NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MOLECULAR MEDICINE

The carrier frequencies of Spinal Muscular Atrophy causing *SMN1* gene mutations in Turkish Cypriot Couples

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APPROVAL

We certify that we have read thesis submitted by "The carrier frequencies of Spinal Muscular Atrophy causing SMN1 gene mutations in Turkish Cypriot Couples" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Health Sciences.

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Prof. Dr. Şanda Çalı

Yakın Doğu Üniversitesi

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Sara Abbasigharei

Abstract

The carrier frequencies of Spinal Muscular Atrophy causing SMN1 gene mutations in Turkish Cypriot Couples

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Master of Science in Molecular Medicine

Thesis Advisors: Assoc.Prof.Dr. Mahmut Cerkez Ergoren

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INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF HEALTH SCIENCE, 2022

Aim

Aim of this study is the evaluating of the carrier frequency of SMN1 mutation causing Spinal Muscular Atrophy(SMA) in Turkish Cypriot Couples. This is the first study to evaluate the SMN1deletion mutations in this population.

Background

Spinal Muscular Atrophy (SMA)

Spinal muscular atrophy (SMA) is a hereditary neuromuscular condition which is defined as an autosomal recessive disorder that has high carrier frequency rate, and significantly one out of each 10,000 live births can be affected by this syndrome. The all atrophy's degree are caused by the gradual loss of alpha motor neurons, however this missing can occur either within the ventral spinal cord or motor nuclei within the lower brainstem.

Classification, Phenotypes and Pathophysiology

Its classification is according to the age of unset, plus its status. Although it is now evident depending on the underlying genotype, phenotypes cover a wide range of traits. Due to mutations and abnormalities in the SMN1 gene sequence which is located on long arm of chromosome number 5 (5q13), SMA is divided into 4 subtypes.that typr 1 is the most severe form of disease.

Causes and Inheritance

Mutations that occur within SMN1 (survival motor neuron) gene sequence lead to SMA. The highly homologous of SMN1 is SMN2 this centromeric element SMN2 consist of over 99 percent nucleotide similarity with SMN1 whereas it has been determined there is an association between milder phenotypes of SMA and greater copy number of SMN2, as a result SMN2 is considered as a modifying factor.

Material and methods

Overall, 94 samples were obtained that the genders were equal (47 individuals female/ 47 individuals male), and the average age of the population was about 31.17 ± 4.18, 29.38 ± 3.94 years old, respectively, for men and women.SNPure Genomic DNA Kit (SNP Biotechnology, Cat. No: 21S-01-250,250 Preps, Ankara, Turkey) is designed particularly for extracting DNA from whole blood by using DNA binding column method that has high accuracy. All 94 samples' DNA was isolated by using this specific kit from prepheral blood. Furthermore, in order to screen deletions of the Exon 7 and/or 8 SMN1 genes and precisely substitution of C/T at nucleotide number 840 within exon 7, the IntRaFast-Q SMA screening kit was chosen because of its optimization and specificity for deletions on exon 7 and/or 8 that cause variety spectrum of SMA disorder.

Detection SMN1 gene mutations by Quantitative Real-Time PCR (RT-qPCR)

The mutation detection of SMN1 gene mutations, Exon 7 and 8 deletions and c.849C/T substation within exon 7 was carried according to manufacturer guidelines (IntRaFast-Q SMA Real Time PCR Screening Kit, Cat. No: 200R-40-20, SNP Biotechnology, Ankara, Turkey).

Findings

In the total of 94 individuals, 1 patient turned out to be a carrier of the SMN1 gene in both exon 7 and 8 (carrier number 1) and another patient (Carrier number2) showed a carrier status of SMN1 gene only in exon 7. Our findings revealed that the carrier frequency of mutation in the SMN1 gene for exon 7 is 2.12% (2:94 healthy individuals) while its mutation prevalence for exon 8 is 1.06% (1:94 healthy individuals).

Conclusion

We have done this research because to prevent the occurrence of such genetic diseases and to make relevant decisions for the population of a country, knowing the abundance of the target gene in the population is the first step toward having a healthy population. One of the treatment process' limitations is the cost-effective therapies for this genetic disorder, such as Onasemnogene abeparvovec (Zolngensma). Screening is the first step in preventing a child from developing SMA. Our findings will be presented to the Ministry of Health, and their decision will be conducted to include SMN1 gene mutation in screenings before marriage, however before having a baby screening for SMA is highly recommended.

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CHAPTER I

Introduction

In Inheritance Condition of monogenic autosomal recessive, both alleles must be aberrant for the disorder characteristic to occur. The offspring [Homozygote] receive a single modified autosomal gene copy from both parents, and recessive trait is displayed again according to the identical gene copy of both alleles.

Spinal muscular atrophy (SMA) is a hereditary neuromuscular condition which is defined as an autosomal recessive disorder that has high carrier frequency rate, and significantly one out of each 10,000 live births can be affected by this syndrome. In the SMA condition the anterior horn cells of the human spinal cord degenerate, ultimately results in motor neuron loss and its classification is according to the age of unset which includes type 0 to IVOne out of two most known medicine for SMA is Nusinersen (Spinraza), which is the first medicine that licensed for the treatment, Spinraza is an antisense oligonucleotide that is able to elevate the rate of SMN protein in the central nervous system of human body and it works through alternative splicing of the SMN2 gene [60]. Onasemnogene abeparvovec (Zolgensma) is the second medicine or method which is specified gene therapy that is able to transfer the functional SMN1 gene into the motor neurons via using a viral vector(adeno-associated) after cystic fibrosis, SMA is the second most prevalent autosomal recessive genetic disorder in humans. Knowing the abundance of the target gene in the population is the initial step toward having a healthy population. The aim of this study was to evaluate the carrier frequency of SMN1 mutation causing Spinal Muscular Atrophy (SMA) in Turkish Cypriot Couples for the first. And ultimately assist the Ministry of Health in considering to include the SMA monitoring test as a requirement before to marriage and pregnancy.

Genetic disorders

A genetic disorder is a health condition that caused by one or more abnormalities in individual’s genome. These abnormalities can be a drawback of a mutation in a single gene (is called monogenic) or numerous genes (is called polygenic) or even cause by a chromosomal anomaly[1][2].

Monogenic disorders

The term "monogenic" is made up of two Greek words: mono implies "single" and genic signifies "gene." "Monogenic diseases or Single-gene Diseases" [MDs] are conditions characterized by the inheritance of a single mutant gene from parent to offspring[3]. Based on whether the mutation is in an autosome or a sex chromosome, these abnormalities are classified as "Autosomal" or "Sex-Linked"[4]. Monogenic diseases are also described as "Mendelian Disorders," since Gregor Mendel, the Father of Genetics, proposed the hypothesis of inheritance of hereditary traits together with genetic material[5]. In addition Mendel developed the concepts of dominant and recessive inheritance.

Inheritance Patterns of Monogenic Disorders

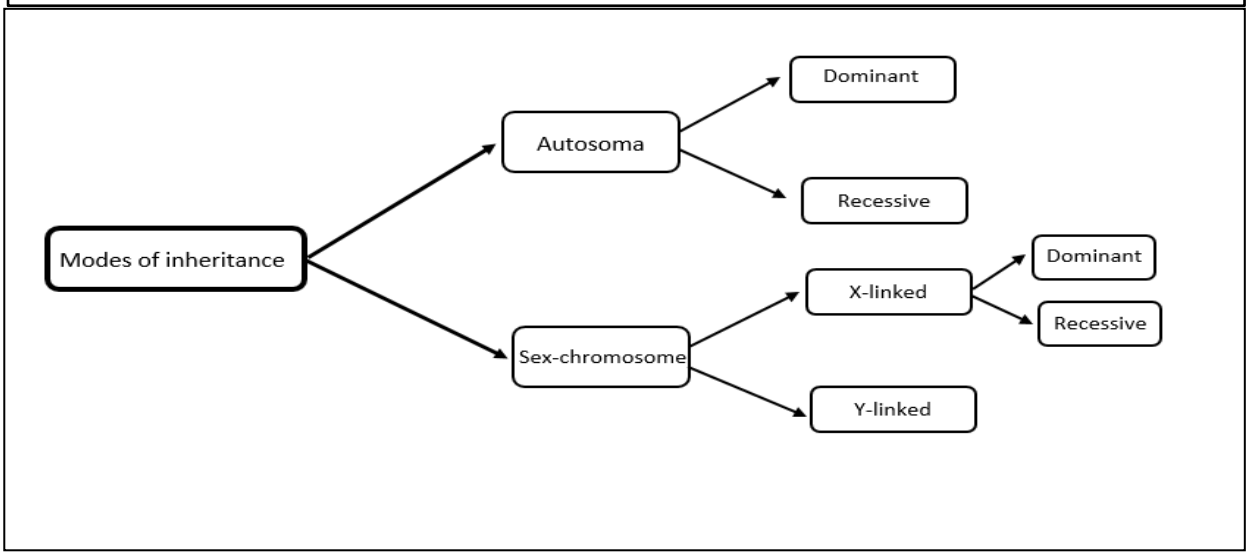
The copy of a mutant gene, or several copies, is inherited in Monogenic Disorders and generates a distinctive phenotype of that gene by following Mendelian Segregating patterns [6].

