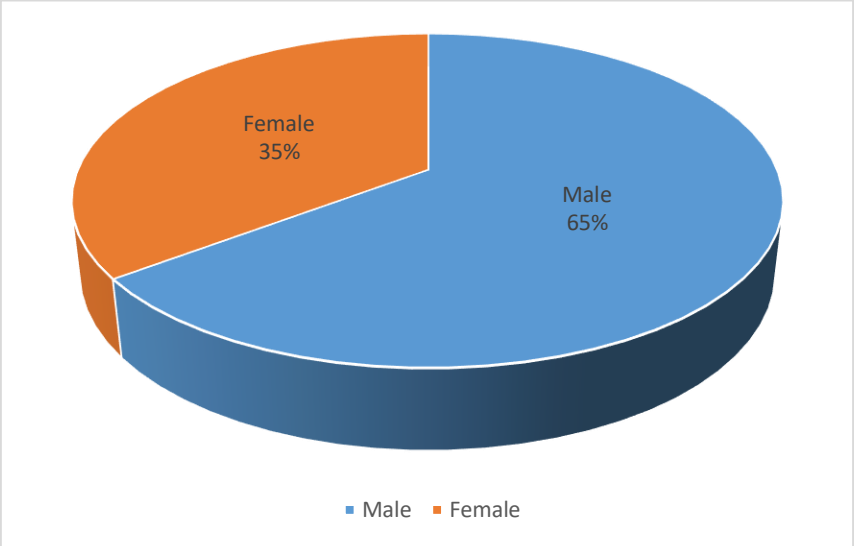


**Figure 17:** Distribution of patients according to gender.

## 1.2. Age distribution

The phenotypic characteristics of NS may vary depending on the age of the patient. In addition, the age range of the patients in our series was from 2 days to 14 years, with a median age of 4 years.

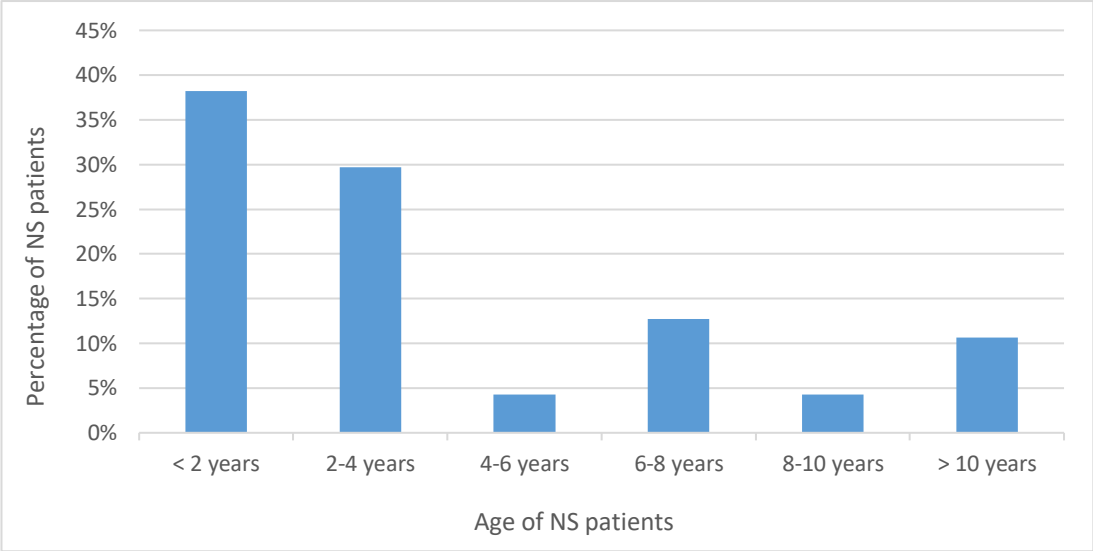


**Figure 18:** Distribution of patients according to diagnosis age.

## 1.3. Distribution according to consanguinity

NS occurs sporadically in 60% of cases or according to an autosomal dominant transmission(Shaw, Kalidas, Crosby, Jeffery, & Patton, 2007; Marco Tartaglia & Gelb, 2005).

Furthermore, 90% of our cohort cases occurred sporadically and, only 10% occurred autosomally.

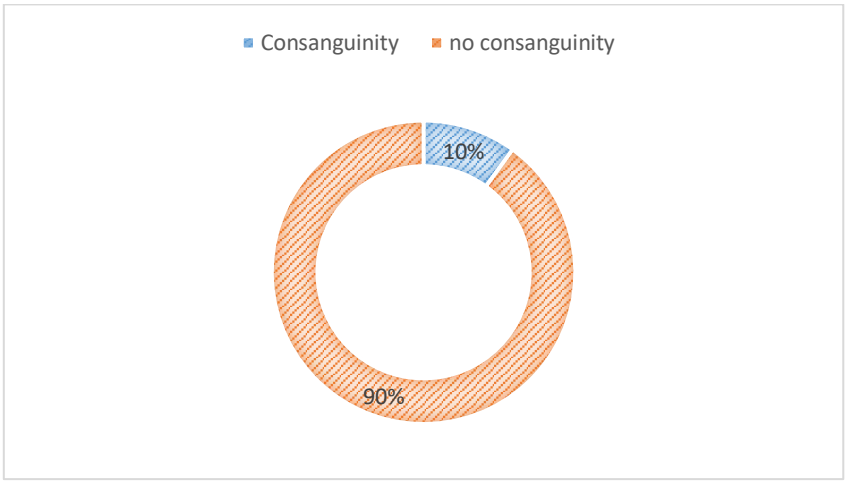


**Figure 19:** Distribution of patients according to consanguinity.

## 2. Clinical data

### 2.1. Distribution according to facial features

The clinical examination of our cohort finds a dysmorphic syndrome made of facial dysmorphia (hypertelorism, ptosis, tall forehead, Short neck**,** Antimongoloid eye slant, micrognathia, Posteriorly rotated ears).

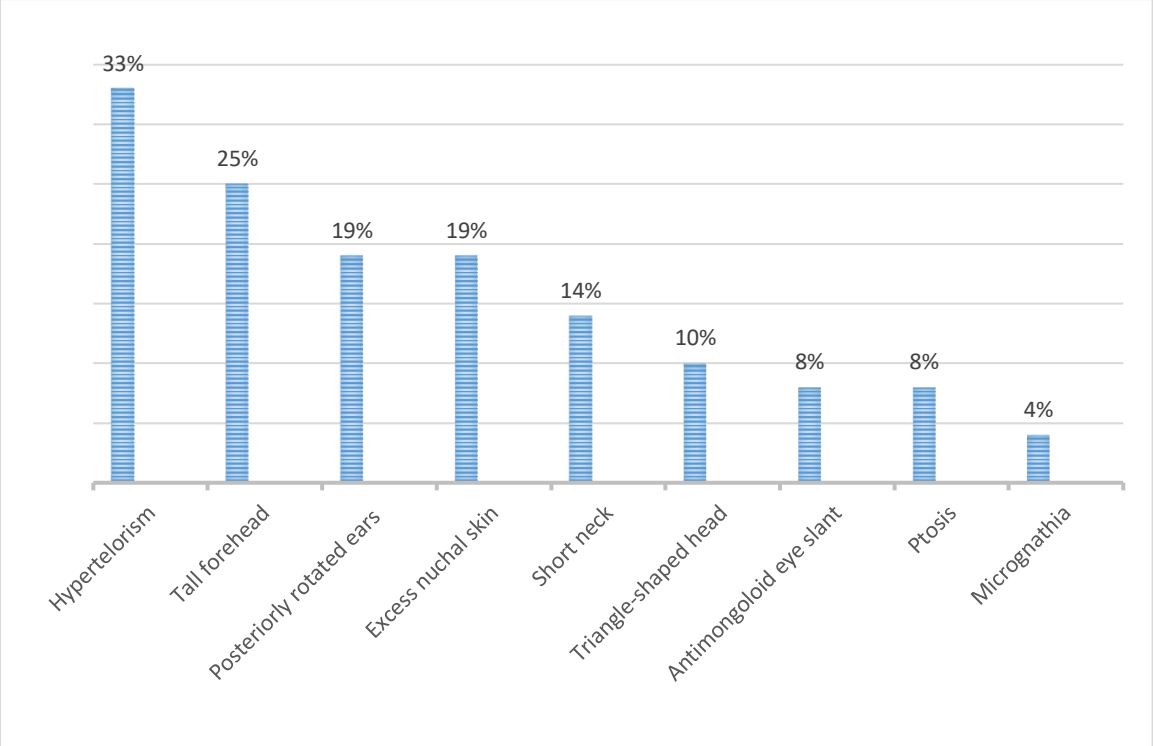


**Figure 20:** Distribution according to facial features.

## 2.2. Distribution according to cardiovascular features

Cardiovascular features are often the diagnostic entry point before dysmorphia or growth retardation. In our cohort, PS was detected in 20 cases (61%) and was usually due to a dysplastic pulmonary valve. HCM was detected in 7 cases (23%), and ASD in 4 cases (16%).

