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SNaPshot Multiplex Assay for Rapid Detection of Mutations Associated with Hereditary Hearing Loss in Palestine

By

Tamara Saber Jaraysa

In Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

Background: Hearing loss is the most common sensory disorder affecting a high rate of newborns especially in Palestine. Hearing loss incidence increases with the high rate of consanguinity observed in the Palestinian population. It is also a highly heterogeneous trait, which is affected by genetic and environmental factors. There are two types of hereditary hearing loss, syndromic hearing loss that accounts 30% of the cases and nonsyndromic hearing loss that accounts for 70% of the cases.

Methods and materials: 29 common sequence variants in 16 different genes associated with hereditary hearing loss were scanned in the Palestinian population. A Snapshot Multiplex assay was used to enable rapid diagnosis for patients especially newborns. Instead of identifying each mutation by using Sanger sequencing method, we can pool all the mutations together in one reaction using multiplex PCR program. The strategy involves the utilization of a single nucleotide primer extension for each mutation and size exclusion using gene scan tool.

Results: A diagnostic technique consisting of four panels of Snapshot Multiplex assay was successfully developed; each panel consists of approximately ten sequence variants. The different genotypes can be determined based on the position and the fluorescent color of the peaks in a single electropherogram. Positive control samples been used as references.

Conclusion: This study demonstrates a robust, cost effective, time saving and a high level of accuracy diagnostic method that detects common causative variants of hearing loss in Palestinian cohort with relatively heterogeneous ethnic background. This relatively inexpensive assay should accelerate genotyping identification, early diagnosis and essential management of hearing loss patients.

ملخص

SNaPshot Multiplex Assay

من أجل الفحص الجيني السريع للطفرات التي تسبب فقدان السمع الوراثي في فلسطين

فقدان السمع من أكثر الامراض الحسية شيوعاً في مجتمعنا الفلسطيني ويصاب به بالعادة الأطفال حديثي الولادة. فقدان السمع هو مرض جيني متنحي يزداد في فلسطين مع زيادة زواج الأقارب. وهو متعدد الأسباب، يتأثر بالعامل الوراثي و أيضا البيئي. هناك نوعان من فقدان السمع الوراثي، إما أن يكون فقدان سمع مصحوب بأعراض أخرى ويشكل هذا النوع ٣٠% من الحالات، أو أن يكون فقدان سمع غير مصحوب بأية أعراض أخرى ويشكل هذا النوع ٧٠% من الحالات.

في هذه الدراسة تم تحليل ٢٩ طفرة في أكثر من ١٦ جين مسؤول عن هذه الصفة الوراثية في المجتمع الفلسطيني. لقد تم العمل على تشكيل تقنية جديدة تسمى (Snapshot Multiplex Assay) من أجل تحقيق تشخيص جيني سريع للمرض خاصة للأطفال حديثي الولادة. أيضا من أجل استبدال استخدام تقنيات أخرى مثل (Sanger Sequencing) التي تقوم بفحص كل طفرة لوحدها، بإمكاننا مزج أكثر من طفرة في فحص واحد.

في نهاية هذه الدراسة تم الحصول على تقنية جديدة تتكون من أربع مجموعات من الفحوص؛ كل فحص يحتوي تقريباً على عشرة طفرات. تحديد وجود الطفرة يعتمد على موقع الإشارة ولونها. تم استخدام عينات متماثلات للطفرة او حاملات للطفرة أو غير حاملات للطفرة كدالة على النتائج.

لقد قامت هذه الدراسة على تطوير تقنية قوية، غير مكلفة، وموفرة للوقت و ذات دقة عالية، من أجل فحص طفرات تؤدي إلى فقدان السمع في المجتمع الفلسطيني ذات أعراق مختلفة وبتكلفة قليلة مقارنة بغيرها. هذه التقنية عملت على تسريع الفحص الجيني ومعرفة السبب الوراثي لفقدان السمع من أجل حسن التعامل مع المرضى وتقديم العلاج المناسب.

DECLARATION

I declare that the Master Thesis entitled “SNaPshot Multiplex Assay for Rapid Detection of Mutations Associated with Hereditary Hearing Loss in Palestine" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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Date: _____

Dedication

I dedicate this thesis to my father, the one who believes in my abilities to achieve my goals in life. The one who supported me though my studies and kept me going to accomplish this mission. Also I dedicate my thesis to my mother, brothers and sisters who are always there for me. Special thanks to my supportive fiancé who never loses faith in every step I take.

Acknowledgment

I would like to thank and give appreciation to Professor Moien Kanaan, the one who was always giving me support and inspiration. He has been the ideal thesis supervisor guiding me throughout my project. I thank him for all his advice and endless patience in writing my thesis. He was more a father than a supervisor leading me through my studies in his laboratory.

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Finally, thanks to my wonderful lab team working in Hereditary Research Laboratory. I really appreciate their help and support during my studies. Thanks to Amal Abu Rayyan, Lara Kamal and Christina Canavati, they made working in this lab such a great experience.