In both the case of autosomes and sex chromosomes, inheritance patterns may be anticipated. They also specify whether a single copy of a gene is inherited and is responsible for severe or whether both copies of the gene are altered, namely dominant and recessive inheritance [7].

The following are critical patterns of inheritance that aid in the identification of monogenic disease.(figure1)

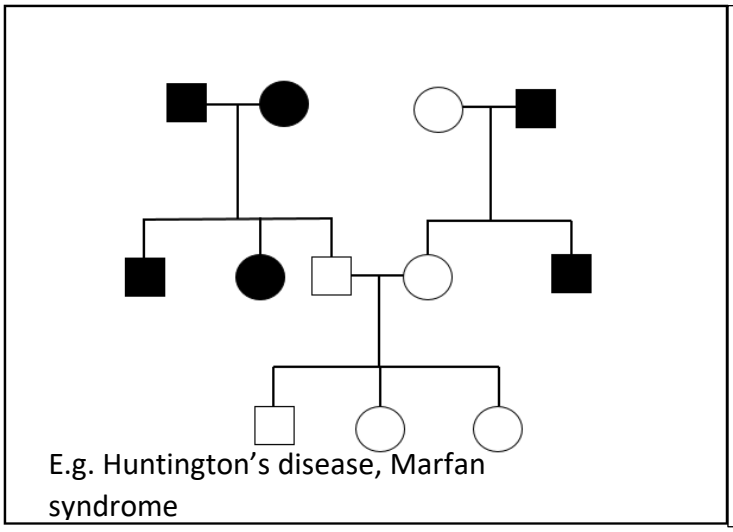
- X-Linked Dominant
- X-Linked Recessive
- Y-Linked
- Autosomal Dominant
- Autosomal Recessive

FIGURE1: Mendelian inheritance of monogenic disorders



Autosomal Dominant

Individuals with autosomal dominant monogenic diseases have a single mutated copy of the disease-associated gene. The existence of a single non-mutant or "wild-type" copy of the gene is insufficient to avoid the illness in this circumstance. Individuals might receive the disease-associated gene's mutated copy from either their mother or their father.[8] Since the mutation located on the autosome, both males and females can be afflicted, and the condition has a 50% chance of being passed along due to existence of a wild type and a mutant allele or gene copy in each parent's offspring[9](figure2).



E.g. Huntington's disease, Marfan syndrome

FIGURE2: Autosomal Dominant pedigree

Wild-type Male
Wild-type Female
Affected Male
Affected Female

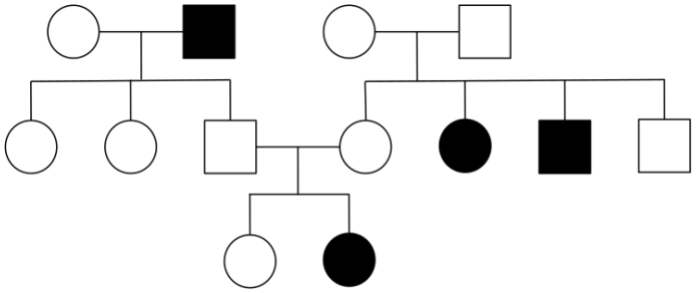
Individuals with single dominant pattern, one of the parents is certainly affected as well.

Autosomal recessive

Inheritance Condition In monogenic autosomal recessive, both alleles must be aberrantfor the disorder characteristic to occur. The offspring [Homozygote] receive a single modified autosomal gene copy from both parents, and recessive trait is displayed again according to the identical gene copy of both alleles[10]. In AR inheritanceonce the individual appears to be healthy phenotypically in another words is asymptomatic, accordingly one of the individual's gene is wild type, but the person is still carrier of the disorder and therefore can pass it on to following generations if continues in consanguinity [11].

Because such diseases may be controlled by avoiding consanguineous relationships, they can only be seen in a single generation. These abnormalities have a high probability of being passed down through consanguineous parents. As a result, for each pregnancy, heterozygous carriers of a recessive mutant allele have a 25% chance of conceiving an offspring with the disease.[12] (figure3).

FIGURE3: Autosomal Recessive pedigree
E.g. Tay-Sachs Disease, Spinal Muscular Atrophy(SMA), Cystic fibrosis, Sickle Cell Anemia(SC)

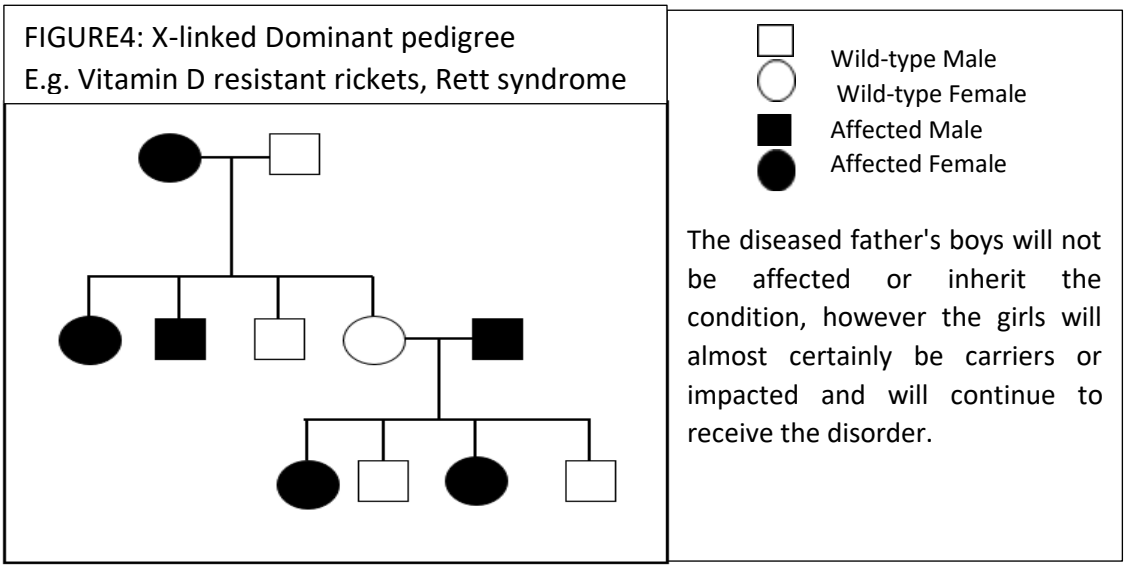


Wild-type Male
Wild-type Female
Affected Male
Affected Female

Non - affected siblings of an affected person have a 67% (two-thirds) probability of being mutant allele carriers.

X-linked dominant

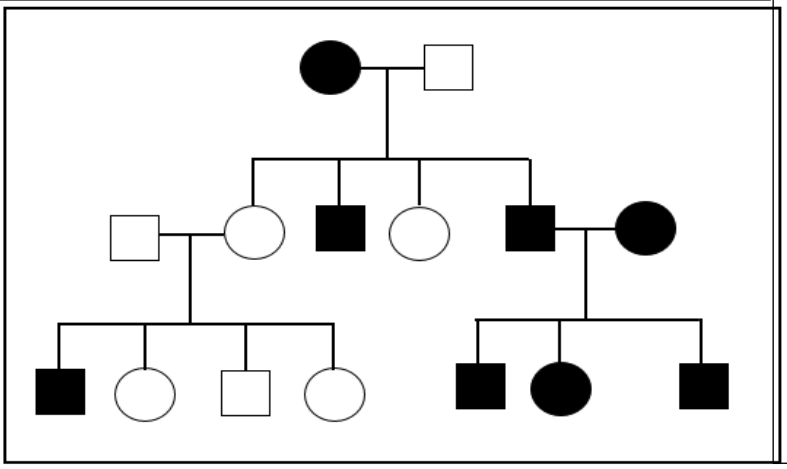
Specific conditions relating sex chromosomes are reflected in X-linked inheritance of traits. X-Linked Diseases are a clinical syndrome caused by mutations in the X-chromosome. Because males have just one X chromosome whereas females have two, males with the mutant allele will be affected[13]. In X-linked dominant condition, Females with an X-chromosome gene mutation will show the disorder appearance but will likely have a milder disease symptoms than men with the mutation. As a result, the mode of inheritance of X-Linked Dominant Disorders is direct; it will either generate affected children or normal ones, with a 50 percent chance of both, and the disease will be passed down through generations because the genetic variant is dominant and must be represented in each condition[14]. Significant point of X-linked inheritance is male-to-male passing is never occur.(figure4)



X-linked recessive

In X-Linked recessive monogenic diseases, the X-Chromosomal genes are modified, and they are not displayed as a distinctive trait under specific heterozygote circumstances, but can only be displayed in homozygote or hemizygote cases[15]. Hemizygotes are predominantly males in X-linked recessive disorders because they contain two distinct genes at separate loci and are more frequently afflicted than females. Due to a possible skewing in the process of ionization or X-inactivation, X-linked recessive diseases often can develop in females. Females' activation of one of the two X-chromosomes is inhibited selectively in each cell early in embryonic development during usual conditions[16]. As a result, the female might be a disease carrier who is affected herself but has the potential to transmit the disease on to coming generations through its children ([17][18].(figure5)