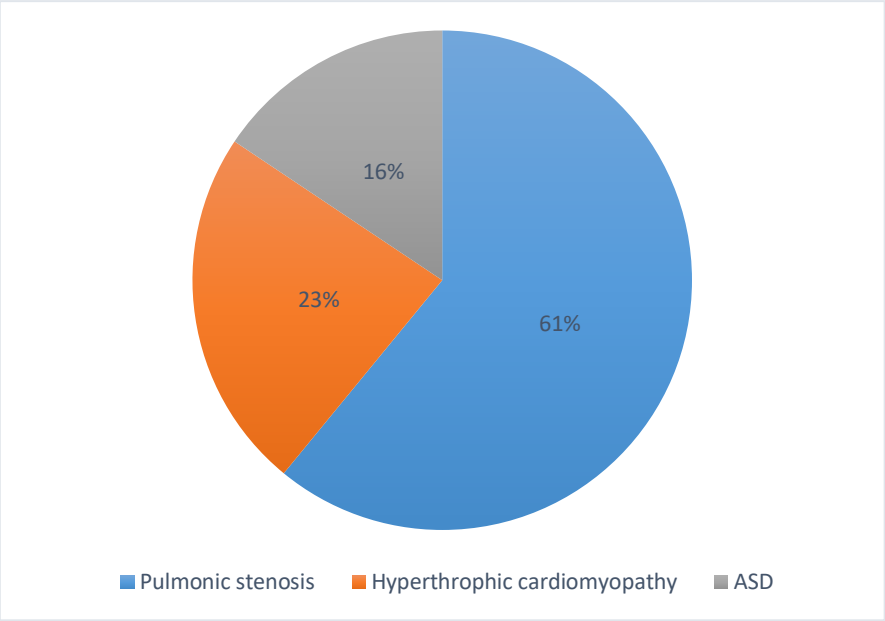


**Figure 21:** Distribution of patients according to cardiovascular features.

## 2.3. Distribution according to other features

In our NS patient, a height percentage less than -2DS was observed in 36% of our cohort.

Ectodermal data obtained for our cohort showed a pigmentation anomaly of the Cafe-au-lait spots type in 9% of our series.

A lower prevalence of cryptorchidism (5%) was observed in the boys of our cohort.

The neurological data obtained for our cohort showed mental retardation in 20% of our series.

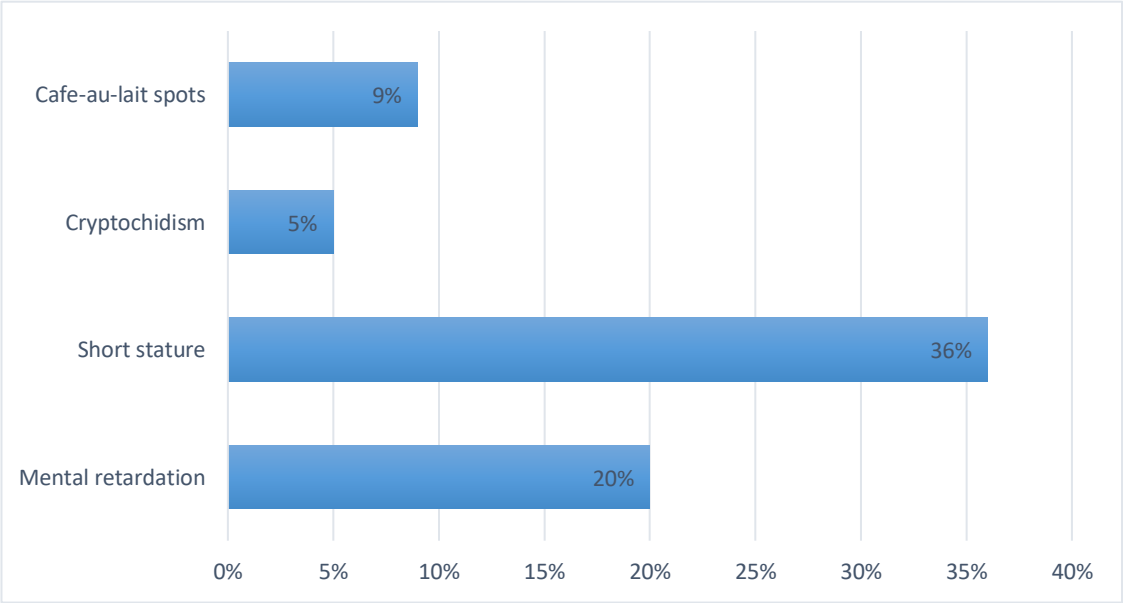


**Figure 22:** Distibution of patients according to other features

# 3. Results of the genetic analysis

## 3.1. Quantification of extracted DNA

After genomic DNA extraction from whole blood of the 54 NS patients using the INVITROGEN kit. DNA purity and concentration are measured using a Nanodrop spectrophotometer suitable for measuring 1 to 2µL of DNA. DNA concentration is estimated by the optical density at 260nm, and the purity is evaluated by the ratio of its optical density (DO260nm/DO280nm).

The concentration result of the extracted DNA in our series ranged from 38.3 ng/µl to 156 ng/µl, the DO 260/280 ratios ranged from 1.84 to 1.98.

Therefore, all of our extracted DNA samples were of good quality, which shows that we were successful in our extraction.

**Table 10:** Extracted DNA concentration of 15 NS patients.

| **Patient** | **DNA concentration ( ng/$\mu$L )** |
|---|---|
| **1** | 138 ng/µl |
| **2** | 119.4 ng/µl |
| **3** | 53.6 ng/µl |
| **4** | 75.8 ng/µl |
| **5** | 94.2 ng/µl |
| **6** | 40.9 ng/µl |
| **7** | 73.5 ng/µl |
| **8** | 84 ng/µl |
| **9** | 60 ng/µl |
| **10** | 75.7 ng/µl |
| **11** | 156ng/µl |
| **12** | 38.3 ng/µl |
| **13** | 50.4 ng/µl |
| **14** | 40.5 ng/µl |
| **15** | 90 ng/µl |

## 3.2. Kras gene amplification by PCR

As part of the Kras gene mutations research, an amplification of exon 2 and 3 of the Kras gene was carried out by the PCR technique after DNA extraction by the commercialized KIT "DreamTaq Green PCR Master Mix".

Then, the quality of the PCR product was verified by agarose gel electrophoresis in the presence of negative control. The visualization of the DNA fragments is done after exposure of the gel to ultraviolet light.

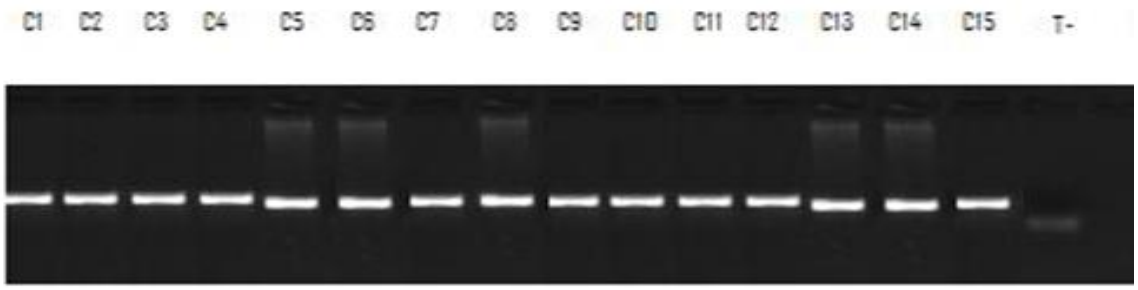


**Figure 23:** 2% agarose gel electrophoresis of PCR amplification products with exon 2 primers in 15 NS patients.

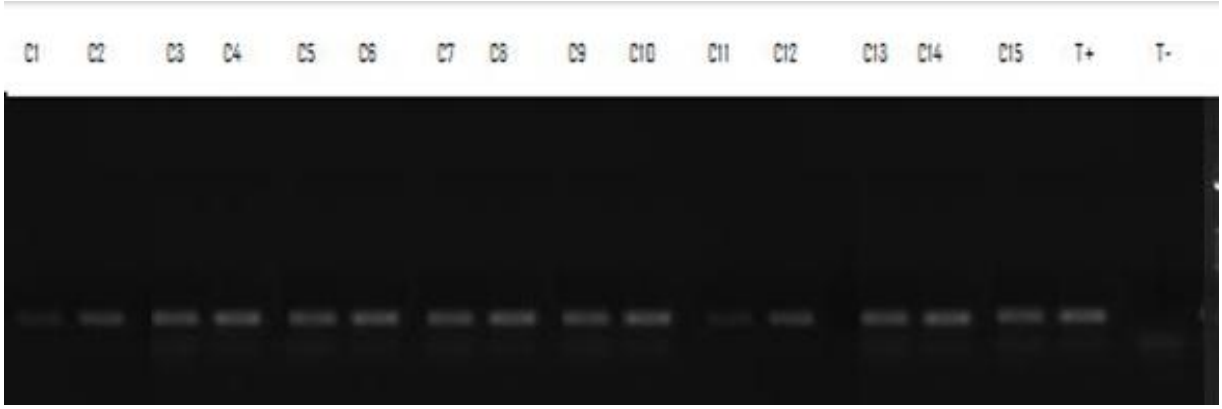


**Figure 24:** 2% agarose gel electrophoresis of PCR amplification products with exon 3 primers in 15 NS patients.

Kras exon 2 and 3 electrophoresis gel profile (Figure 23 and figure 24) results; show that amplified PCR products do not exhibit contamination. This observation is confirmed by the negative control which, shows no band on the gel.