Abbreviations

ABR	Auditory Brain Stem Response
ANSD	Auditory Neuropathy Spectrum Disorder
BRAF	B-RAF proto oncogene, serine/threonine kinase
CDH23	Cadherin related 23
CVS	Chorionic Villus
ddNTPs	didoexynucleotide triphosphates
EGFR	Epidermal Growth Factor Receptor
GJB2	Gap Junction Protein Beta 2
GPSM2	G Protein Signaling Modulator 2
HER2	Human Epidermal Growth Factor Receptor 2
HL	Hearing Loss
HRL	Hereditary Research Laboratory
IVF	In Vitro Fertilization
KCNQ2	Potassium voltage-gated channel subfamily KQT member 4
KRAS	Kirsten Rat Sarcoma viral oncogene homolog
120 LIZ®	In lane Size Standard
MPS	Massively Parallel Sequencing
Myo7A	Unconventional Myosin-VIIa
NGS	Next Generation Sequencing
NSHL	Non Syndromic Hearing Loss
OAE	Otoacoustic Emissions

OTOF	Otoferlin
PCR	Polymerase Chain Reaction
PGD	Pregestational Diagnosis
RFLP	Restriction Fragment Length Polymorphism
SHL	Syndromic Hearing Loss
SLC26A4	Solute carrier family 26(Anion exchanger) member 4
TECTA	Alpha-Tectorin
TMPRSS3	Transmembrane Protease Serine 3

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CHAPTER 1

Introduction

1.1 Background

Hearing loss is one of the most common sensory disorders and birth defects in many developed countries. Hearing loss affects approximately 1 out of 1000 newborns each year in the United Kingdom (Fortnum et al., 2001). Due to high consanguinity in Palestine we have even more cases of newborn hearing loss cases (Dror and Avraham, 2009). The complexity of the genetic inheritance of the hearing loss phenotype is highly heterogeneous, which means that many genes are involved in the hearing pathway (Parker and Bitner-Glindzicz, 2015).

Hearing loss can be divided to many categories depending on phenotypic manifestation, severity, age of onset, audiometric profile and etiology (Parker and Bitner-Glindzicz, 2015). There are three types of hearing loss: sensorineural, conductive, and mixed hearing loss. Sensorineural hearing loss means that the damage occurred in the inner ear or the auditory nerve, while conductive means that the hearing loss occurred due to the damage in the outer or middle ear (Petit and Richardson, 2009). Severity of hearing loss can be mild, moderate, severe or profound. Some genes are associated only with profound hearing loss, while other genes are related to moderate hearing loss problems (Parker and Bitner-Glindzicz, 2015). Recent studies suggest that the profound hearing loss occurred as a results of many disorders during cochlear development rather than hair cell degeneration, while late onset hearing loss occurred as a result of the reduction of the amplification in active cochlea (Wingard and Zhao, 2015).

1.2 Etiology

Hearing loss etiology can be due to environmental factors or genetic defects. Genetic defects are divided to two main types: syndromic hearing loss (SHL) which accounts for 30% of the cases and non-syndromic hearing loss (NSHL) which accounts for the other 70% (Parker and Bitner-Glindzicz, 2015). SHL means that the patient has hearing loss with another phenotype that affects another body organ, such as Usher syndrome which leads to hearing loss and retinitis pigmentosa, or the Pendred syndrome which includes hypothyroidism. NSHL means that the hearing loss is not due to any syndromic or environmental conditions and it occurs due to inner ear defects including cochlea and middle ear defects as well. NSHL occurs as a result of any mutation in all genes that are involved in the hearing pathway or somehow related. Eighty percent of the NSHL cases are autosomal recessive and the remaining are either autosomal dominant, X linked, mitochondrial inheritance or digenetic inheritance (Liu et al., 2009, Yang et al., 2009). Some environmental factors that could lead to hearing loss include exposure to loud noise, viral infection, or certain drugs (toxicity).

1.3 Ear Structure and Hearing Process

Different frequencies or intensities of sound waves are generated in the environment and our ear catches these waves and passes them to the brain. The ear consists of three main compartments: outer, middle and inner ear. Each of them has its own function during hearing process starting from when sounds are captured by the outer ear till they reach the brain by the inner ear. The outer ear captures the sound waves and passes them through ear canal to the eardrum membrane, then to the oval window of the fluid filled inner ear by the middle ear. Middle ear is composed of three small bones that are placed in the tympanic cavity and serve as a link between the eardrum and the oval window of the fluid filled inner ear.

Vibration of the eardrum which amplifies the sounds, leads to the movement of the middle ear bones and the fluid of the inner ear.

The inner ear contains the cochlea which contains the cochlear duct that is divided to three compartments: The scala media, scala vestibule and scala tympani. Scala media and scala tympani are separated by the organ of Corti which is the sensory epithelium of the auditory network. The organ of Corti contains specialized cells, called hair cells, which act as sensory receptors. These hair cells detect the mechanical signal and convert it to electrical one in order to be transmitted to the brain (Raviv et al., 2010). These cells contain actin rich projections called stereocillia, which play an important role in stabilizing the hair cells. As described above, cochlea is a complex mammalian organ that requires a large number of protein-protein interactions and many genes are involved in these pathways. Cochlea is the most organ in the ear that is subjected to be damaged by many mutations (Dror and Avraham, 2009).