FIGURE5: X-linked Recessive pedigree
E.g. Hemophilia, Duchenne muscular dystrophy, Fragile X syndrome



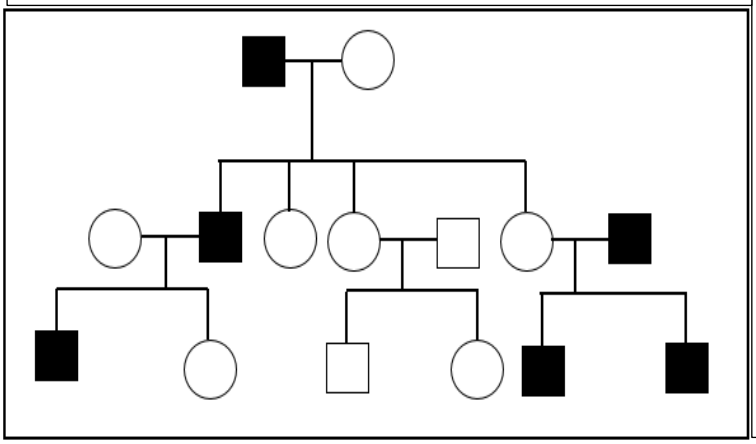
Wild-type Male
Wild-type Female
Affected Male
Affected Female

X-linked recessive conditions are generally restricted to males. Affected female has 50% chance of having an affected son and 50% to have an affected daughter

Y-linked

Y linkage, also known as holandric inheritance, it's a type of sex linkage that refers to traits created by genes on the Y chromosome. Y-Linked Genetic Disorders are caused by a mutation in a gene on the Y-chromosome. Because the Y-Chromosome is substantially smaller and only contains a few genes. As a result, there are only a few Y-Linked genetic disorders that are extremely rare[19], which primarily end up causing fertility issues and are not generally taken to the offspring. However, sometimes in cases, they may be inherited but only affect the sons, while the daughters are completely healthy because they do not take the Y-Chromosome. As a result, it is clearly distinguishable[20].(figure6)

FIGURE6: Y-linked pedigree
E.g. Hypertrichosis of the ears, webbed toes, Porcupine



Wild-type Male
Wild-type Female
Affected Male
Affected Female

Only father to son transmission of Y-linked genes is possible.

Non-Mendelian Disorders

Non-Mendelian syndromes are characterized by the inheritance of traits that do not fulfill Mendel's Law of Segregation (some main differences are listed in table1) , which states that each fertilized egg or sperm receives just one copy of each gene pair.Currently we are noticed that traits may be controlled by several genes and genetic

material can be transferred from parents to children in ways other than those indicated by Mendel in his segregation law[21].

In the non-Mendelian form of inheritance different genes cooperate to express one phenotype, or several traits arise from one gene. Sometimes the features can be detected in the phenotypes that derive from both the distinct alleles. The interaction of several genes or one gene influencing an individual's physiology might lead in disorders that are occasionally severe. The most substantial instances of Non-Mendelian disorders include Multifactorial disorders, Mitochondrial disorders and Dynamic mutations (Repeat number extension)[22].

Multifactorial Disorders:

The most frequent type of genetic condition is multifactorial inherited disorders. Various gene-gene interactions and multiple gene-environment interactions play a dynamic role in the pathophysiology and clinical manifestations of these syndromes, rather than following the Mendelian theory of inheritance pattern. As a result, multifactorial illnesses are caused by numerous genetic and/or environmental variables cooperating[23].

Common birth defects such as congenital heart disease, neural tube defects, facial clefting, and common medical problems such as diabetes, autoimmune disease, cancer, and most cases of autistic spectrum disorder are included in multifactorial disorders subtype[24].

Mitochondrial Disorders

Mitochondria are energy-producing organelle, they convert the energy in food molecules into ATP, which activates most cell functions. Its circular genome contains 13 polypeptides and transcribes 22 tRNAs, 2 rRNAs, as well as parts of the mitochondrial pathway and oxidative phosphorylation machinary. This organelle can be found in all human cells except red blood cells [21]. Mitochondrial disorder is a catch-all phrase for a variety of conditions caused by mitochondrial dysfunction. Mitochondrial disorders (MD) are a group of mitochondrial dysfunction-related genetic abnormalities that are either maternally inherited (The ovum essentially provides the cytoplasm and consequently the entire complement of mtDNA), autosomal dominant, or recessive [25]. MD can develop at any age, and many cases present with a broad variety of symptoms due to heteroplasmy[26]

Repeat expansion disorders

The instability of tandem nucleotide repeats causes repeat expansion diseases. There are over one million identified tandem repeats (TRs) in the human genome. TRs have the greatest mutational frequency in the genome because of their repetitiveness, and they are frequently polymorphic and multiallelic. TRs are divided into subtypes and Microsatellites and Minisatellites ((1–9 bp repeats, 10–99 bp repeats respectively) are the most common subtypes of TRs, that both represent the wide range of tandem repeats, moreover satellites which are 100 bp repeats and particularly comprise centromeres, and heterochromatin are the another examples of TRs.short triplet repeats

can occasionally be discovered in the coding sequence of genes,However this sequences are normally detected within non-coding regions[27][28].

The inheritance pattern for repeat expansion conditions is Mendelian, The repetitive mutation process, unlike static mutations in Mendelian conditions, is dynamic. [21]. The following section summarizesa presence of particular molecular characteristics of repeat expansion diseases.

To begin, depending on where the repetitions are located, repeat expansions have distinct genetic features.[29].Additionally, in comparison to their afflicted forebears, following generations of afflicted persons have a more serious symptoms as well as early age of onset. [30]. Furthermore, in certain clinically asymptomatic people, the number of repetitions appears to be higher than in the general population, a condition known as premutation.

TABLE1: Mendelian Versus Non-Mendelian Inheritance

Mendelian Versus Non-Mendelian Inheritance	
Mendelian	Non-Mendelian
This inheritance is the process through which genes and their associated qualities are handed on from parents to their children via dominant and recessive alleles.	The patterns of heredity that do not follow the Mendelian inheritance.
Involve only 2 alleles	Involve multiple alleles or polygenes
Theoretically, phenotypic proportions can be predicted.	Phenotypic proportions are not the same as theoretical proportions.
Autosomal Dominant (AD) disorders Autosomal Recessive (AR) disorders X-linked Dominant disorders X-linked Recessive disorders Y-linked disorders	Multifactorial disorders Mitochondrial disorders Repeat number extension disorders
The two alleles of a gene are either dominant or recessive	The two alleles are neither dominant nor recessive

Chromosomal Abnormalities

Genomic instability is caused by chromosomal abnormalities, which include both numerical and structural abnormalities[31].(table2&3)

Numerical Abnormalities

Aneuploidy and polyploidy, which is defined by changes in chromosomal value (gain or loss) in other word consequent of chromosomal segregation errors, are the most common numerical chromosomal abnormalities[32].The gains and losses of chromatid or chromosomal segments is known as aneuploidy. Aneuploidy is classified as entire chromosomal aneuploidy or segmental aneuploidy based on the mechanisms of generation, Nondisjunction is a cell division error in which the chromosomes do not correctly distributed during anaphase. Usually in meiosis anaphase I or II, but infrequently in mitosis.E.g. monosomy (Turner syndrome 45X), trisomy (down syndrome 47XX+21).[33].Polyploidy, on the other hand, is a phenomenon in which a diploid cell includes over 2n chromosomes than usual (extra set of chromosome).The non-disjunction of sister chromatids is one of the most likely reasons of ploidy. This mainly happens during meiotic cell divisions,throughout gamete production, the number of chromosomes is duplicated prior meiosis e.g. triploidy(3n) and tetraploidy(4n)[34]

1.2.3.2 Structural Abnormalities

When the structure or sections of a chromosome alter, structural chromosomal abnormalities arise. In most human cells, there are 46 chromosomes overall. When a portion of a chromosome is missing, an additional part is present, or a part has traded places with another section, structural chromosomal abnormalities appear. These changes may involve either one chromosome or two even more chromosomes as a result these abnormalities lead to some birth problems. The two basic categories of structural anomalies are balanced and unbalanced alterations. Each one is further subdivided into smaller groups. There are excess or missing chromosomal segments in unbalanced structural defects. In contrast, balanced structural abnormalities include chromosomal rearrangement with no gain or loss of chromosome parts, Individuals with these defects are normally unaffected, but their offspring may acquire imbalanced chromosomal disorders[35]. DNA damage results in chromosome structural anomalies ranging from chromosome arm-level deletions or amplifications to multiple chromosome changes[36].