## 3.3. Sequencing results of purified PCR products of the Kras gene

The sequencing reaction of the 54 NS patients, as well as the bioinformatics analysis of the sequences obtained (alignment by BLAST and correction of the sequences obtained by the Sequencing Analysis Software v5.4) of the NS patients, showed an absence of mutations in exon 2 and 3 of the Kras gene in these patients.

An example of an electropherogram of a patient without a mutation in exon 2 of the Kras gene is showed below :

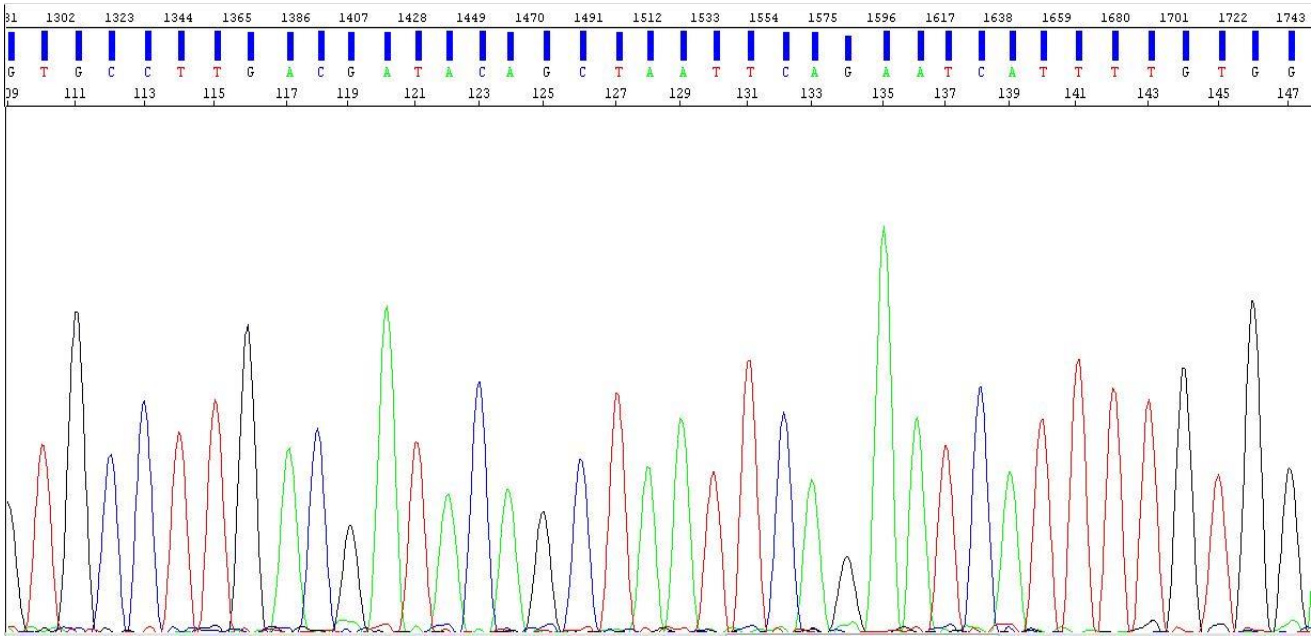


**Figure 25:** Electropherogram of an exon 2 sequence of the Kras gene of a non-mutated NS patient.

Alignment with a BLAST database sequence confirmed the result obtained ( Figure 26):

Download GenBank Graphics

**Homo sapiens chromosome 12, GRCh38.p13 Primary Assembly**

Sequence ID: NC_000012.12 Length: 133275309 Number of Matches: 1

Range 1: 25227195 to 25227400 GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 381 bits(206) | 1e-103 | 206/206(100%) | 0/206(0%) | Plus/Minus |

Features: gtpase kras isoform b
gtpase kras isoform b

```
Query  1         AAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGTCAA  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  25227400  AAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGTCAA  25227341

Query  61        GAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGTA  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  25227340  GAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGTA  25227281

Query  121       TTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGGTGGGTTTAAATT  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  25227280  TTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGGTGGGTTTAAATT  25227221

Query  181       GAATATAATAAGCTGACATTAAGGAG  206
               ||||||||||||||||||||||||||
Sbjct  25227220  GAATATAATAAGCTGACATTAAGGAG  25227195
```

**Figure 26:** Sequence alignment of exon 2 in a non-mutated NS patient.

An example of an electropherogram of a patient without a mutation in exon 3 of the Kras gene is showed below :

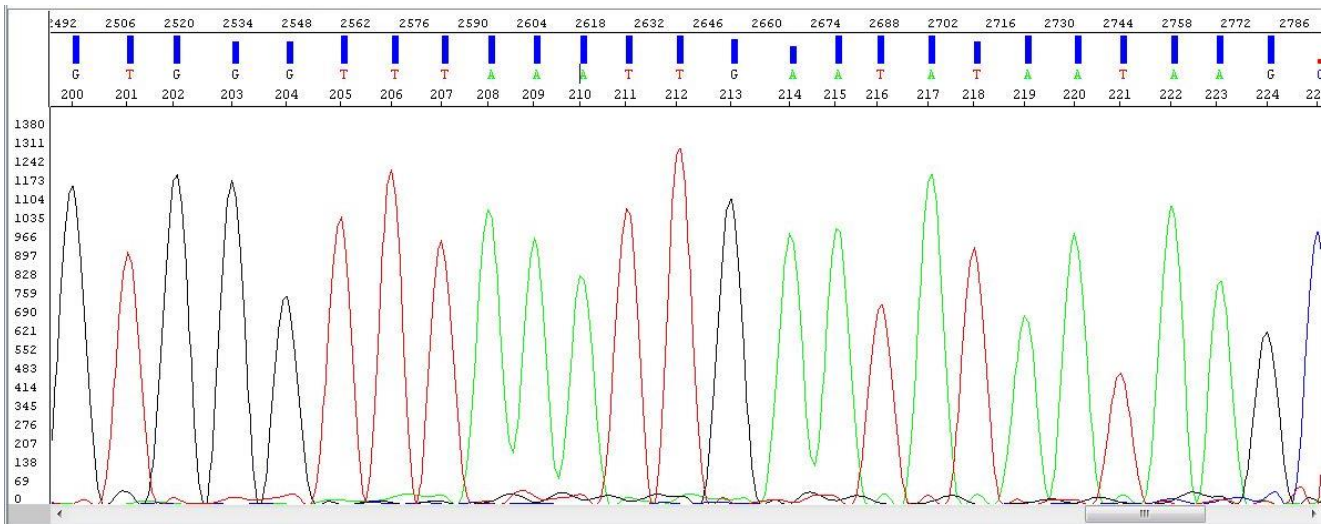

**Figure 27:** Electropherogram of an exon 3 sequence of the Kras gene of a non-mutated NS patient.

Alignment with a BLAST database sequence confirmed the result obtained ( Figure 28):