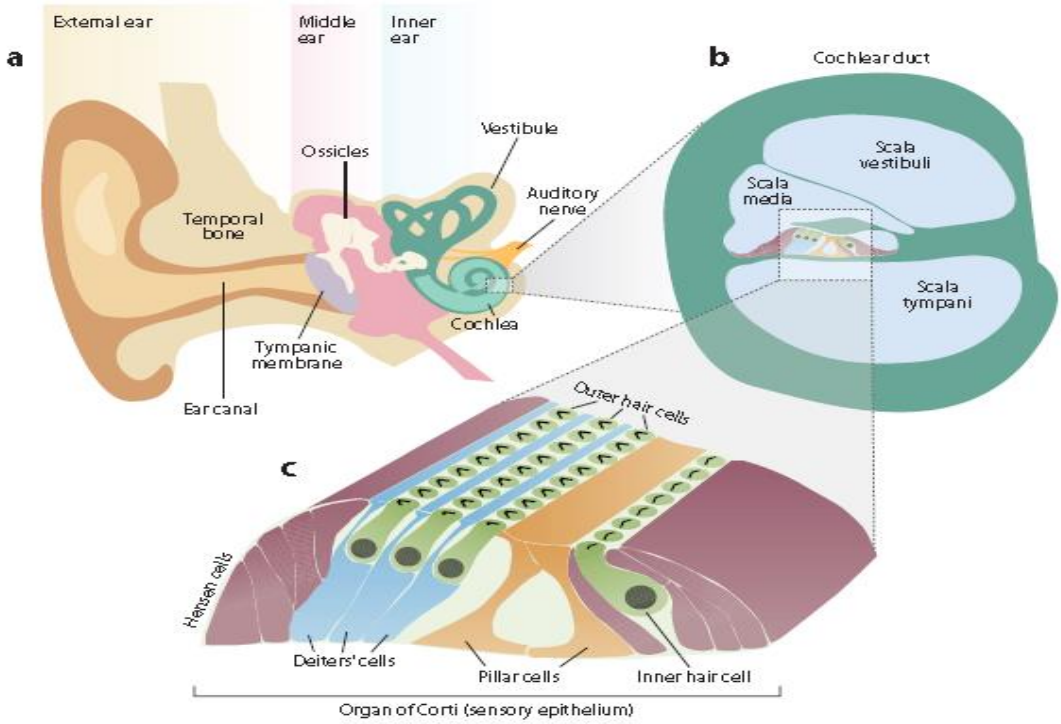

Figure 1.1: (A) Human ear diagram; outer, middle and inner ear. (B) Cochlear duct. (C) Organ of Corti. (Dror and Avraham, 2009)

1.4 Heterogeneity of Hearing Loss

The complexity of the auditory system leads to the involvement of many genes and proteins in the hearing pathways. Mutation in these genes such as GJB2, Myo7A, TECTA, GPSM2, OTOF, CDH23, SLC26A4, KCNQ4, TMPRSS3 and many others lead to deafness. These genes are divided to many categories depending on its function in the auditory network. It could be divided to motor proteins, cell-cell junction proteins, transporter proteins, ion interaction proteins, regulatory elements and many other categories depending on the function (Dror and Avraham, 2009).

The first category of the genes in the hearing pathway is the genes translated to Motor proteins, which includes myosins protein (Dror and Avraham, 2009). Myo7A, a member of

the myosins protein family, is expressed in stereocilia and any homozygous recessive mutation in the gene of this protein will lead to loss of stereocilia structure and disorganization of the remaining hair bundle (Riazuddin et al., 2008). Myo6 protein is another example of the myosins protein family that moves toward the actin to the base of stereocilia and any damage in the gene translated to this protein stops the movement along the actin (Dror and Avraham, 2009).

The second category includes cell-cell junction proteins such as Cadherin related 23 (CDH23), Connexin 26 (GJB2), and Otoancorin (OTOF). CDH23 gene codes for proteins that are essential for maintaining hair bundle development. Mutations in CDH23 gene affect the cell polarity and lead to disorganization of stereocilia as well as the tip links (Di Palma et al., 2001). Mutations in OTOF gene are likely the cause of auditory neuropathy spectrum disorder (ANSD) that leads to the damage of the inner hair cell (Norrix and Velenovsky, 2014). The GJB2 gene if mutated, accounts for more than 50% of the NSHL cases (Gasparini et al., 2000). It provides cochlear amplification by intercellular communication that allows ions passage and cell signaling molecules (Wingard and Zhao, 2015).

The third category includes genes coding for transporter proteins such as SLC26A4. SLC26A4 is the second common gene that causes hearing loss after GJB2 (Everett et al., 1997, Scott et al., 1999). SLC26A4 encodes an anion transporter in the hearing pathway (chloride and iodide) and mutations in this gene lead to the enlargement of the vestibular aqueduct and sacs followed by head trauma (Colvin et al., 2006).

Other mutations in genes involved in the hearing pathway contribute to the hearing loss phenotype. Biallelic mutations in TMPRSS3 gene may lead to reduction of the high frequencies compared to the low ones, severe mutations in this gene lead to prelingual HL

while mild mutations lead to postlingual HL (Weegerink et al., 2011). GPSM2 gene encodes a protein called G-protein signaling modulator expressed in the hair cells. Mutations in GPSM2 gene will affect cell polarity and the asymmetric organization of the apical (organ of the Corti) (Walsh et al., 2010). Another example of these genes is TECTA gene which codes for alpha tectorin protein of the tectorial membrane that surrounds the outer hair cells and is responsible for the amplification of the sound. Mutation in this gene leads to HL as well (Verhoeven et al., 1998).

1.5 Importance of Newborns Screening for Hearing Loss

Every child that fails the Otoacoustic Emissions (OAE) test and auditory brainstem response (ABR) test and then diagnosed with non syndromic hearing loss should be screened for hearing loss mutations as fast as possible. OAE and ABR are hearing tests that used to diagnose newborns hearing. OAE measures the function of the outer hair cells in the cochlea whereas ABR measures the function of the auditory nerve. Since it's very important to know that the hearing loss is due genetic defects or not. In Palestine they should be screened first for GJB2 gene mutations which account more than 50% of hearing loss cases as mentioned. Pediatrician should be aware of hearing loss in patients early in order to offer proper treatment, for example cochlear implantation as this effects speech and other development milestones. Genetic counseling of the parents is very important in case they want to have healthy children , for example they could do PGD on the particular variant that they have (Parker and Bitner-Glindzicz, 2015). Early identification of the genetic defects using molecular techniques alleviates to do other clinical tests to the patients in order to know the cause of hearing loss, so they know that the hearing loss is due genetic defects not due to any environmental factors , thus reducing the cost .For example, in the case of SHL such as Usher

syndrome so the parents will be aware about the blindness issues before happening (Brownstein and Avraham, 2009).