Inversion:

Balanced Structural chromosomal abnormalities (inversion) this chromosomal anomaly occurs when the order of genes in a chromosomal section is 180 degrees inverted.Inversion usually involves two breaks in distinct parts of the chromosome. The freshly produced segments are then switched out. In 1921, inversion was found. Although we do not yet understand why inversion occurs, we do know that it is the most fundamental mechanism for genomic reorganization. Pericentric inversion generates deletions, insertions, or malformed centromeres; paracentric inversion is the more frequent variety and is less detrimental to its carrier[37]

Translocation:

The phenomenon that a region of chromosome or entire arms transfer across two different chromosomes known as Translocation. Robertsonian translocations and Reciprocal translocations are the most known translocation forms, while 2 acrocentric chromosomes fuse together by their long arms and rearrange 1 chromosome (either metacentric or sub-metacentric), which occur in 1 in 1000 population. But at the other

hand, once 2 segments of chromosome exchange between two, (that might involve any of the chromosomes) this rearrangement is called Reciprocal translocations [21].

Deletion:

The loss of a chromosomal segment is known as deletion. For the interstitial portion to be deleted, two breaks must occur. One break is enough to delete the terminal region (telomere). The genes present in segments that have been removed from the chromosome are lost because they are unable to "live" under their own. There is a unique case of deletion. It's known as the "ring chromosome." It occurs when a chromosome loses both of its ends. The arms then join along, forming a ring-shaped chromosome [38]

Duplication:

Duplication is an un-balanced rearrangement of the chromosome once region of the chromosome is doubled, giving in an overabundance of genetic material.Ectopic recombination, Retrotransposition,Replication slippage, Aneuploidy, Polyploidy are mechanisms that involve chromosomal. E.g. Chromosome 12p duplication (Pallister Killian syndrome[39]

Insertion:

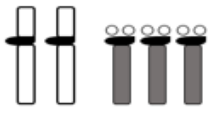
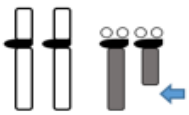
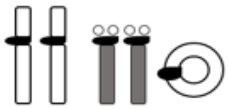
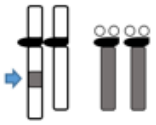
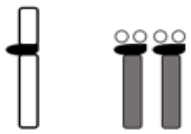
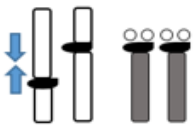
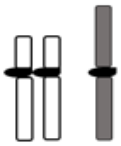
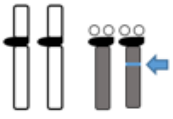
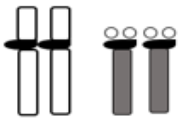
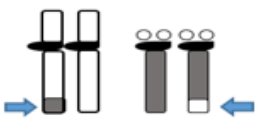
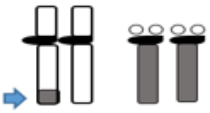
Insertion describes the presence of anything attaches inside.Insertion is a sort of aberration in which genetic material is increased. An insertion mutation might be as small as a single additional DNA base pair or as large as a whole chromosome. Insertional mutations can be either frameshift or non-frameshift, a frameshift mutation changes all of the codons downstream of the mutation,if the insertion is inside a multiple of three, and therefore, it will have no influence on the coding downstream. Fragile x syndrome, Huntington's disease are examples of insertion mutations. [40]

Isochromosome

Isochromosomes are malformed chromosomes that have two copies of one specific arm (it can be either a short or long arm).Isochromosomes are formed whenever the centromere divides abnormally. The centromere typically splits vertically. Whereas it splits horizontally in this scenario, and one arm is generally lost as a result. It signifies that a newly formed chromosome only includes two q arms or two p arms that are ordinarily joined by the centromere. It is relatively common in the X chromosome. It is a major issue during fertilization. Since the individual develops trisomic in one arm while monosomic in the other[41].

TABLE2&3: CHOROMOSOMAL ABNORMALITIES CLASSIFICATION

CHROMOSOMAL ABNORMALITIES	Numerical	Aneuploidy	Monosomy (45X)	
			Trisomy (47XXY, 21...)	
		Polyploidy	Triploidy (3n)	
			Tetrapoidy (4n)	
	Structural	Balanced	Inversion (inv)	Pericentric
				paracentric
			Translocation (t)	Robertsonian
				Reciprocal
		Un-Balanced	Deletion (del)	
			Duplication (dup)	
			Isochromosome (I, iso)	
			Ring chromosome (r)	

Numerical aberrations	Structural aberrations		
 Trisomy	 Deletion	 Ring chromosome	 Insertion
 Monosomy	 Inversion	 Robertsonian Translocation	 microdeletion
 Wild-Type	 Balanced Translocation	 Unbalanced Translocation	

Spinal Muscular Atrophy (SMA)

Spinal muscular atrophy (SMA) is a hereditary neuromuscular condition which is defined as an autosomal recessive disorder that has high carrier frequency rate, and significantly one out of each 10,000 live births can be affected by this syndrome. In the SMA condition the anterior horn cells of the human spinal cord degenerate, ultimately results in motor neuron loss. This deficiency depending on the genotype can cause Hypotonia as well as muscular weakness. The all atrophy's degree are caused by the gradual loss of alpha motor neurons, however this missing can occur either within the ventral spinal cord or motor nuclei within the lower brainstem[42, 43].

Classification, Phenotypes and Pathophysiology

SMA like the most genetic disorders includes severity of the condition and its classification is according to the age of unset, plus its status[44].Although it is now evident depending on the underlying genotype, phenotypes cover a wide range of traits. Due to mutations and abnormalities in the SMN1 gene sequence which is located on long arm of chromosome number 5 (5q13), the most devastating form of SMA is referred to type 1. Once an Infant is affected by type 1 SMA typically develops the condition early after the birth and in this situation never gains the ability to sit alone [45, 46]. Recent studies have been suggested a classification of type one with 3 subtypes, One of them is based on age of onset : for instance type 1a SMA refers to newborns who show obvious and rush clinical indications early just after the birth (during the first two weeks of life); in additionin type 1b which is included infants weakness appears by three months of age; and the last subtype is 1c SMA which is described the infants who represent weakness by six months of age, without pharmacological therapy, the expectancy for respiratory muscle failure is less than two years [47]. Patients with Type II manifest with growing proximal weakness, mainly in the legs, at the age of 6–18 months [48] .Patients can sit but not walk independently, and infrequently develop respiratory problems that need non-invasive ventilation before maturity, similarly as orthopedic problems such severe scoliosis and joint contractures[49]. Type III manifests at 18 months, with individuals experiencing growing proximal weakness but reaching significant milestones and being able to walk unassisted at first [50]. After the age of 20–30 years, Type IV manifests with a milder phenotype, with SMA type IV rarely leading to death [51].

Causes and Inheritance

Mutations that occur within SMN1 (survival motor neuron) gene sequence lead to SMA. [46] However SMN1 (also in some papers is referred to as SMNT, where T stands for telomere) is located within the telomeric region of long arm of chromosome number 5 that includes 20-kb , which is highly vulnerable to rearrangements and deletions. The highly homologous of SMN1 is SMN2 (or SMNC, that C stands for centromere) this centromeric element SMN2 consist of over 99 percent nucleotide similarity with SMN1 [52, 53] whereas it has been determined there is an association between milder phenotypes of SMA and greater copy number of SMN2, as a result SMN2 is considered as a modifying factor[54]. 9 exons within the SMN geneare the sequence templates that encode the SMN protein (294-amino-acid)which is required for motor neuron survival [55]. This RNA-binding

proteinparticipates in a variety of cellular functions and pathways, most notably within the cytoplasmic assembly of snRNP complexes [56].

Diagnosis and Screening Methods

Initial step in diagnostic testingfor this disorder is screening for exon 7 deletion. In 95 percent of afflicted subjects the deletion of SMN1 (homozygous) is seen. MLPA (or multiplex ligation-dependent probe amplification) is convenient, extremely sensitive, and capable of assessing copy number of either SMN1, SMN2 or both, this procedure isthe foremost method thatcan detect deletions of these genes in laboratories [57, 58]moreover diagnosis can be affectively achieved through quantitative techniques such as real-time PCR, according to a recent result by François Boemer[59].

1.3.5 Treatment and Limitations

One out of two most known medicine for SMA is Nusinersen (Spinraza), which is the first medicine that licensed for the treatment, Spinraza is an antisense oligonucleotide that is able to elevate the rate of SMN protein in the central nervous system of human body and it works through alternative splicing of the SMN2 gene [60]. Onasemnogene abeparvovec (Zolgensma) is the second medicine or method which is specified gene therapy that is able to transfer the functional SMN1 gene into the motor neurons via using aviral vector(adeno-associated)[61], according to FDA it has been demonstrated to enhance motor function in newborns with severe SMA type 1 [62] When taken prior to the onset of symptoms, such therapies can limit or prevent disease progression; nonetheless, management and support are necessary to address the disorder's consequences [63].

Work in this thesis

After cystic fibrosis, SMA with autosomal recessive inheritance pattern is in second place of most prevalent genetic disorder in humans. SMA affects roughly one in every 6,000 to 10,000 live births, with significant carrier frequency about one in every 40-80 individuals.

Turkey's Health Minister stated on December 2021 that by the end of the month, screening for Spinal Muscular Atrophy (SMA) disorder will be mandatory prior to marriage in the country. As a result to prevent the occurrence of such genetic diseases and to make relevant decisions for the population of a country. Knowing the abundance of the target gene in the population is the first step toward having a healthy population.