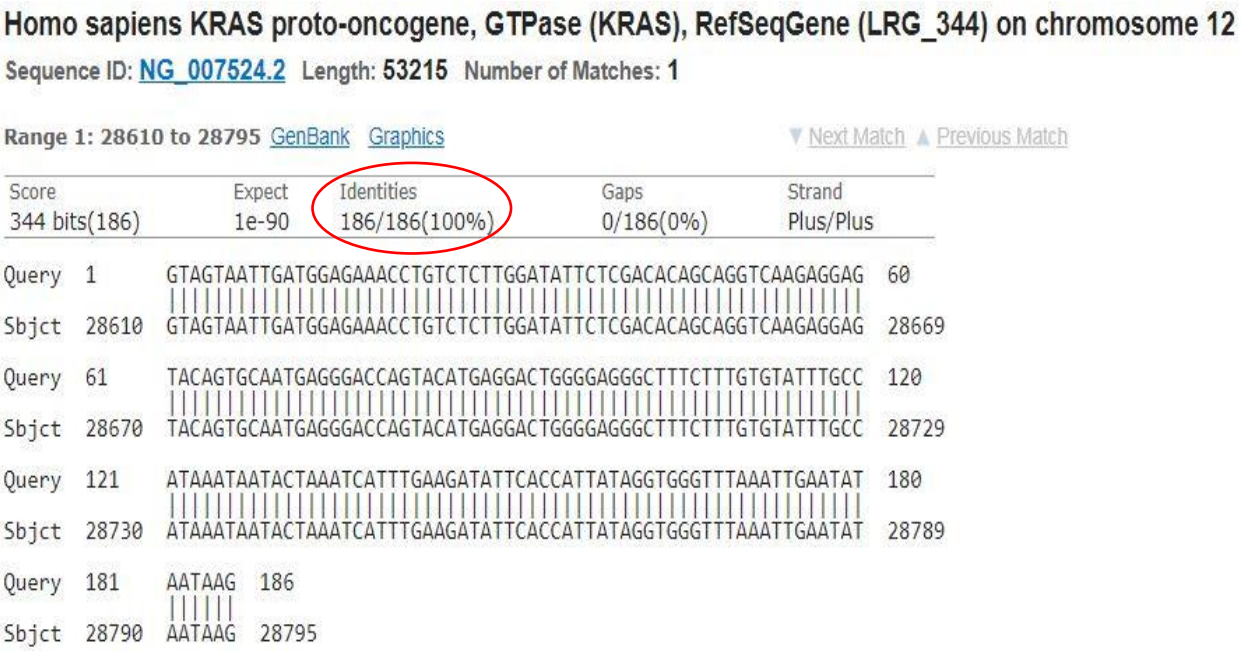


**Figure 28:** Sequence alignment of exon 3 in a non-mutated NS patient.

In summary, sequence analysis of exons 2 and 3 for the Kras gene in our cohort revealed the absence of mutations at these two exons in the Kras gene.

## II. Discussion

In our cohort, more than 50% of the cases present morphological features classically described in the literature(J. E. Allanson, 1987; Marco Tartaglia et al., 2002) with ear anomalies ( posterior rotation, thick ears, and large lobes), a triangular face, eye anomalies (hypertelorism, down-slanting palpebral fissure, and ptosis), low capillary implantation, a short neck, an excess nuchal skin, and micrognathia.

The 3 most common cardiac anomalies found in our cohort were pulmonary valve stenosis, HCM, and ASD. These anomalies appear isolated or most often associated with each other. These results are relevant to those of the literature. However, if we look at the order of frequency of the 3 main heart diseases in our series, we find, in decreasing order, pulmonary valve stenosis, which is the most frequent cardiopathy, followed by HCM and then ASD (61% > 23% > 16%). These results are in agreement with the data in the literature(Sharland, Burch, McKenna, & Paton, 1992; Van Der Burgt, 2007).

A lower prevalence of height was observed in our cohort. Women were 154 cm and, men 161 cm. These results are consistent with the reference data in the literature, which show a mean final height in adulthood for NS patients at 152.7 cm for women and 162.5 cm for men(Ranke et al., 1988).

Children with NS usually have mental retardation characterized by visual constructional problems and verbal performance discrepancy; it is present in 15-35% of cases(Van Der Burgt, 2007). Our results are consistent with those of the literature. Mental retardation was observed in 30% of our series.

A lower prevalence of cryptorchidism in males of our cohort was found with an incidence of around 5%. The result of our series is in disagreement with the literature(J. E. Allanson, 1987).

The discrepancy observed for this criterion in our cohort compared to the literature may be related to the low clinical data obtained.

Ectodermal anomalies, including cafe-au-lait spots, are found in 10% of NS patients(J. E. Allanson, 1987). This anomaly was found in 9% of our cohort. Therefore this result is associated with the literature data.

Moreover, Moroccan studies were previously conducted concerning only the *PTPN11* gene; the first one consisted of a molecular exploration of the DNA of two brothers with NS syndrome, in whom the article reports a mutation of *PTPN11* in the form of germline mosaicism (Elalaoui et al., 2010). As well as a study conducted at the Department of Medical Genetics of the CHU Hassan II, Fez, in a cohort of 16 NS patients that revealed the presence of 3 heterozygous mutations (Asp61Gly, Tyr63Cys, and Asn308Ser) in 4 individuals(El Bouchikhi et al., 2015). In addition, a recent study performed in the Medical Genetics Department by EL Bouchikhi et al., in a cohort of 31 patients with Noonan syndrome reported the presence of five pathogenic mutations, one synonymous variant with a potential altering effect on splicing function, and three novel intronic duplications in seven hotspot exons (2, 3, 4, 7, 8, 12, and 13) of the PTPN11 gene(El Bouchikhi et al., 2020). Therefore, the present work constitutes the first experience in Morocco concerning molecular study of a population (a series of patients) with NS for the *Kras* gene.

The second work presented in this project was, thus, to search for *Kras* gene mutations responsible for NS in our cohort. Since we could not receive the primers for exons 4, 5, and 6 of the Kras gene, we were able to look just for mutations at exons 2 and 3 of the *Kras* gene. The amplification step for those two exons was done by simplex PCR followed by sequencing based on the sanger principle.

So far, mutations screening for exons 2 and 3 of the Kras gene; revealed; the presence of a novel heterozygous mutation in exon 3 of the KRAS gene (c.214A>C) in a mother and two siblings included in an ongoing cohort study of NS in Brazil(Brasil et al., 2012). Another study performed on a cohort of 59 Korean patients showed a (V14I) mutation in exon 2 of the Kras gene in one patient(Ko et al., 2008).

However, The results of our study showed the absence of mutations at exons 2 and 3 for the *Kras* gene in the 54 patients studied at the Department of Medical Genetics of the CHU Hassan II, Fez. This result confirms the minor contribution of this gene in the NS(Nava et al., 2007; Schubbert et al., 2006).

# CONCLUSION & FUTURE PERSPECTIVE

Congenital heart diseases are the most common congenital malformation. They are associated with high mortality and spontaneous abortion rates.

These malformations occur during cardiogenesis and are caused by genetic and environmental factors. Genetic factors include chromosomal aberrations (number or structure) and gene alterations (substitution, duplication, insertion, or deletion of one or more base pairs) that mainly affect the locus involved in the cardiogenesis process.

The genetic etiology of congenital heart disease has been extensively studied in several populations notably, Chinese, American, Italian, and Japanese populations. Whereas on the African population few studies have been reported.

In addition to isolated congenital heart disease, we were interested in the molecular exploration of a Moroccan population affected by Noonan syndrome since it contains high rates of congenital heart disease, ranging from 78% to 100% in some studies.

NS is a genetic disorder with an autosomal dominant inheritance that can also occur sporadically. It is characterized by typical facial features, congenital heart defect, short stature, and mental retardation.

The classic malformation of cardiovascular anomalies is pulmonary stenosis, often accompanied by valvular dysplasia, followed by hypertrophic cardiomyopathy, atrioventricular canal defect, and ventricular septal defect.

The mutations that cause NS alter genes encoding proteins with roles in the RAS-MAPK pathway, leading to deregulation of this pathway, the first gene described was *PTPN11* located on chromosome 12, followed by *SOS1, RAF1, KRAS, BRAF, NRAS, MAP2K1,* and *RIT1,* and recently *SOS2, LZTR1*, and *A2ML1*, among others.

Diagnosis can be made based on clinical features. However, molecular genetic testing can confirm the diagnosis in 60% of cases.

In light of the discovery progress of genetic factors causing NS, we considered it relevant to review the literature that focuses on the molecular screening of series of patients with this syndrome and with no mutation in the *PTPN11* gene. The aim was to specify; the degree of involvement of each of the most involved genetic factors. This analysis allowed us to deduce that the main components of the genetic etiology are *SOS1, Kras, RAF1,* and *RIT1* genes. These genes deserve to be systemically explored in all patients with Noonan syndrome.

Based on this analysis, we deduce that mutations in the Kras gene account for about 3% of NS cases and can be associated with other Rasopathies such as CFC and CS. However, according to Schubert et al, *KRAS* was the second reported gene for NS, and its mutation was first reported in a minority (five patients, ~4%) of 124 individuals with NS without *PTPN11* mutations. Moreover, Kras gene mutations have been reported in NS patients with severe phenotypes and mental retardation.

This work constitutes the first experience in Morocco concerning the molecular study of the *Kras* gene in a series of patients with congenital heart disease notably, with suspicion of NS and was tested negatively of the *PTPN11* gene at the Genetics and Oncogenetics Unit of the Hassan II University Hospital Center in Fez.

This project aimed to approximate the genetic etiology of the main congenital cardiopathies through molecular research of 54 Moroccan patients diagnosed with NS and tested negatively of the *PTPN11* gene.