1.6 Hereditary Research Laboratory (HRL) Work

Since 2002, the HRL team has examined more than 60 variants in more than 20 genes that lead to hearing loss. These variants and genes are identified by using different molecular techniques such as: Linkage analysis, Sanger Sequencing and massively parallel sequencing. Functional assays are carried using mouse models since mouse genome is very similar to the human genome. These assays include cell biology assays and protein localization in the ear (immunohistochemistry), which confirms the major cause of these identified genes in hearing loss phenotype.

The best methods to genetically diagnose hearing loss phenotype are next generation sequencing (NGS) or whole exome sequencing. Some families may not have a known genetic defect, thus the best solution is to go for NGS. However the cost of this test is high.

The routine test done in HRL is to exclude genetic variants mentioned in table 1.1, and if the result is negative, HRL team go to NGS. Therefore, developing a rapid, accurate and cost effective technique is very critical in order to exclude known variants or even solve these families.

	Gene Symbol (name)	Number of mutated alleles	Initial Experiment	Phenotype	Publication
1	CACNA1D NM_000720	A376V 1127T>C	MPS2	Moderate SNHL	Novel-unpublished
2	CDH23 NM_022124.2	P346S 1427C>T	Linkage analysis	Severe to Profound	Shahin et al , 2010
		P346L 1428C>T	Linkage analysis	Moderate SNHL	Shahin et al , 2010
		P559S 2065C>T	Linkage analysis	Severe to Profound	Shahin et al , 2010
		p.D273V c.A818T	MPS3	Severe to Profound	Novel-unpublished
		E1059K 3181G>A	Linkage analysis	Moderate SNHL	Shahin et al , 2010
		L495P	MPS4	congenital profound	Novel-unpublished
		E1917K	MPS4	HL with retinitis pigmentosa	Novel-unpublished
3	CLDN14 NM_144492	P28L 83T>C	MPS2	Severe to Profound	Novel-unpublished
4	ESRRB NM_004452	R182H 545 A>G	MPS2	Severe to Profound	Novel-unpublished
		G263S c.787G>A	MPS4	Profound	Novel-unpublished
5	GJB2 NM_004004.5	IVS1+1 G to A -3172G->A	Sanger sequencing	Severe to Profound	Brownstein, 2009
		c.35delG	Sanger sequencing	Severe to Profound	Sobe et al, 2000
		E120del 358_360delGAG	Sanger sequencing	Severe to Profound	Mani et al , 2009
		235DelC	Sanger sequencing	Severe to Profound	Fuse et al , 1999
		167delT	Sanger sequencing	Severe to Profound	Sobe et al, 1999
		W77R 229T->C	Sanger sequencing	Severe to Profound	Carraquillo,1997
6	GPR98 NM_032119	G1182R 3544G>A	MPS2	Moderate to Severe SNHL	Novel-unpublished
7	GPSM2 NM_013296.4	R127X 875C>T	WES	Severe to Profound	Walsh et al , 2010
		W326X 977A>G	MPS2	Severe to Profound	Novel-unpublished
8	MYH9 NM_002473	p.S1713G c.A5137G	MPS3	Moderate	Novel-unpublished
9	MYO15A NM_016239.3	Asp2403fs X2414 c.7545G>T	Linkage analysis	Severe to Profound	Shahin et al , 2010
		R3191H c.8183G>A	Linkage analysis	Severe to Profound	Novel-unpublished
		Splice mutation in hypothetical new	Linkage analysis	Moderate	Novel-unpublished

		exon 2A Chr17: 18,026,708, G>A			
		del Glu2769 in exon 45 8309delAGG	Linkage analysis	Severe to Profound	Novel-unpublished
		A408V 1223T>C	MPS2	no audiograms	Brownstein ,2013
		E1414K 4240G>A	Linkage analysis	Severe to Profound SNHL	Novel-unpublished
10	MYO6 NM_004999	E299D 897T>G	MPS2	Late onset (>30), progressive, SNHL	Brownstein et al ,2013
		L926Q 2777A>T	MPS2	Severe to Profound	Brownstein et al ,2013
11	MYO7A NM_000260.3	G2123S 6487G>A	Linkage analysis	Severe to Profound	Shahin et al , 2006
		p.Q2066X c.C6049T	MPS3	Severe to Profound	Novel-unpublished
		Splice mutation in hypothetical new exon 2A Chr17: 18,026,708, G>A 4153-2A>G	MPS2	Severe to Profound	Brownstein et al ,2013
		Q2071X 6211C>T	MPS2	Severe to Profound	Brownstein et al ,2013
		N769fs (c.2307delC)	MPS4	severe to profound	Novel-unpublished
12	OTOA NM_001161683	D356V 788A>T	Linkage analysis	Moderate	Walsh et al , 2006
		Δ 320-550Kb	Linkage analysis	Moderate to severe	Walsh et al , 2006
13	OTOF NM_194248.2	R577X 4157C>T	Linkage analysis		Shahin et al ,2010
		p.R1583C c.C4747T	MPS3	Severe to Profound	Novel-unpublished
14	PAX3 NM_181459.3	S84F 251T>C	MPS2	Waardenburg Syndrome	Zlotogora, 1995
15	POU3F4 NM_000307	R282L 858G>T	Sanger Sequencing	Severe to Profound	Novel-unpublished
16	PTPRQ NM_001145026	Q429X 1285C>T	Linkage analysis	Moderate to severe	Walsh et al , 2010
17	SLC26A4 NM_000441.1	1001G>T	Linkage analysis	Severe to Profound SNHL	Walsh et al , 2006
		ivs11(+1)delG	Linkage analysis	Severe to Profound SNHL	Walsh et al , 2006
		F683S 2048C>T	MPS2	Moderate	Gardner et al , 2006
		S399fs c.1197delT	MPS2	Moderate to Severe	Novel-unpublished