The aim of this study was to evaluate the carrier frequency of SMN1 mutation causing Spinal Muscular Atrophy in Turkish Cypriot Couples for the first time. A total of 100 Turkish-Cypriot couples who are planning to have their first child tested for these investigations. Turkish Cypriot were defined as three generation is in Cyprus. DNA isolated from peripheral blood using Invitrogen Genomic DNA Isolation Kit. To analyze the sequences Quantitative Real Time PCR (RT-qPCR) has been performed.

CHAPTER II

Literature Review and Theoretical Framework

Spinal Muscular Atrophy (SMA)

Spinal muscular atrophy (SMA) is a hereditary neuromuscular condition which is defined as an autosomal recessive disorder that has high carrier frequency rate, and significantly one out of each 10,000 live births can be affected by this syndrome. In the SMA condition the anterior horn cells of the human spinal cord degenerate, ultimately results in motor neuron loss. This deficiency depending on the genotype can cause Hypotonia as well as muscular weakness. The all atrophy's degree are caused by the gradual loss of alpha motor neurons, however this missing can occur either within the ventral spinal cord or motor nuclei within the lower brainstem.

Classification, Phenotypes and Pathophysiology

SMA like the most genetic disorders includes severity of the condition and its classification is according to the age of unset, plus its status. Although it is now evident depending on the underlying genotype, phenotypes cover a wide range of traits. Due to mutations and abnormalities in the SMN1 gene sequence which is located on long arm of chromosome number 5 (5q13), the most devastating form of SMA is referred to type 1. Once an Infant is affected by type 1 SMA typically develops the condition early after the birth and in this situation never gains the ability to sit alone. Recent studies have been suggested a classification of type one with 3 subtypes, One of them is based on age of onset : for instance type 1a SMA refers to newborns who show obvious and rush clinical indications early just after the birth (during the first two weeks of life); in addition in type 1b which is included infants weakness appears by three months of age; and the last subtype is 1c SMA which is described the infants who represent weakness by six months of age, without pharmacological therapy, the expectancy for respiratory muscle failure is less than two years. Patients with Type II manifest with growing proximal weakness, mainly in the legs, at the age of 6–18 months .Patients can sit but not walk independently, and infrequently develop respiratory problems that need non-invasive ventilation before maturity, similarly as orthopedic problems such severe scoliosis and joint contractures. Type III manifests at 18 months, with individuals experiencing growing proximal weakness but reaching significant milestones and being able to walk unassisted at first. After the age of 20–30 years, Type IV manifests with a milder phenotype, with SMA type IV rarely leading to death.

Causes and Inheritance

Mutations that occur within SMN1 (survival motor neuron) gene sequence lead to SMA. However SMN1 (also in some papers is referred to as SMNT, where T stands for telomere) is located within the telomeric region of long arm of chromosome number 5 that includes 20-kb, which is highly vulnerable to rearrangements and deletions. The highly homologous of SMN1 is SMN2 (or SMNC, that C stands for centromere) this centromeric element SMN2 consist of over 99 percent nucleotide similarity with SMN1 whereas it has been determined there is an association between milder phenotypes of SMA and greater copy

number of SMN2, as a result SMN2 is considered as a modifying factor. 9 exons within the SMN gene are the sequence templates that encode the SMN protein (294-amino-acid) which is required for motor neuron survival. This RNA-binding protein participates in a variety of cellular functions and pathways, most notably within the cytoplasmic assembly of snRNP complexes.

Screening Methods

Initial step in diagnostic testing for this disorder is screening for exon 7 deletion. In 95 percent of afflicted subjects the deletion of SMN1 (homozygous) is seen. MLPA (or multiplex ligation-dependent probe amplification) is convenient, extremely sensitive, and capable of assessing copy number of either SMN1, SMN2 or both, this procedure is the foremost method that can detect deletions of these genes in laboratories [57, 58] moreover diagnosis can be affectively achieved through quantitative techniques such as real-time PCR, according to a recent result by François Boemer [59].

AIM

To evaluation of the carrier frequency of SMN1 mutation causing Spinal Muscular Atrophy (SMA) in Turkish Cypriot Couples. This will be the first study to evaluate the SMN1 deletion mutations in this population.

Material and methods

Subjects

A total of 100 Turkish-Cypriot couples who are planning to have their first child will be tested for these investigations. Turkish Cypriot will be define as three generation is in Cyprus.

DNA isolation

DNA will be isolate from peripheral blood using Invitrogen Genomic DNA Isolation Kit.Quantitative Real Time PCR (RT-qPCR)In order to determine the carrier and homozygous types, DNA extracted from blood wasevaluated by Quantitative Real Time PCR (qPCR), by following the manufacturer'sprocedures (IntRaFast-Q SMA SCREENING KIT Cat. No: 200R-40-01) to identify exon 7& 8 deletion and C/T substitution at nucleotide 840 of exon 7 within the SMN1 gene. FAMfor SMN1 Exon 7 & C/T substitution at nucleotide 840, HEX for SMN1 Exon 8, andTEXAS RED for reference gene dyes are used in the IntRaFast-Q master mix. The data was collected and analyzed using IntRa-Q software (SNP Biotechnology, Gebze, Istanbul)

Analysis

Hardy-Weinberg Equilibrium will be use to evaluate the mutation frequency in the studied population ($P < 0.05$ will consider as statistically significant).

Related Researches

Since incidence of Spinal Muscular Atrophy has a high rate, screening for carrier frequency of SMA in variety population have been performed. SMA has an incidence about 1 into 6000 however it can be reached to 10000 , depends on the location and consanguineous marriage prevalence. For instance according to a study that accomplished in Thailand within 505 adult Thai individuals the researchers found the carrier frequency at 1.8% by performing MLPA[64], moreover cohort study in Iran showed the prevalence of SMA in Iranian population 5% due to high level of consanguineous marriage[65]. Similar research was performed in north India and notably their research revealed carrier frequency of SMA in north Indian population without any family history 16.5% [66]. Additionally a pilot study in Saudi Arabia on 2015 showed the prevalence rate is about 5% in Middle East[67]. Another study in Greece represented that the incidence in this countryis between 1:6,000 and 1:11,000 live births, with a range of prevalence between 1/38 and 1/50, furthermore 2% is the range of being a carrier of SMA in Turkey.

To sum up, to prevent the occurrence of such genetic diseases and to make relevant decisions for the population of a country. Knowing the abundance of the target gene in the population is the first step toward having a healthy population. Since the prevalence of the SMA in North Cyprus has not been reported, this research was performed for the first time to inquire the rate of SMA carrier frequency in Turkish Cypriote population.

CHAPTER III

Methodology

Subjects and DNA Isolation

Proposal of this survey handed to the ethics committee of Near East University Hospital and ethical concern approved, to perform this study an announcement called the Cypriot Turkish couples for screening *SMN1* gene mutations and deletions. (Turkish Cypriot defined as three-generation is in Cyprus).

The individuals' peripheral blood was taken and collected at +4 degrees C to proceed DNA isolation. Overall, 94 samples were obtained that the genders were equal (47 individuals female/ 47 individuals male), and the average age of the population was about 31.17 ± 4.18, 29.38 ± 3.94years old, respectively, for men and women. In this study any α or β-Thalassemia status for all patients is considered as a comparative criteria since up-to-date there is no reportregarding a patient as a carrier for both mutation in either*HBA* or *HBB* and *SMN1* genesAccording to their consent forms, eight females and ninemales had carrier status of β-Thalassemia that eight out of them were partner (4 couples) its carrier frequency in our study population was 18.08%, no family history of SMA was found in this group however some genetic disorders such as Down syndrome, Neurofibromatosis, Chromhidrosis and etcetera were reported in eight cases' family history. ~83% of this study population was Cypriot 7.4% was from Turkey and ~ 5.3% was born in other countries but their parents were Cypriot . General characteristics are shown in table 4.

Table 4: General Characteristics of the population

N= 94 couples=47	
Age Mean age ±SD	Male 31.17 ± 4.18
	Female 29.38 ± 3.94
	Total 30.27 ± 4.16
Country of origin	Northern-Cyprus 78
	Turkey 7
	Other 5
Thalassemia Status	Male 9
	Female 8
	Couples 4
	Total carrier frequency of β-Thalassemia18.08%

SNPure Genomic DNA Kit (SNP Biotechnology, Cat. No: 21S-01-250,250 Preps, Ankara, Turkey) is designed particularly for extracting DNA from whole blood by using DNA binding column method that has high accuracy. All 94 samples' DNA was isolated by using this specific kit from prepheral blood. Furthermore, in order to screen deletions of the Exon 7 and/or 8 *SMN1* genes and precisely substitution of C/T at nucleotide number 840 within exon 7, the IntRaFast-Q

SMA screening kit was chosen because of its optimization and specificity for deletions on exon 7 and/or 8 that cause variety spectrum of SMA disorder.

Detection SMN1 gene mutations by Quantitative Real-Time PCR (RT-qPCR)

The mutation detection of *SMN1* gene mutations, Exon 7 and 8 deletions and c.849C/T substation within exon 7 was carried according to manufacturer guidelines (IntRaFast-Q SMA Real Time PCR Screening Kit, Cat. No: 200R-40-20, SNP Biotechnology, Ankara, Turkey).