Our study approach consisted of molecular identification and bioinformatics characterization of the Kras gene variants likely to disrupt the mechanism of cardiogenesis in the population concerned.

Through this study, and because we were unable to receive exon 4, 5, and 6 primers to perform a complete screening for the Kras gene in our population, we have identified the absence of mutations at exons 2 and 3 for the Kras gene in our cohort.

This work opens several research perspectives; in particular, it is necessary to look for mutations in exons 4, 5, and 6 of the Kras gene for the NS population studied at the Department of Medical Genetics of the CHU Hassan II, Fez to elucidate and clarify the sequence variants or rather the polymorphisms that can be detected in the Kras gene causing NS.

Finally, it would also be very interesting to study mutations in the SOS1 gene which is responsible for almost 20% of NS patients.

## References

Addissie, Y. A., Kotecha, U., Hart, R. A., Martinez, A. F., Kruszka, P., & Muenke, M. (2015). Craniosynostosis and Noonan syndrome with KRAS mutations: Expanding the phenotype with a case report and review of the literature. *American Journal of Medical Genetics, Part A*, *167*(11), 2657–2663. https://doi.org/10.1002/ajmg.a.37259

Allanson, J. E. (1987). Noonan syndrome. *Journal of Medical Genetics*, Vol. 24, pp. 9–13. https://doi.org/10.1136/jmg.24.1.9

Allanson, J. E. (2009). The clinical phenotype of Noonan syndrome. *Monographs in Human Genetics*, *17*, 9–19. https://doi.org/10.1159/000160047

Allanson, Judith E., & Roberts, A. E. (2021). NOONAN SYNDROME. In *Cassidy and Allanson's Management of Genetic Syndromes* (pp. 651–669). https://doi.org/10.1002/9781119432692.ch41

Amanda Salem Brasil,1 Alexandre C. Pereira,2 Luciana Turolla Wanderley,1 Chong Ae Kim,1 Alexsandra C. Malaquias,3 Alexander A.L. Jorge,3 Jose´ Eduardo Krieger, 2 and De´ bora Romeo Bertola1, & Noonan. (1981). PTPN11 and KRAS Gene Analysis in Patients with Noonan and Noonan-Like Syndromes. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.

Baban, A., Olivini, N., Lepri, F. R., Calì, F., Mucciolo, M., Digilio, M. C., … Drago, F. (2019). SOS1 mutations in Noonan syndrome: Cardiomyopathies and not only congenital heart defects! Report of six patients including two novel variants and literature review. *American Journal of Medical Genetics, Part A*, *179*(10), 2083–2090. https://doi.org/10.1002/ajmg.a.61312

Bartlett, J. M. S., & Stirling, D. (2003). A Short History of the Polymerase Chain Reaction. In *PCR Protocols* (pp. 3–6). https://doi.org/10.1385/1-59259-384-4:3

Brasil, A. S., Malaquias, A. C., Kim, C. A., Krieger, J. E., Jorge, A. A. L., Pereira, A. C., & Bertola, D. R. (2012). KRAS gene mutations in Noonan syndrome familial cases cluster in the vicinity of the switch II region of the G-domain: Report of another family with metopic craniosynostosis. *American Journal of Medical Genetics, Part A*, *158 A*(5), 1178–1184. https://doi.org/10.1002/ajmg.a.35270

Carcavilla, A., Suárez-Ortega, L., Sánchez, A. R., Gonzalez-Casado, I., Ramón-Krauel, M., Labarta, J. I., … López-Siguero, J. P. (2020). Noonan syndrome: genetic and clinical update and treatment options. *Anales de Pediatría (English Edition)*, *93*(1), 61.e1-61.e14. https://doi.org/10.1016/j.anpede.2020.04.009

Carta, C., Pantaleoni, F., Bocchinfuso, G., Stella, L., Vasta, I., Sarkozy, A., … Tartaglia, M. (2006). Germline missense mutations affecting KRAS isoform B are associated with a severe Noonan syndrome phenotype. *American Journal of Human Genetics*, *79*(1), 129–135. https://doi.org/10.1086/504394

Cirstea, I. C., Kutsche, K., Dvorsky, R., Gremer, L., Carta, C., Horn, D., … Zenker, M. (2010). A restricted spectrum of NRAS mutations causes Noonan syndrome. *Nature Genetics*, *42*(1), 27–29. https://doi.org/10.1038/ng.497

Cordeddu, V., Di Schiavi, E., Pennacchio, L. A., Ma'ayan, A., Sarkozy, A., Fodale, V., … Tartaglia, M. (2009). Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. *Nature Genetics*, *41*(9), 1022–1026. https://doi.org/10.1038/ng.425

Denayer, E., Peeters, H., Sevenants, L., Derbent, M., Fryns, J. P., & Legius, E. (2012). NRAS mutations in Noonan syndrome. *Molecular Syndromology*, *3*(1), 34–38. https://doi.org/10.1159/000338467

Drabik, A., Bodzoń-Kułakowska, A., & Silberring, J. (2016). Gel Electrophoresis. In *Proteomic Profiling and Analytical Chemistry: The Crossroads: Second Edition* (pp. 115–143). https://doi.org/10.1016/B978-0-444-63688-1.00007-0

El Bouchikhi, I., Belhassan, K., Moufid, F. Z., Iraqui Houssaini, M., Bouguenouch, L., Samri, I., … Ouldim, K. (2016). Noonan syndrome-causing genes: Molecular update and an assessment of the mutation rate. *International Journal of Pediatrics and Adolescent Medicine*, *3*(4), 133–142. https://doi.org/10.1016/j.ijpam.2016.06.003

El Bouchikhi, I., Bouguenouch, L., Moufid, F. Z., Samri, I., Abdouss, F., Melhouf, M. A., … Ouldim, K. (2020). Molecular and environmental characterization of Noonan syndrome in Morocco reveals a significant association with consanguinity and advanced parental age. *Egyptian Journal of Medical Human Genetics 2020 21:1*, *21*(1), 1–9. https://doi.org/10.1186/S43042-020-0047-9

El Bouchikhi, I., Samri, I., Iraqui Houssaini, M., Trhanint, S., Bouguenouch, L., Sayel, H., … Ouldim, K. (2015). The first PTPN11 mutations in hotspot exons reported in Moroccan children with Noonan syndrome and comparison of mutation rate to previous studies. *Turkish Journal of Medical Sciences*, *45*(2), 306–312. https://doi.org/10.3906/sag-1310-50

Elalaoui, S. C., Kraoua, L., Liger, C., Ratbi, I., Cavé, H., & Sefiani, A. (2010). Germinal mosaicism in Noonan syndrome: A family with two affected siblings of normal parents. *American Journal of Medical Genetics, Part A*, *152*(11), 2850–2853. https://doi.org/10.1002/AJMG.A.33685

*ExoSAP-IT ® PCR Cleanup reagent.* (n.d.).

Gelb, B. D., & Tartaglia, M. (2006). Noonan syndrome and related disorders: Dysregulated RAS-mitogen activated protein kinase signal transduction. *Human Molecular Genetics*, *15*(SUPPL. 2). https://doi.org/10.1093/hmg/ddl197

Hüffmeier, U., Zenker, M., Hoyer, J., Fahsold, R., & Rauch, A. (2006). A variable combination of features of Noonan syndrome and neurofibromatosis type I are caused by mutations in the NF1 gene. *American Journal of Medical Genetics, Part A*, *140*(24), 2749–2756. https://doi.org/10.1002/ajmg.a.31547

Jafry, M., & Sidbury, R. (2020). RASopathies. *Clinics in Dermatology*, *38*(4), 455–461. https://doi.org/10.1016/j.clindermatol.2020.03.010

Jongmans, M. C. J., Hoogerbrugge, P. M., Hilkens, L., Flucke, U., Van Der Burgt, I., Noordam, K., … Hoogerbrugge, N. (2010). Noonan syndrome, the SOS1 gene and embryonal rhabdomyosarcoma. *Genes Chromosomes and Cancer*, *49*(7), 635–641. https://doi.org/10.1002/gcc.20773

Jorge, A. A. L., Malaquias, A. C., Arnhold, I. J. P., & Mendonca, B. B. (2009, April). Noonan syndrome and related disorders: A review of clinical features and mutations in genes of the RAS/MAPK pathway. *Hormone Research*, Vol. 71, pp. 185–193. https://doi.org/10.1159/000201106

Ko, J. M., Kim, J. M., Kim, G. H., & Yoo, H. W. (2008). PTPN11, SOS1, KRAS, and RAF1 gene analysis, and genotype-phenotype correlation in Korean patients with Noonan syndrome. *Journal of Human Genetics*, *53*(11–12), 999–1006. https://doi.org/10.1007/s10038-008-0343-6