		V239D 716T > A	Linkage analysis	Severe to Profound	Walsh et al , 2006
		K447fs	MPS4	severe to profound w/endolymphatic sac	Novel-unpublished
		Q383fs	MPS4	severe to profound w/endolymphatic sac	Novel-unpublished
		R373C	WES	severe-profound	Novel-unpublished
18	TECTA NM_005422	C1619X 4857C>A	Linkage analysis	Moderate to severe	Shahin et al , 2010
19	LHFPL5 NM_182548	M1V 1A>G	Linkage analysis		Shahin et al , 2010
20	TMPRSS3 NM_024022	C194X 783T>A	Linkage analysis	Severe to Profound	Shahin et al , 2010
		1190delA	Linkage analysis	Severe to Profound	Walsh et al , 2006
		Ins(β-sat)+ del	Linkage analysis	Severe to Profound	Walsh et al , 2006
21	TRIOBP NM_007032	R347X 1039C>T	Linkage analysis	Severe to Profound	Shahin et al , 2006
		Q581X 1741C>T	Linkage analysis	Moderate to severe	Shahin et al , 2006
		G1019R 3055G>A	Linkage analysis	Moderate to severe	Shahin et al , 2006
22	PTRH2 NM_016077	Q85P	WES	bilat severe w/mild MR and motor delay	Alazami et al , 2015

Table 1.1: Palestinian hearing loss causing genes and variants seen in HRL HL families.

CHAPTER 2

Literature Review

2.1 Choosing SNapShot Technique

Many molecular techniques have been used to test for mutations including direct sequencing, real time PCR allelic discrimination, RFLP, pyrosequecing...etc. The sensitivity and specificity of the technique depend on many factors, such as DNA quality and the type of tissue (blood sample or paraffin embedded tissue) that is available for the test. In order to choose which technique to use, the following factors are taken into account: cost, flexibility, time, equipment, workload, result interpretation and man power (Table 2.1).

Comparison Method	**Sanger sequencing**	**Snapshot**
Flexibility	No	Yes
Time	2 working days	1.5 working days
Equipments	Sequencer	Capillary electrophoresis
Workload	Laborious	Less Laborious(multiplex)
Result interpretation	Time consuming	Easy
Multiplex	No	Yes

Table 2.1: Comparing the performance of Snapshot and Direct sequencing (Farina Sarasqueta et al., 2011).

2.2 Snapshot Multiplex Assay

Snapshot is a multiplex assay used for simultaneous multigene screening of up to 10 variants of DNA sequences in one reaction. (Wu et al., 2009).

2.3 Snapshot Reaction

Snapshot reaction involves two stages. First, multiplex Polymerase Chain Reaction (PCR) and single base primer extension and termination. During multiplex PCR reaction of the variants, the snapshot probes must anneal to their complementary templates in the presence of fluorescently labeled didoexynucleotide triphosphates (ddNTPs), the annealing occurs adjacent to the site of the variant nucleotide. To analyze these products, capillary electrophoresis is used. Four fluorescent dyes allow labeling each nucleotide with four different colors that are specific to each nucleotide i.e. silver for Cytosine (C), blue for Guanine (G), green for Adenine (A) and red for Thymine (T). The genotype analysis of the variant is dependent on two categories: the color and the size of the peak. One or two peaks could result depending on the genotype (one peak indicating homozygosity whereas two peaks indicate heterozygosity). The molecular weight of the alleles might be shifted since the mobility of the fluorophores is different from each other. Thus, Snapshot reaction can be divided to four steps: 1) PCR amplification; 2) purification of the product; 3) annealing of the probes; 4) analysis using Genemapper software (Applied Biosystems)(Bardien et al., 2009).(See figure 2.1)

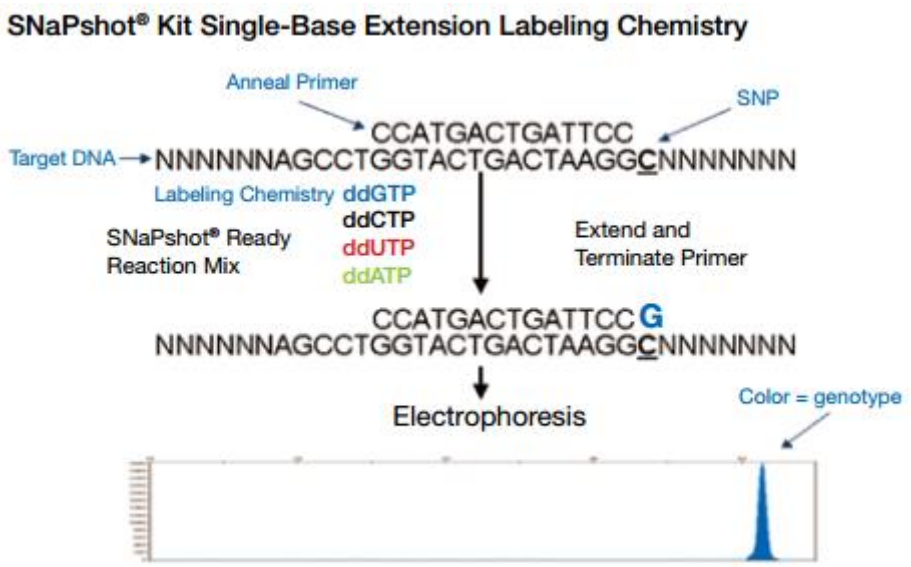

Figure 2.1: Snapshot reaction chemistry labeling (Applied Biosystems by life Technologies PRODUCT BULLETIN SNaPshot® SNP Analysis manual)