The master mix including precise probes and primers is also included fluorescence dyes, FAM has been designed for specifying exon 7 of the *SMN1* gene as well as C/T substitution at nucleotide 840, and TEXAS RED is allocated for reference genes and HEX dye is capable of determining exon 8 of desire gene (*SMN1*).

<table>
<tr><td colspan="3">Selected FAM, HEX and TEXAS RED as florescent dyes.</td></tr>
<tr><td>96C</td><td>2 Sec.</td><td rowspan="2">32 × Cycle</td></tr>
<tr><td>60 C</td><td>40Sec.</td></tr>
</table>

By following the kit instruction 2 µl of any isolated DNA of the population were pipetted into 23µl of the master mix and in order to compare and detect any mutations/deletions in samples genome, homozygous deletion specimen, wild-type as well as 1 carrier samples that were the kit components and another carrier sample that has been proved and reported as an SMA patient were comprised qPCR.

Statistics

Hardy-Weinberg Equilibrium was used to evaluate the mutation frequency in the studied population ($P< 0.05$ will consider statistically significant). Quantitative Real-Time PCR (RT-qPCR) was performed and analyzed by IntRa-Q software(SNP Biotechnology, Ankara, Turkey).

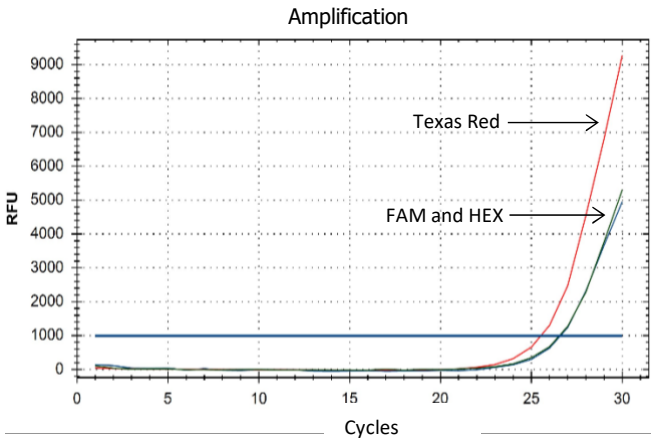
The IntRa-Q Software is based on IR method calculates a ratio of “reference gene quantification / *SMN1* gene exon 7 and exon 8 quantification. Both the quantifications of the reference gene and the *SMN1* gene were detected by means of embedded slope value in the software, and the ratio of these quantification values determined the IR value. The software gives the results automatically based on these values.

Chapter IV

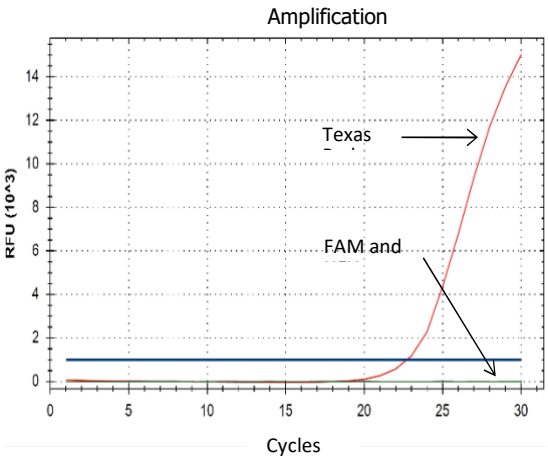
Results

Findings for Research "The carrier frequencies of Spinal Muscular Atrophy causing SMN1 gene mutations in Turkish Cypriot Couples"

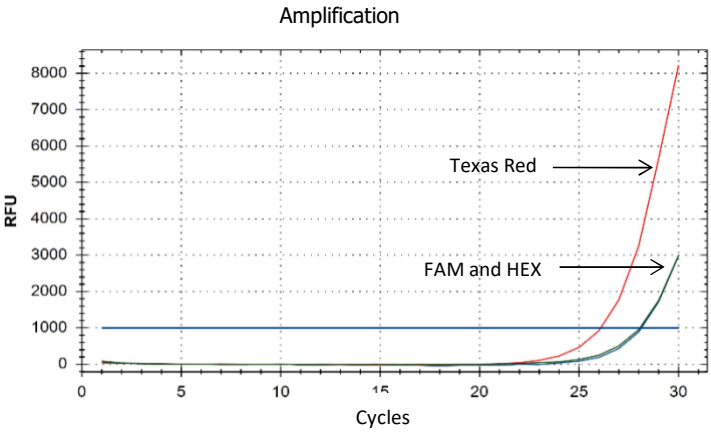
To calculate IR values for data analysis we used IntRa-Q Software (SNP Biotechnology, Ankara, Turkey). Figure 7 illustrates all the amplification plots possibilities in any sort of sample (wild-type, carrier, homozygous) , the mutation detection was used according to manufacturer guidelines (IntRaFast-Q SMA Real-Time PCR Screening Kit, Cat. No: 200R-40-20, SNP Biotechnology, Ankara, Turkey). To reach to reliable results all 94 samples were compared to the homozygous, carrier, and wild-type specimens that were provided by the considered kit.



SMN1 Wild-Type Sample from blood



SMN1 Homozygous Deletion from blood



SMN1 Exon 7 and Exon 8 Carrier State from blood

Figure7 comparative charts for wild-type, carrier, homozygous samples, illustrated by Kit(IntRaFast-Q SMA Real Time PCR Screening Kit, Cat. No: 200R-40-20, SNP Biotechnology, Ankara, Turkey)(copy-right)

To determine amplification plots of SMA carrier states and homozygous deletion, *SMN1* exon 7 and exon 8 as well as reference genes analyzed together. In all the charts FAM (represents *SMN1* Exon 7 – is showed by blue plot), HEX (determines *SMN1* Exon 8 - marked as green plot) and TEXAS RED (Proofs reference gene and demonstrated by red plot) are fluorescence dyes that are chosen to be used in this study.

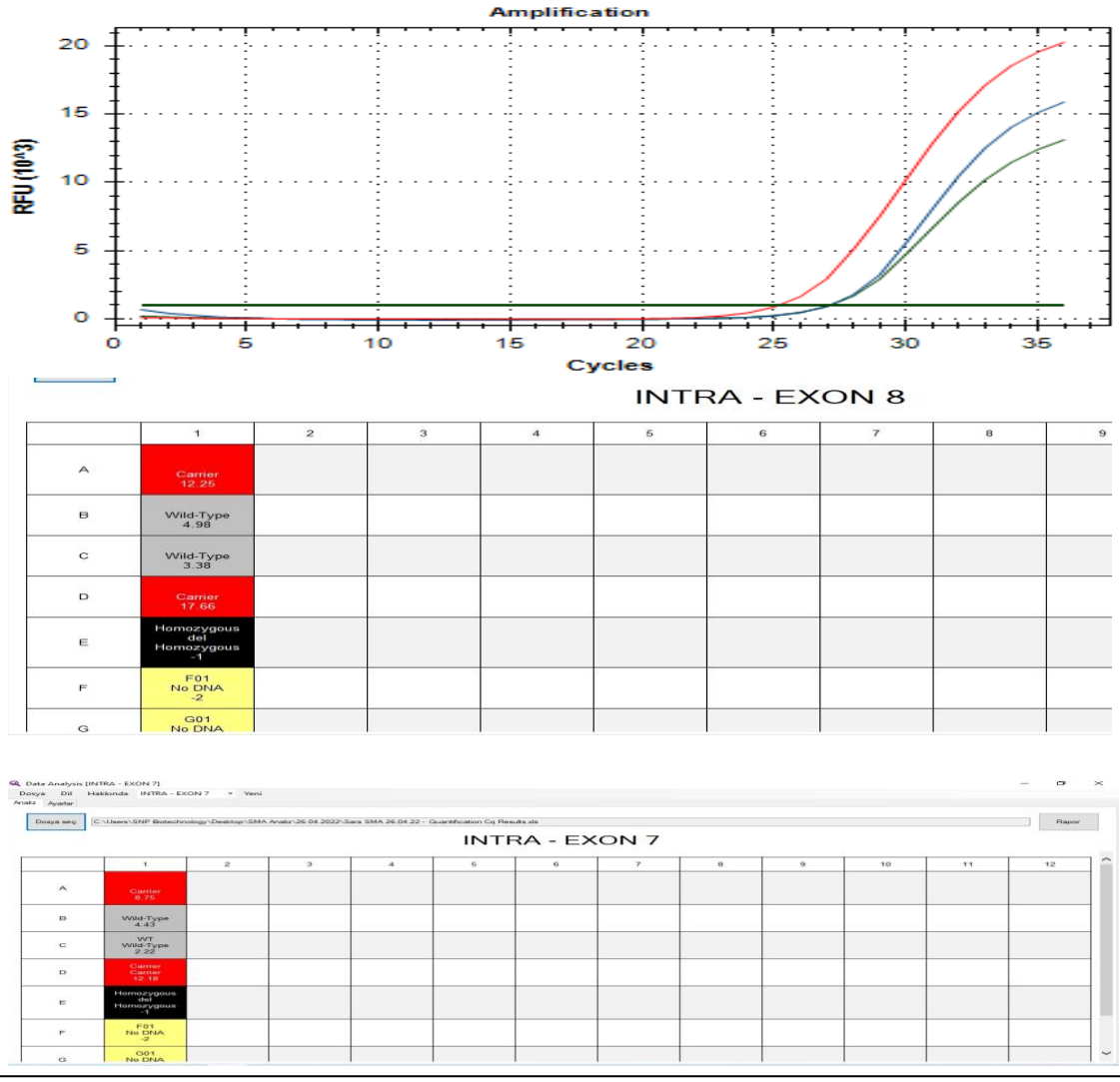
In the total of 94 individuals, 1 patient turned out to be a carrier of the *SMN1* gene in both exon 7 and 8 (carrier number 1) and another patient (Carrier number2) showed a carrier status of *SMN1* gene only in exon 7.