Kobayashi, T., Aoki, Y., Niihori, T., Cavé, H., Verloes, A., Okamoto, N., … Matsubara, Y. (2010). Molecular and clinical analysis of RAF1 in Noonan syndrome and related disorders: Dephosphorylation of serine 259 as the essential mechanism for mutant activation. *Human Mutation*, *31*(3), 284–294. https://doi.org/10.1002/humu.21187

Kratz, C. P., Zampino, G., Kriek, M., Kant, S. G., Leoni, C., Pantaleoni, F., … Zenker, M. (2009). Craniosynostosis in patients with noonan syndrome caused by germline KRAS mutations. *American Journal of Medical Genetics, Part A*, *149*(5), 1036–1040. https://doi.org/10.1002/ajmg.a.32786

Kruszka, P., Porras, A. R., Addissie, Y. A., Moresco, A., Medrano, S., Mok, G. T. K., … Muenke, M. (2017). Noonan syndrome in diverse populations. *American Journal of Medical Genetics, Part A*, *173*(9), 2323–2334. https://doi.org/10.1002/ajmg.a.38362

Kuslich, C. D., Chui, B., & Yamashiro, C. T. (2019). Overview of PCR. *Current Protocols in Essential Laboratory Techniques*, *18*(1). https://doi.org/10.1002/cpet.27

Lee, B. H., Kim, J. M., Jin, H. Y., Kim, G. H., Choi, J. H., & Yoo, H. W. (2011). Spectrum of mutations in Noonan syndrome and their correlation with phenotypes. *Journal of Pediatrics*, *159*(6), 1029–1035. https://doi.org/10.1016/j.jpeds.2011.05.024

Lepri, F., De Luca, A., Stella, L., Rossi, C., Baldassarre, G., Pantaleoni, F., … Tartaglia, M. (2011). SOS1 mutations in Noonan syndrome: molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. *Human Mutation*, *32*(7), 760–772. https://doi.org/10.1002/humu.21492

Lepri, F. R., Scavelli, R., Digilio, M. C., Gnazzo, M., Grotta, S., Dentici, M. L., … Dallapiccola, B. (2014). Diagnosis of Noonan syndrome and related disorders using target next generation sequencing. *BMC Medical Genetics*, *15*(1). https://doi.org/10.1186/1471-2350-15-14

Li, X., Yao, R., Tan, X., Li, N., Ding, Y., Li, J., … Wang, X. (2019). Molecular and phenotypic spectrum of Noonan syndrome in Chinese patients. *Clinical Genetics*, *96*(4), 290–299. https://doi.org/10.1111/cge.13588

Malaquias, A. C., & Jorge, A. A. L. (2021, January 1). Activation of the MAPK pathway (RASopathies) and partial growth hormone insensitivity. *Molecular and Cellular Endocrinology*, Vol. 519. https://doi.org/10.1016/j.mce.2020.111040

Nava, C., Hanna, N., Michot, C., Pereira, S., Pouvreau, N., Niihori, T., … Cavé, H. (2007). Cardio-facio-cutaneous and Noonan syndromes due to mutations in the RAS/MAPK signalling pathway: Genotype-phenotype relationships and overlap with Costello syndrome. *Journal of Medical Genetics*, *44*(12), 763–771. https://doi.org/10.1136/jmg.2007.050450

Noonan, J. A. (2009). History of Noonan syndrome and related disorders. *Monographs in Human Genetics*, *17*, 1–8. https://doi.org/10.1159/000158550

Noonan, Jacqueline A. (1968). Hypertelorism with Turner Phenotype: A New Syndrome with Associated Congenital Heart Disease. *American Journal of Diseases of Children*, *116*(4), 373–380. https://doi.org/10.1001/archpedi.1968.02100020377005

Noonan, Jacqueline A. (1994). Noonan Syndrome: An Update and Review for the Primary Pediatrician. *Clinical Pediatrics*, *33*(9), 548–555. https://doi.org/10.1177/000992289403300907

Noonan Syndrome. (n.d.). Retrieved April 6, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4099190/

OMIM Entry - * 164790 - NRAS PROTOONCOGENE, GTPase; NRAS. (n.d.). Retrieved May 10, 2021, from https://www.omim.org/entry/164790

Pandit, B., Sarkozy, A., Pennacchio, L. A., Carta, C., Oishi, K., Martinelli, S., … Gelb, B. D. (2007). Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nature Genetics*, *39*(8), 1007–1012. https://doi.org/10.1038/ng2073

PureLink® Genomic DNA Kits. (n.d.). Retrieved July 1, 2021, from http://tools.thermofisher.com/content/sfs/manuals/purelink_genomic_man.pdf

Ranke, M. B., Heidemann, P., Knupfer, C., Enders, H., Schmaltz, A. A., & Bierich, J. R. (1988). Noonan syndrome: growth and clinical manifestations in 144 cases. *European Journal of Pediatrics*, *148*(3), 220–227. https://doi.org/10.1007/BF00441408

Razzaque, M. Abdur, Nishizawa, T., Komoike, Y., Yagi, H., Furutani, M., Amo, R., … Matsuoka, R. (2007). Germline gain-of-function mutations in RAF1 cause Noonan syndrome. *Nature Genetics*, *39*(8), 1013–1017. https://doi.org/10.1038/ng2078

Razzaque, Md Abdur, Komoike, Y., Nishizawa, T., Inai, K., Furutani, M., Higashinakagawa, T., & Matsuoka, R. (2012). Characterization of a novel KRAS mutation identified in Noonan syndrome. *American Journal of Medical Genetics, Part A*, *158 A*(3), 524–532. https://doi.org/10.1002/ajmg.a.34419

Roberts, A. E., Allanson, J. E., Tartaglia, M., & Gelb, B. D. (2013). Noonan syndrome. *The Lancet*, Vol. 381, pp. 333–342. https://doi.org/10.1016/S0140-6736(12)61023-X

Rohrer, T. (2009). Noonan syndrome: Introduction and basic clinical features. *Hormone Research*, *72*(SUPPL. 2), 3–7. https://doi.org/10.1159/000243772

Romano, A. A., Allanson, J. E., Dahlgren, J., Gelb, B. D., Hall, B., Pierpont, M. E., … Noonan, J. A. (2010, October). Noonan syndrome: Clinical features, diagnosis, and management guidelines. *Pediatrics*, Vol. 126, pp. 746–759. https://doi.org/10.1542/peds.2009-3207

Runtuwene, V., Van Eekelen, M., Overvoorde, J., Rehmann, H., Yntema, H. G., Nillesen, W. M., … Den Hertog, J. (2011). Noonan syndrome gain-of-function mutations in NRAS cause zebrafish gastrulation defects. *DMM Disease Models and Mechanisms*, *4*(3), 393–399. https://doi.org/10.1242/dmm.007112

Sarkozy, A., Carta, C., Moretti, S., Zampino, G., Digilio, M. C., Pantaleoni, F., … Tartaglia, M. (2009). Germline BRAF mutations in noonan, LEOPARD, and cardiofaciocutaneous Syndromes: Molecular diversity and associated phenotypic spectrum. *Human Mutation*, *30*(4), 695–702. https://doi.org/10.1002/humu.20955

Schubbert, S., Zenker, M., Rowe, S. L., Böll, S., Klein, C., Bollag, G., … Kratz, C. P. (2006). Germline KRAS mutations cause Noonan syndrome. *Nature Genetics*, *38*(3), 331–336. https://doi.org/10.1038/ng1748

Selfors, L. M., Schutzman, J. L., Borland, C. Z., & Stern, M. J. (1998). soc-2 encodes a leucine-rich repeat protein implicated in fibroblast growth factor receptor signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(12), 6903–6908. https://doi.org/10.1073/pnas.95.12.6903

Sharland, M., Burch, M., McKenna, W. M., & Paton, M. A. (1992). A clinical study of Noonan syndrome. *Archives of Disease in Childhood*, *67*(2), 178–183. https://doi.org/10.1136/adc.67.2.178

Shaw, A. C., Kalidas, K., Crosby, A. H., Jeffery, S., & Patton, M. A. (2007). The natural history of Noonan syndrome: A long-term follow-up study. *Archives of Disease in Childhood*, *92*(2), 128–132. https://doi.org/10.1136/adc.2006.104547

Tajan, M., Paccoud, R., Branka, S., Edouard, T., & Yart, A. (2018, October 1). The RASopathy family: Consequences of germline activation of the RAS/MAPK pathway. *Endocrine Reviews*, Vol. 39, pp. 676–700. https://doi.org/10.1210/er.2017-00232

Tartaglia, M., & Gelb, B. D. (2009). Molecular genetics of Noonan syndrome. *Monographs in Human Genetics*, *17*, 20–39. https://doi.org/10.1159/000163018

Tartaglia, Marco, & Gelb, B. D. (2005). Noonan syndrome and related disorders: Genetics and pathogenesis. *Annual Review of Genomics and Human Genetics*, Vol. 6, pp. 45–68. https://doi.org/10.1146/annurev.genom.6.080604.162305