2.4 Snapshot Probes

Snapshot probes contain DNA sequence that is complementary to the sequence of interest with one base pair missing at its 3 prime end. Snapshot probes contain a tail that assists in the differentiation between the variants in the same panel according to size. The probes are not labeled, thus decreasing the cost of this technique. Snapshot probes must be tested for many categories, e.g, secondary structure, complementary sequence, hairpin formation and specificity (Hurst et al., 2009). These categories require to be tested in order to not affect the reaction negatively. Many softwares are available online to test these categories, the one that was chosen in this study is called: Oligo Cal: Oligonuecleotide Properties Calculator (http://www.basic.nothwestern.edu/biotools/ Oligocalc.html).

For designing Snapshot probes there are some guidelines that should be taken into account:

A. No hairpin and secondary structure formation exist.
B. Four to six base pairs difference between each probe length.
C. GC content is approximately ~50%.
D. Probes must contain a tail at its 5 prime end in order to make the analysis according to the size as previously mentioned, for example $(T)_n$ nucleotide tail.
E. Minimum length of the probe is 20 base pair.
F. The probes must not have a complement with another snapshot probes.

2.5 Why Snapshot?

The main advantage of using Snapshot technique rather than another technique is time saving. Snapshot is a very rapid screening technique. So by knowing the genes and the variants that leads to HL in the patients we can manage hearing loss and its consequences (Dror and Avraham, 2009).

Snapshot allows us to do fast genetics screening during pregnancy, by using CVS (Chorionic villus) samples which can be done at 11–13 weeks of pregnancy. Upon gene identification, the pediatrician can know the diagnosis or the type of hearing loss that the fetus will develop. Since specific genes can lead to congenital HL compared to other genes causing different levels of hearing loss.

Pregestational Genetic Diagnosis (PGD) could be done on the embryo after doing *in-vitro* fertilization (IVF) so we can test the embryo for the variant detected by Snapshot and reduce the percentage of having deaf people in the population(Liss et al., 2011).

2.6 Advantages of Snapshot Technique: ((Wu et al., 2009, Magnin et al., 2011, van Oers et al., 2005, Hurst et al., 2009, Farina Sarasqueta et al., 2011)**)**

1. Snapshot technique does not require additional sequencing step.
2. More Cost effective than any other technique such as Sanger sequencing, it cost 0.16 euro per sample versus 2.59 euro per sample for Sanger (Hurst et al., 2009).
3. High sensitivity compared to Sanger sequencing in case of cells that contain 10% tumor cells (Farina Sarasqueta et al., 2011).(See figure 2.2)
4. Can give results while using partially degraded DNA e.g extracted from paraffin embedded tissues.
5. More than one variant can be detected by single snapshot probe.
6. Flexible technique, since we can extend the number of variants tested in snap panel according to the specific population that has specific variants, so it is not a closed method.
7. Very effective screening and routine analysis technique for relatively heterogeneous ethnic background population.

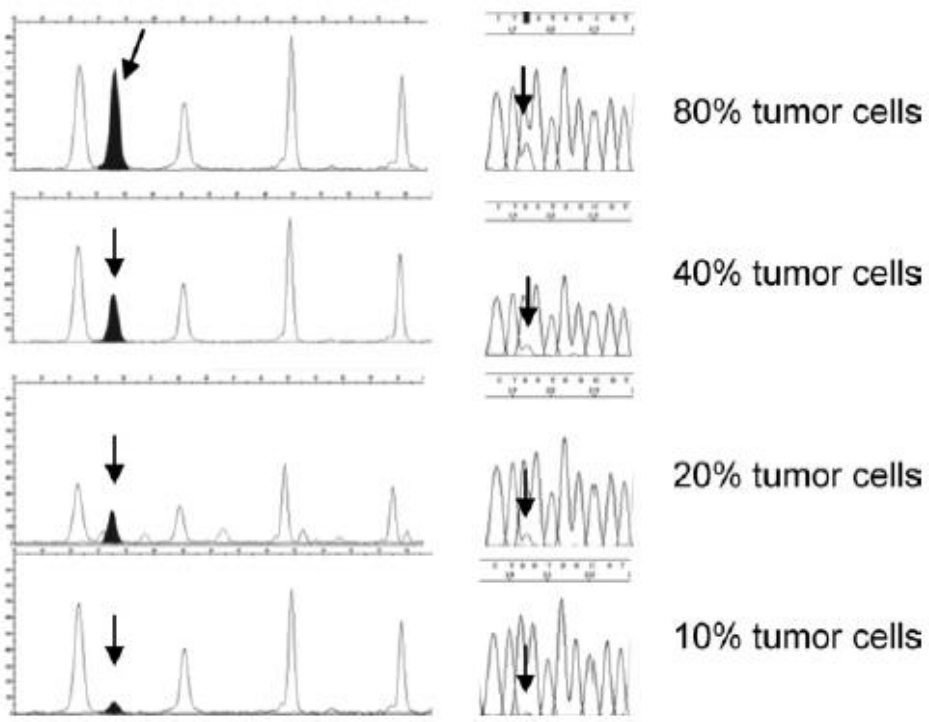

Figure 2.2: Sensitivity of Snapshot over Sanger sequencing in case of tumor cells that are present in colon cancer patients(Farina Sarasqueta et al., 2011).