Carrier1, a 32 years old man who is originally from Northern Cyprus, with wild-type Thalassemia Status, has one sibling, No family history or any known genetic disease and no known miscarriage of the mother is reported.

Carrier2 is 26 years old man from Northern Cyprus with Wild-Type status of Thalassemia, 2 siblings and no family history of known genetic disorders, and no miscarriage of the mother is reported.

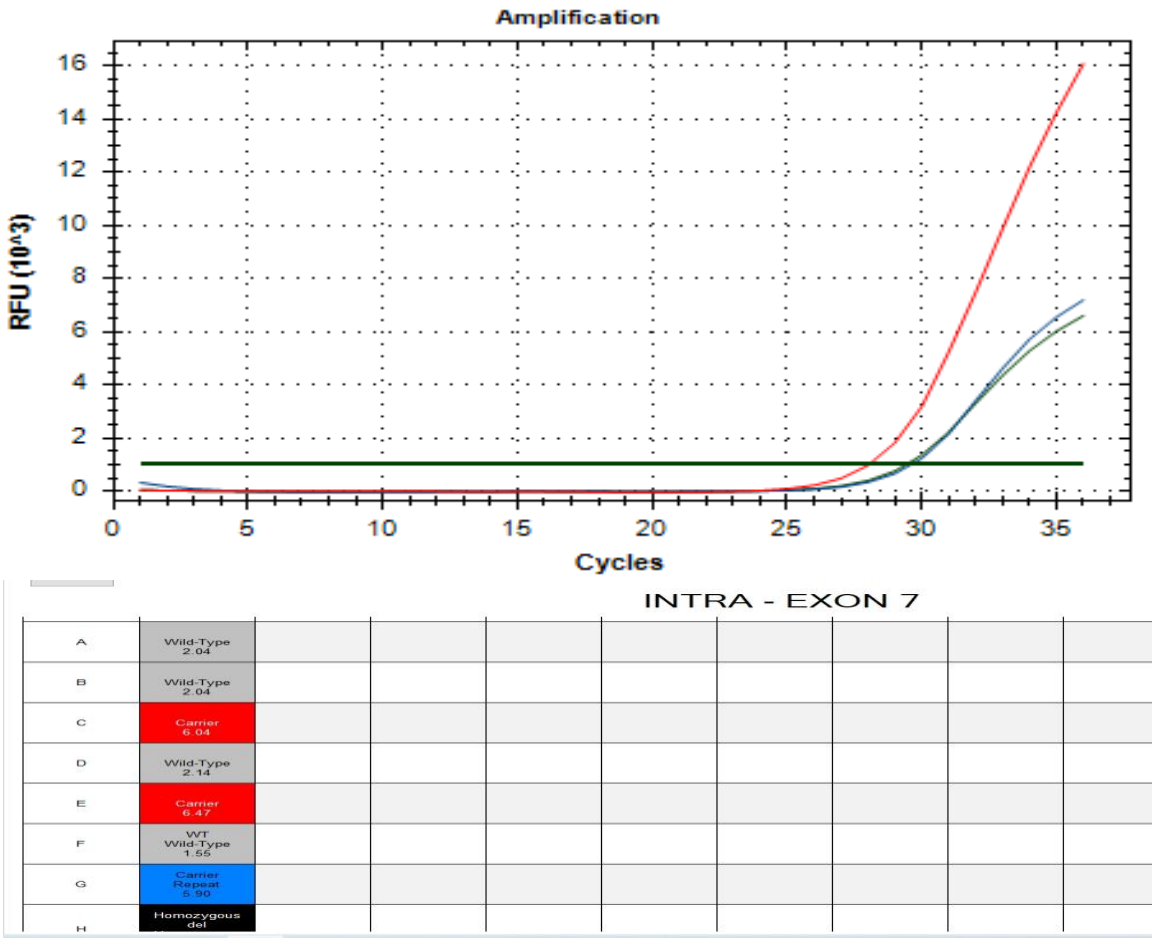
Carrier1 showed a ratio of 12.25 for exon 8 and 8.75 for exon 7 whilst the ratio for wild-type status is shown as 1.55 for exon 7 and 3.38 for exon 8. Figure 8 shows the results revealed by IntRa-Q Software, decaling of the FAM and HEX curves for both corresponding exons is quite indicative which proves the carrier status of *the SMN1* gene for patient number 1(Carrier1). Figure 8 shows the IR results for SMN1 gene exon 7 and 8 Carrier1.

Figure 8 IR results for *SMN1* gene exon 7 and 8 Carrier1
(FAM (SMN1 Exon 7 - blue plot), HEX (SMN1 Exon 8 - green plot) and TEXAS RED (Reference gene - red plot)



Furthermore Carrier2 showed ratio 6.04 for exon 7 while the wild-type sample represented a ratio about 1.55, figure 9 presents general data that disclosed by IntRa-Q Software and determinesreduction of FAM and HEX thus it confirms carrier status of exon 7, *SMN1* genein patient number 2(Carrier2).

Figure 9 IR results for *SMN1* gene exon 7 Carrier2
(RFU= relative fluorescence unit, FAM ((SMN1 Exon 7 - blue plot)), HEX ((SMN1 Exon 8 - green plot)) and TEXAS RED
((Reference gene - red plot)).)



Discussion

Spinal Muscular Atrophy with a high prevalence and incidence rate is the 2nd common autosomal recessive genetic disease which has a remarkable rate of infant mortality that causes by a monogenic condition [68].

According to a report that NCBI has been published its incidence is about 1:6000-10000 live birth with a significant prevalence in the asymptomatic population of about 1:40-60[69, 70].SMA's carriers by having a heterozygous status of *SMN1* gene are asymptomatic, one study conducted in the US showed prevalence 1 in every 47 white individuals, however, it revealed carrier frequency in the black population is 1 in 72 individuals[69], and research reported a carrier frequency of SMA in China ~2% (1 in 50 individuals)[71], moreover, cohort study in Iran showed the prevalence of SMA in Iranian population 5% due to high level of consanguineous marriage[65].Similar research was performed in north India and notably, their research revealed carrier frequency of SMA in the north Indian population without any family history 16.5% [66].Additionally, a pilot study in Saudi Arabia in 2015 showed the prevalence rate is about 5% in the Middle East[67]. Another research in Greece represented that the incidence in this country is between 1:6,000 and 1:11,000 live births, with a range of prevalence between 1/38 and 1/50, furthermore, 2% is the range of being a carrier of SMA in Turkey.

SMA subtypes are classified according to the severity of the conditions, thus the most severe form of this disease is the type I shows obvious and rushed clinical indications early just after the birth that degeneration of anterior horn cells of the spinal cord leads to death a few weeks after the birth due to respiratory failures and muscle weakness.

Even though SMA symptoms were previously thought to be unbreakable, two recently discovered drugs—Nusinersen (Spinraza) and Onasemnogene abeparvovec (Zolngensma)—have altered the condition and abilities of afflicted newborns. Regarding the experience of treatment by Zolngensma in Qatar treatments should be begun at the pre-symptomatic stage of SMA to optimize the effectiveness of this therapy.

We have done this research because to prevent the occurrence of such genetic diseases and to make relevant decisions for the population of a country, knowing the abundance of the target gene in the population is the first step toward having a healthy population.

Our findings revealed that the carrier frequency of mutation in *the SMN1* gene for exon 7 is 2.12% (2:94 healthy individuals) while its mutation prevalence for exon 8 is 1.06% (1:94 healthy individuals).

As a result, wild-type status of α and β-Thalassemiaof these two traced subjects is confirmed, resembling other research that has been done up-to-date in our study population no mutation /deletion of *SMN1* gene along with *HBA* or *HBB* genes is recognized. these two 32 and 26 years old men, do have not any family history of SMA, and no Known genetic disorders are reported as a result carrier frequency of 2:94 healthy individuals with no previous positive family history has significant policy implications.

It means the carrier could pass the mutated copy of *the SMN1* gene to the next generation and in case their partner is also a carrier for the corresponding gene their risk of having a child with SMA disorder is 25% and the risk of having a baby with a carrier status is 50%.