Tartaglia, Marco, Gelb, B. D., & Zenker, M. (2011, February). Noonan syndrome and clinically related disorders. *Best Practice and Research: Clinical Endocrinology and Metabolism*, Vol. 25, pp. 161–179. https://doi.org/10.1016/j.beem.2010.09.002

Tartaglia, Marco, Kalidas, K., Shaw, A., Song, X., Musat, D. L., Van der Burgt, I., … Gelb, B. D. (2002). PTPN11 mutations in noonan syndrome: Molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *American Journal of Human Genetics*, *70*(6), 1555–1563. https://doi.org/10.1086/340847

Tartaglia, Marco, Pennacchio, L. A., Zhao, C., Yadav, K. K., Fodale, V., Sarkozy, A., … Gelb, B. D. (2007). Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. *Nature Genetics*, *39*(1), 75–79. https://doi.org/10.1038/ng1939

Tidyman, W. E., & Rauen, K. A. (2009, June). The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Current Opinion in Genetics and Development*, Vol. 19, pp. 230–236. https://doi.org/10.1016/j.gde.2009.04.001

Turner, A. M. (2014, October 1). Noonan syndrome. *Journal of Paediatrics and Child Health*, Vol. 50, pp. E14–E20. https://doi.org/10.1111/j.1440-1754.2010.01970.x

Van Der Burgt, I. (2007, December 14). Noonan syndrome. *Orphanet Journal of Rare Diseases*, Vol. 2, p. 4. https://doi.org/10.1186/1750-1172-2-4

Yart, A., & Edouard, T. (2018, February 1). Noonan syndrome: An update on growth and development. *Current Opinion in Endocrinology, Diabetes and Obesity*, Vol. 25, pp. 67–73. https://doi.org/10.1097/MED.0000000000000380

Zadeh, N. (2019). Noonan syndrome: Phenotypic variations and molecular genetics. In *Noonan Syndrome: Characteristics and Interventions* (pp. 1–14). https://doi.org/10.1016/B978-0-12-815348-2.00010-4

Zenker, M., Horn, D., Wieczorek, D., Allanson, J., Pauli, S., Van Der Burgt, I., … Kutsche, K. (2007). SOS1 is the second most common Noonan gene but plays no major role in cardio-facio-cutaneous syndrome. *Journal of Medical Genetics*, *44*(10), 651–656. https://doi.org/10.1136/jmg.2007.051276

## Appendix 1 : Noonan Syndrome Clinical Sheet


# ETUDE DU GENE *Kras*
## (Noonan)

Prescription et renseignements clinico-biologiques

| PATIENT | MEDECIN PRESCRIPTEUR |
|---|---|
| Nom .....<br>Prénom.....<br>Date de naissance .....<br>Adresse.....<br>.....<br>.....<br>..... | SIGNATURE : |

**RENSEIGNEMENTS CLINIQUES**

Retard staturo-pondéral : Taille: ..... (.....DS) Poids:..... (.....DS)

Période prénatal : □ hydramnios □ clarté nucale augmentée

Dysmorphie faciale : □ front haut □ hypertélorisme □ oreilles bas implantées □ ptosis □ micrognathie □ narines antéversées
□ cou court □ pterygium coli □ excès de peau nucale □ constriction bitemporale
□ Macrocéphalie relative □ Microcéphalie □Face triangulaire □FP anti mongoloïdes
□ Epicanthus □ □ Rotation post des oreilles □Lèvres épaisses □ Traits épais

Cardiovasculaire : □ sténose valve pulmonaire □ sténose artère pulmonaire
□ coarctation de l'aorte □ canal atrio-ventriculaire □ cardiomyopathie hypertrophique

Peau et phanères : □ hyperkératose □ tâches café au lait

Neurologie :

| | | |
|---|---|---|
| Retard mental | □ non | □ oui : □ léger □ modéré □ sévère |
| Retard de langage | □ non | □ oui |
| Retard à la marche | □ non | □ oui |
| Traits autistiques | □ non | □ oui |

Divers : □ pectus excavatum/carinatum □ scoliose □ cubitus valgus □ clinodactylie □ hyperlaxité des doigts
□ cryptorchidie □ troubles oculaires □ surdité □ lymphoedème □ hépato-splénomégalie □ troubles de l'hémostase

Autres signes et informations (précisez) :
.....
.....

Arbre généalogique ☐ Consanguinité Père symptomatique : □ oui □ non Mère symptomatique :□ oui □ non

Unité de Génétique Médicale et d'Oncogénétique. Laboratoire Central d'Analyses Médicales.

CHU HASSAN II, B.P.1835, Fès – Tél: 0 5 53 08 70 03 Fax: 0 5 35 61 37 44

## Appendix 2 : Exon 2, 3, 4, 5, and 6 primers of Kras gene

Primers derived from the work of (Carta et al., 2006):

## Exon 2

**F** GATACACGTCTGCAGTCAACTG
**R** GGTCCTGCACCAGTAATATGC

GATAAAAGAACTGTCTATGTAGCATTTATGCATTTTTCTTAAGCGTCGATGGAGGAGTTT
GTAAATGAAGTACAGTTCATTACGATACACGTCTGCAGTCAACTGGAATTTTCATGATTG
AATTTTGTAAGGTATTTTGAAATAATTTTTCATATAAAGGTGAGTTTGTATTAAAAGGTA
CTGGTGGAGTATTTGATAGTGTATTAACCTTATGTGTGACATGTTCTAATATAGTCACAT
TTTCATTATTTTTATTATAAG**GCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTG**
**GAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGG**
**ACGAATATGATCCAACAATAGAG**GTAAATCTTGTTTTAATATGCATATTACTGGTGCAGG
ACCATTCTTTGATACAGATAAAGGTTTCTCTGACCATTTTCATGAGTACTTATTACAAGA
TAATTATGCTGAAAGTTAAGTTATCTGAAATGTACCTTGGGTTTCAAGTTATATGTAACC

## Exon 3

**F** GGTGCACTGTAATAATCCAGACT
**R** CATGGCATTAGCAAAGACTCA

GATGTTGAGCATCTTTTCAGGTGCTTAGTGGCCATTTGTCCGTCATCTTTGGAGCAGGAA
CAATGTCTTTTCAAGTCCTTTGCCCATTTTTAAATTGAATTTTTTGTTGTTGAGTTGTAT
ATAACACCTTTTTTGAAGTAAAAGGTGCACTGTAATAATCCAGACTGTGTTTCTCCCTTC
TCAG**GATTCCTACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCT**
**CGACACAGCAGGTCAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGA**
**GGGCTTTCTTTGTGTATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTA**
**TAG**GTGGGTTTAAATTGAATATAATAAGCTGACATTAAGGAGTAATTATAGTTTTTATTT
TTTGAGTCTTTGCTAATGCCATGCATATAATATTTAATAAAAATTTTTAAATAATGTTTA
TGAGGTAGGTAATATCCCTGTTTTATAAATGAAGTTCTTGGGGGATTAGAGCAGTGGAGT
AACTTGCTCCAGACTGCATCGGTAGTGGTGGTGCTGGGATTGAAACCTAGGCCTGTTTGA
CTCCACAGCCTTCTGTACTCTTGACTATTCTACAAAAGCAAGACTTTAAACTTTTTAGAT
ACATCATTAAAAAAGAAAACCATAAAAAAGAATATGAAAAGATGATTTGAGATGGTGTCA

## Exon 4

**F** GGTGTAGTGGAAACTAGGAATTAC

**R** GACATAACAGTTATGATTTTGCAG

AGAAGGAAGGAAAATTTGGTGTAGTGGAAACTAGGAATTACATTGTTTTCTTTCAGCCAA
ATTTTATGACAAAAGTTGTGGACAGGTTTTGAAAGATATTTGTGTTACTAATGACTGTGC
TATAACTTTTTTTTCTTTCCCAG**AGAACAAATTAAAAGAGTTAAGGACTCTGAAGATGTA**
**CCTATGGTCCTAGTAGGAAATAAATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAG**
**GCTCAGGACTTAGCAAGAAGTTATGGAATTCCTTTTATTGAAACATCAGCAAAGACAAGA**
**CAG**GTAAGTAACACTGAAATAAATACAGATCTGTTTTCTGCAAAATCATAACTGTTATGT
CATTTAATATATCAGTTTTTCTCTCAATTATGCTATACTAGGAAATAAAACAATATTTAG
TAAATGTTTTTGTCTCTTGAGAGGGCATTGCTTCTTAATCCAGTGTCCATGGTACTGCTT
TTGGCTTTGGTTTCTTTCTACATTGAAAATTTCTCTTCAATTCTGAGCACATGTTAACAT
TTAGAATTCAAGAGGTGGGGATTTTTTTTTCCCATGGTTACATATATATATATATATATA
TATATATATATATATATATATATATATATAAAGAACAGGGCAACAAATTTTTGCGTTTTC
TATTTCGGTAGTACTTTTAAACCATTATGTCATGTTTCTAGGTTAAACGTTGTTGTATTT
GAAGAATTTTACTTTGGCAGAATTTTTTTGAGGATGTGTTTATTTCTGGAGAAAGGTCTC
ATTAAAGAAAGACAATACCCAGAAAGCCAACAGAAATTCTGTTACTCATTTAATGCATTT
TTCTGACAAAAATTATTGCCAGAGAGAACCTGAATTTTGTTTCAAAAATCATCTTTGTTT
TAAAAATGACTTTTTCTTCAGGTAAAATAAAATAATTTCAGTTGCTATTATTTAACCTGT
TTGTATGAAGAGTTTAACATATAGGAAATGAATACATAAAGATAGGAAGGAATTAATTGT
TATATGTAGTCATATGTCTCTTAATGACAGGGATACTTTCTAAGAAATACATTGTTAGGT