2.7 Snapshot Applications

Snapshot technique is widely used to do multigene screening for different diseases, such as colon cancer. Most of the colon cancer patients have variants or mutations in KRAS and BRAF genes. These patients are resistant to the therapy compared to patients that have colon cancer but do not have these variants, therefore, it is very important to do the screening in a minimal cost and time (Magnin et al., 2011). Also, this application is useful in screening many variants that are related to many cancer types such as non-small cell lung cancer, that are associated in many genes such as EGFR and HER2 genes (Su et al., 2011) and many other diseases that are associated with different known variants that should be test. Snapshot technique used also to screen hearing loss patients other than Palestinian such as Taiwanese

patients. This technique is capable to screen patients with idiopathic sensorineural hearing impairment for GJB2, SLC26A4 and mitochondrial variants (Wu et al., 2009).

CHAPTER 3

Materials and Methods

3.1 Data Recruitment

Since 2002, HRL have been working on identifying variants related to hearing loss phenotype. Most of these variants were distributed on the Snapshot panels.

3.2 Snapshot Four Panels

3.2.1 Panel One:

Gene	Variant	Forward Primer	Reverse Primer	Snap probe	Size(bp)
TECTA	C1619X	GAGGGGTTTCTGGTGATTGA	CCCTCGCAAGGTCACATAAT	GAGGCTGCAGAACAAAGTGTG	21 bp
SLC26A4	V 239D	GGCCCAGACTCAGAGAATGA	CTTGGCAGATCCTTTGGTTG	T(18)TTTTGGTTGAAACATTGAGG	38bp
MYO15A	E1414K	AGGCTTGGGCTTGTATGTGT	GGCACTCACCTGGTTCAGAT	T(24)AGGAATTACCACATCTTCTAC	45bp
TMSH1	M1V	CTTCTAGGCCTCCATCCACA	GAAGAGGGCCATGACCAGTA	T(31)CCCAGGGCCTGCTGCCCTACC	52bp
Otoancorin	D356V	ACCACCATCCCTCCTCTTCT	CCCAGCTGTACCTTCTGGAC	T(45)ACAATGACCTGGAATTGCTGG	66bp
CDH23	E1059K	AACGTGGATGGGAAGTTCAG	CTGCCCAGATCACTGTGTTG	T(50)GAACCGTGGTGGGCCTGGACCGG	73bp
TMPRS33	C194X	CTGAGGGCAAGGAGATAGGA	ATCTGGGGCATTTTTCACAG	T(51)CTGCAAGGTAACCACGTGGCCAGAGGC	78bp

Table 3.2.1: Panel one primers and probes.

3.2.2 Panel two:

Gene	Variant	Forward Primer	Reverse Primer	Snap probe	Size(bp)
SLC26A4	1001	TATGGAGCCAACCTGGAAAA	TGTTTCTTCCAGATCACACACA	T(12)GTTAAATCCATCCCAAGGG	31bp
TRIOPB	R347X	GGGCCTCATCTACACAGTGG	AGGGTGTCCTGGGGTTTTC	T(16)CCCCAGAGCTTCCTCTCCCTCA	38bp
CDH23	P559S	GCTTCACCCTGACGATCATT	TGACAGAAGGCTCGTTCTCC	T(24)GGATGTCAACGACAACGTGCC	45bp
MYO7A	IVS-2 4120	GAGCCTTTGGTGGTGTGG	ATGTAGGTGGGCACGAGGT	T(39)GACCGAGGCCTCCCCCCACCT	60bp
GPR98	G3476R	TCAAGAGGTGCCTGTCAGTG	AAAATTATTGCAAGAGCATTTTCAT	T(43)TATTTGCCGAAAATGTCTTTCTA	66bp
CLDN14	P28L	ATCGGTAGATCTGGCACTGG	GCTTCCTGCTCAGCTTCCT	T(55)ACGTGCGCTGTCCTCCGCCAGTG	78bp

Table 3.2.2: Panel two primers and probes.

3.2.3 Panel three:

Gene	Variant	Forward Primer	Reverse Primer	Snap probe	Size(bp)
CDH23	P346S	GCTTCACCCTGACGATCATT	TGACAGAAGGCTCGTTCTCC	GTGTTGGATGTCAACGACAACGTGCC	26bp
MYO7A	G2163S	CTTCTGTGAGGGCATGTGTG	GACGTCTCGCAGAGCAGTTT	T(13)GATCTCCAACTGGAGCAGC	32bp
OTOF	R577X	GAGGAGGCAGAGTTCCAGGT	CACAATTTGGGGTCAACAGA	T(18)TCCAGAGGGCTCTTGTTGTC	38bp
MYO6	E299D	TTAAACCGAGGCTGCACTAGA	AGAACTCTTACTTGGGCTCTAAAA	T(23)ACAGAACCGCAAAAGTCCTGA	44bp
ESRRB	R182H	TCAGTGCTTCTACCCTGGTG	ATGGCTTTTTAGCAGGTGGA	T(29)TTGATCGAGTGCGTGGAGGCC	50bp

MYO7A	Q2071X	GGTCAAGTTCGAGGAGGACA	CTGCTGGCCAGTGTCTGC	T(35)GTGCCCCAGGACCTTATCCGG	56bp
SLC26A4	F683S	TCCTGAGCAAGTAACTGAATGC	GAAAGGGCTTACGGGAAAGT	T(41)ATTTTTAGATTGTCAAAGAAT	62bp
TMPRS33	1190delT	GACTCCGAATCTTGGCTTCA	TGTTTTCTCGGACTCCTGCT	T(59)ACTTTTCCATCGGGGAAGTTC	80bp

Table 3.2.3: Panel three primers and probes.