Conclusion

In this study, we have described our experience of carrier frequency of SMA and estimated the incidence of SMA in north Cyprus. , to date no data was available on the prevalence of SMA in North Cyprus. Thus, we have estimated the SMA carrier frequency in North Cyprus population using a reliable quantitative real-time PCR to quantify the *SMN1* gene (common variant, deletion of SMN1 exon 7 and 8).

Whereas the prevalence of this disease is 1 in 10,000 live births globally, and after cystic fibrosis is known as second most common fetal autosomal recessive disorder, however no data was reported on its incidence, we have been performed this research for the first time in north Cyprus and our findings revealed that the carrier frequency of deletion in the *SMN1* gene for exon 7 is 2.12% (2:94 healthy individuals) while its deletion prevalence for exon 8 is 1.06% (1:94 healthy individuals).

Notably upon comparing our findings to data that reported from other countries, the estimated prevalence of *SMN1* gene deletion is lower than that reported in some countries such as Iran (5%), India (16.5%) and Saudi Arabia (5%) which could be due to consanguineous marriage however its prevalence is similar to US, China Turkey which is about 2%.

Ultimately, this is the first research in North Cyprus to address the estimate of SMA carrier frequency. With such a high frequency of SMA associated to the deletion of the *SMN1* gene, health precautions should be taken. Individuals with a positive family history may benefit from carrier testing as a tool for genetic counseling. One of the treatment process' limitations is the cost-effective therapies for this genetic disorder, such as Onasemnogene abeparvovec (Zolngensma). Screening is the first step in preventing a child from developing SMA. Our findings will be presented to the Ministry of Health, and their decision will be conducted to include *SMN1* gene mutation in screenings before marriage, however before having a baby screening for SMA is highly recommended.This data are important for uncovering potential local profiles of SMA and defining the disease clearly as well as for determining premarital protection programs.

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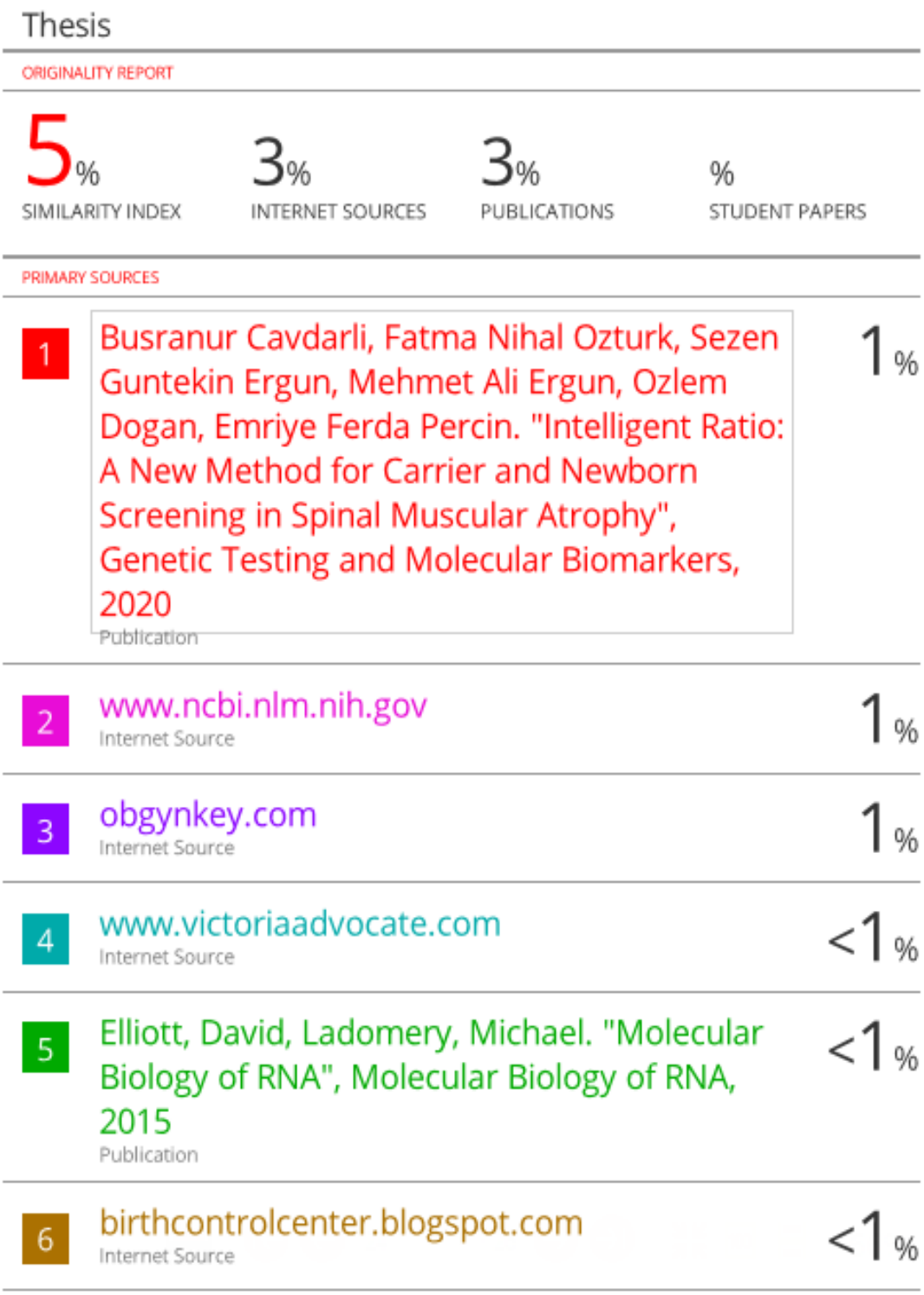

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SUMMARY

My name is Sara I was born in 1996 in Iran , and until finishing my bachelor program I lived there, I studied molecular cell biology and genetic as my Bachelor degree and graduated with GPA 3.6.
During this time I attended some internships and workshops to improve my laboratory skills specifically areas related to genetic such as PCR, cell culture ,DNA, RNA and protein extraction, probe and primer designing and etc. at Iran Pasteur institute.
Afterwards, I have started my master's program in molecular medicine , I've just defended my thesis research on first of the June 2022 which was "carrier frequency of SMN1 mutation causing Spinal Muscular Atrophy (SMA) in Turkish Cypriot Couples" in title.

CURRENT RESEARCH:

"New-Born Prevalence of Spinal Muscular Atrophy in Northern-Cyprus"
Genome Sequencing of rare genetic disease.

EDUCATION

MSc. Molecular Medicine
Near East University – North Cyprus
2021 – 2022
GPA: 3.78

BSc. Cell Molecular Biology-Genetics
Azad University - Iran
2014 - 2018
C.P.G.A: 17 out of 20
G.P.A: 3.6

WORK EXPERIENCE

Voluntarily works and internship
2017-2018 at research center of Shiraz Iran as an internship
2018-2019 at Research Tower of Shiraz as an internship.
2020-21at Covid_19 laboratory part time in Nicosia, North Cyprus.

Congresses
international congress of biomedicine (ICB) 2017
International congress of biomedicine 2020 (online)
Northern-Cyprus pediatric congress 2022
NEU International student congress 2022

Workshops
Cell culture , DNA Extraction, Protein extraction, Bioinformatics (R programming) at Pasteur Institute of Iran.
Clinical molecular diagnostic and genetic technologies.

LANGUAGES

English	Advance
German	Intermediate
Turkish	Intermediate
Farsi	Native

HOBBIES

Writing and reading poem
Playing chess
Photography
Watching documentaries

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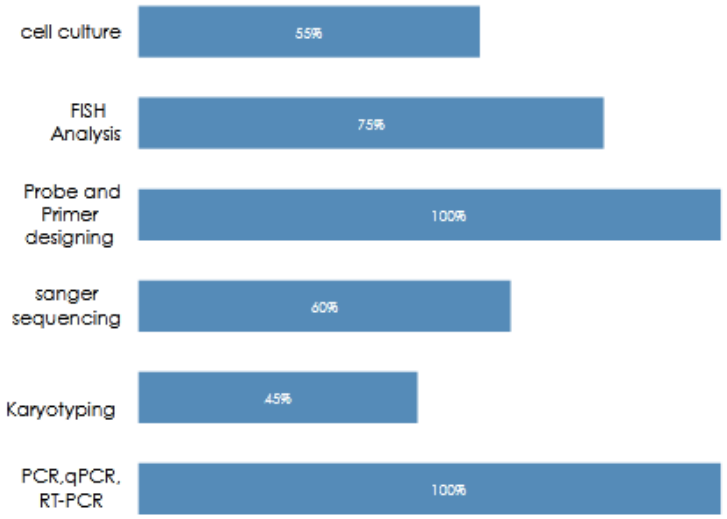

SELECTED COURSES DURING MASTER PROGRAM

Molecular Pathology	AA
Cell Culture	BA
Cytogenetic	AA
Molecular Genetics	AA
Molecular Techniques in Molecular Medicine	AA
Current Topics in Cell and Molecular Medicine	AA

*GPA 3.78

SELECTED MAIN COURSES DURING BACHELOR PROGRAM

Human Genetic
Cell and Molecular biology
Oncology
Biochemistry
Tissue and Embryology
Immunology
Microbiology