## Exon 5

**F** CTCAAGCTCATAATCTCAAACTTCT

**R** GTAGTTCTAAAGTGGTTGCCACC

AAAACTGCATCCTCAGTTTTATTCAAACAGTCTGATGTCTGTTTAAAAAAAAAAAAAATCT
CAAGCTCATAATCTCAAACTTCTTGCACATGGCTTTCCCAGTAAATTACTCTTACCAATG
CAACAGACTTTAAAGAAGTTGTGTTTTACAATGCAG**AGAGTGGAGGATGCTTTTTATACA**
**TTGGTGAGAGAGATCCGACAATACAGATTGAAAAAAATCAGCAAAGAAGAAAAGACTCCT**
**GGCTGTGTGAAAATTAAAAAATGCATTATAATGTAATCTG**GTAAGTTTAAGTTCAGCACA
TTAATTTTGGCAGAAAGCAGATGTCTTTTAAAGGTAACAAGGTGGCAACCACTTTAGAAC
TACTTAGGTGTAGTATTCTAACTTGAAGTATTAAAAGATAAGAAACTTGTTTCCATAATT
AGTACATTTATTTTTAATCTAGTGGGAATTAATTATAATTGAGACAATTTTGATGGCTGT
AGTAGACTAATCTATATTTGGCATAAAGTCTAATGATTTAATGAGTCTTAAGTAAACTAA
ATATTTGGAAACTGATATTTACCTTTATTTTTAAGGGAAAAGTTTTGAGATAATCAGCAG

## Exon 6

**F** GACAAAACACCTATGCGGATGA

**R** GCTAACAGTCTGCATGGAGCA

TTAAGTTCTCCTGTGAAAAAGTCGTTACCTTATTTAAAATTCTGTGCCATTGGTTATCCT
TGTCTTTTGTGAAAATTAGTGTTCCTGTTTATAATATTGACAAAACACCTATGCGGATGA
CATTTAAGAATTCTAAAAGTCCTAATATATGTAATATATATTCAGTTGCCTGAAGAGAAA
CATAAAGAATCCTTTCTTAATATTTTTTCCATTAATGAAATTTGTTACCTGTACACATGA
AGCCATCGTATATATTCACATTTTAATACTTTTTATGTATTTCAG**GGTGTTGATGATGCC**
**TTCTATACATTAGTTCGAGAAATTCGAAAACATAAAGAAAAGATGAGCAAAGATGGTAAA**
**AAGAAGAAAAAGAAGTCAAAGACAAAGTGTGTAATTATGTAAATACAATTTGTACTTTTT**
**TCTTAAGGCATACTAGTACAAGTGGTAATTTTTGTACATTACACTAAATTATTAGCATTT**
**GTTTTAGCATTACCTAATTTTTTTCCTGCTCCATGCAGACTGTTAGCTTTTACCTTAAAT**
**GCTTATTTTAAAATGACAGTGGAAGTTTTTTTTTCCTCTAAGTGCCAGTATTCCCAGAGT**
**TTTGGTTTTTGAACTAGCAATGCCTGTGAAAAAGAAACTGAATACCTAAGATTTCTGTCT**
**TGGGGCTTTTGGTGCATGCAGTTGATTACTTCTTATTTTTCTTACCAATTGTGAATGTTG**
**GTGTGAAACAAATTAATGAAGCTTTTGAATCATCCCTATTCTGTGTTTTATCTAGTCACA**
**TAAATGGATTAATTACTAATTTCAGTTGAGACCTTCTAATTGGTTTTTACTGAAACATTG**
**AGGGAACACAAATTTATGGGCTTCCTGATGATGATTCTTCTAGGCATCATGTCCTATAGT**
**TTGTCATCCCTGATGAATGTAAAGTTACACTGTTCACAAAGGTTTTGTCTCCTTTCCACT**
**GCTATTAGTCATGGTCACTCTCCCCAAAATATTATATTTTTTCTATAAAAAGAAAAAAAT**
**GGAAAAAAATTACAAGGCAATGGAAACTATTATAAGGCCATTTCCTTTTCACATTAGATA**
**AATTACTATAAAGACTCCTAATAGCTTTTCCTGTTAAGGCAGACCCAGTATGAAATGGGG**

**Appendix 3: DNA Extraction protocol by the Pure Link™ Genomic DNA Kit.**

**Cell lysis with proteinase K and lysis buffer**

Set a water bath or heat block at 55°C. In a 1.5 ml Eppendorf tube:

• Add 200 µl of blood + 20 µl of proteinase K + 20 µl of RNase A.

• Mix well by vortexing for about 15 seconds.

• Incubate for 2 minutes at room temperature.

• Add 200 µl of Pure Link™ Genomic Lysis / Binding Buffer and mix well by vortexing for about 15 seconds to obtain a homogenous solution.

• Incubate for 10 minutes at 55°C to promote protein digestion.

• Add 200µl of absolute ethanol. Mix well by vortexing for about 5 seconds to yield a homogenous solution.

**Binding DNA**

• Add the lysate (~640 µL) prepared with PureLink® Genomic Lysis/Binding Buffer and ethanol to the PureLink® Spin Column.

• Centrifuge the column at 8000rpm for 1 minute.

• Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.

**Washing DNA**

•Add 400 µL Wash Buffer 1 to the column, and centrifuge the column at 8000rpm for 1 minute.

•Discard the collection tube and place the spin column into a clean PureLink® collection tube supplied with the kit.

•Add 400 µL Wash Buffer 2 to the column, and centrifuge the column at 1400rpm for 3 minutes. Discard collection tube.

**Eluting DNA**

•Place the spin column in a sterile 1.5-mL microcentrifuge tube.

•Add 70 µL of PureLink® Genomic Elution Buffer to the column.

•Incubate at room temperature for 1 minute. Centrifuge the column at 8000rpm for 1 minute. The tube contains purified genomic DNA.

•To recover more DNA, perform a second elution step using the same elution buffer volume as the first elution in another sterile, 1.5-ml microcentrifuge tube. Centrifuge the column at 8000rpm for 1 minute.

**Storing DNA**

•To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store it at –20°C for long-term storage.

# ملخص


متلازمة نونان (NS) هي اضطراب وراثي جسمي سائد. ومع ذلك ، فإن معظم الحالات متفرقة. يصيب 1000/1 - 2500/1 فرد. يتميز بخلل في الوجه وتشوه القلب وصغر الحجم والتخلف العقلي. التشخيص سريري بشكل أساسي .وتشترك الطفرات الجينية المحددة في مسار نقل الإشارات RAS/MAPK ، وهي تمثل حالياً 60% من حالات NS. الجين الأول الموصوف كان.PTPN11

سلط تقريرنا الضوء على دراسة أجريت على 54 من المرضى المغاربة المصابين بمتلازمة نونان ، وتم فحصهم سلبا على الجينPTPN11 في وحدة علم الوراثة وعلم الأورام التابعة لمركز مستشفى الحسن الثاني الجامعي في فاس

90٪ من مجموعتنا حدثت بشكل متفرق وفقط في 10٪ كانت هناك الدرجة الأولى من القرابة. كانت المظاهر التي أدت إلى التشخيص هي الوجوه المميزة في 100٪ من الحالات ، واعتلال القلب من نوع التضيق الرئوي في 61٪ من الحالات ، وخصائص الخصية في 5٪ ، وقصر القامة في 36٪ ، والتخلف العقلي في 20٪ من الحالات.

في ضوء هذه الملاحظات السريرية ، ركزت دراستنا على البحث عن الطفرات في exon 2 و exon 3 من جين Kras بواسطة PCR متبوعًا بتسلسل Sanger لفوجنا. كشفت هذه الدراسة عن عدم وجود طفرات في exon 2 و exon 3 من جين Kras في مجموعتنا.



***كلمات دلالية*** **:** متلازمة نونان؛ تضيق رئوي؛ قصرالقامة؛ التأخرالعقلي