3.2.4 Panel four:

Gene	Variant	Forward Primer	Reverse Primer	Snap probe	Size(bp)
OTOF	R1583C	TAGAGTGGAGGCAAAGCAGG	GCTGTGTATGACTGGGACCT	CAGGTGGCGCGGTGCTTGCTGTAGAA	26 bp
CDH23	D228V	CAAGACAAGACCAGGCCTCT	GGAAGTCCCTCTGAGAAGGG	(T)11ACTTGGCCATCATCATCACAG	33bp
MYH9	S1317G	GGAGAAGTGAGGGGCCTAC	CACACTCTGGGTCTTTGTGG	T(18)AGCTCACCCTTTGCCGCTGC	38bp
GJB2	IVS1+1	CCAAGGACGTGTGTTGGTC	CAGCGCAGAGACCCCAAC	(T)24AGTCCGGGGCCGGCGGGCTCA	45bp
GPR98	E299E	GTGTATCATGGGTGGTTAGTCC	CACTGTCACACCTGAGTTCT	(T)31CATTTACTCCCTTCCAGATGA	52bp
GPSM2	W326X	GGACCTCTTTTCAAATAACTGCA	CCTCTCTTGAAATTTCCAAGTGC	(T)39TTGGTGAAGGAAGAGCATGTT	60bp
MYO6	L926Q	TCCACTATGATGACGCAGGA	TCCTCTTCCTTTCTTCGACGT	(T)45AGGAAGAGGAAGCAGAAAGGC	66bp
SLC26A4	S399fs	AGGATCGTTGTCATCCAGTCT	AGGCTGTTGTTCCTACCTGT	(T)52CATCTTCTCAGGATTCTTCTC	73bp

Table 3.2.4: Panel four primers and probes.

3.3 Primer Design

Primers were designed for each variant, using primer3 software. In-silico PCR tool in the UCSC genome browser was used to review each designed primers to ascertain that it doesn't overlap with any known SNPs or repeats especially at the primers junction site. The PCR product of each variant should not exceed 190 bp (according to the Snapshot reaction manual kit).

3.4 Probe Design

Snapshot probes were designed to be DNA sequences that are complementary to the template, missing one base pair at the 3' end site as described before. While designing the Snapshot probes, many categories should be tested:

1. Probe specificity
2. Hairpin formation
3. Self-complementary
4. GC content

All these items were tested by using software called OligoCalc: Oligonucleotide Properties Calculator (http://www.basic.northwestern.edu/biotools/oligocalc.html).

3.5 Probe Preparation

Each probe arrived to HRL from the Hylabs company should be diluted to 100 pmol by using nuclease free water (Ambion® Cat#AM9932), then the working solutions of these probes

were prepared from these stocks with different concentrations upon request (0.01-2.00pmol/ul).

3.6 Polymerase Chain Reaction (PCR) Protocol and Program

PCR reaction was done to each variant separately by using the basic touchdown PCR protocol indicated below and carried out using thermocycler Geneamp PCR System 9700 (Applied Biosystems).

Items	**Volume**
2X PCR Ready Master Mix (Abgene-Cat# AB-0575-DC-LD)	12.5ul
Primers (Forward and reverse)(10pM)	0.5ul each primer
N.F.H2O (Ambion® Cat#AM9932)	10.5ul
DNA (100ng/ul)	1ul
Total volume per sample	25ul

Basic PCR touchdown program (T.D 60)

Step1: **95** ºC for 5 min.

Step2 (3 cycles): **94** ºC for 30 sec, **68** ºC for 30 sec, **72** ºC for 30 sec

Step3 (3 cycles): **94** ºC for 30 sec, **66** ºC for 30 sec, **72** ºC for 30 sec

Step4 (3 cycles): **94** ºC for 30 sec, **64** ºC for 30 sec, **72** ºC for 30 sec

Step5 (3 cycles): **94** ºC for 30 sec, **62** ºC for 30 sec, **72** ºC for 30 sec

Step6 (35 cycles): **94** ºC for 30 sec, **60** ºC for 30 sec, **72** ºC for 30 sec

Step7: **72** ºC for 5 min, **4**ºC for 7 min.

3.7 Gel Electrophoresis of PCR Products

Gel electrophoresis was done on the PCR products of all variants using 1.5% agarose gel containing 0.01% ethidium bromide (Amresco-Cat# E406-5ML) using 1X TAE running

buffer at 120V for 20 minutes. The resulting bands were seen under UV light using BioRad ultraviolet imaging system.

3.8 SnapShot Reaction

3.8.1 PCR Product Cleaning of Each Variant

PCR products that gave a clear band on agarose gel were cleaned using two enzymes Antarctic Phosphatase (BioLabs, Cat#M0289L) and Exonuclease I (BioLabs, Cat#M0293L). This step was done in order to get rid of both; the remaining dNTPs in the reaction and primer dimers.

PCR Cleaning protocol:

Items	Volume
Antaractic phosphatase enzyme	0.25ul
Exonuclease I enzyme	0.25ul
N.F.H2O	1.5ul
PCR product	5ul
Total volume per sample	7ul

PCR cleaning program

Step1: **37** ºC for 30 min.

Step 2: **80** ºC for 20 min.

Step 3: **4** ºC for ∞

3.8.2 Multiplex PCR Reaction

PCR products were pooled for all the variants in the same panel together in a single tube. All probes for these variants are also pooled together in a single tube. Snapshot Mix (Applied Biosystems, Cat#4323159) are added to these items making 10 uls reaction as follow:

Multiplex PCR protocol:

Items	Volume
Pooled PCR products	3ul
Pooled Probes	1ul
Snapshot Mix	5ul
N.F.H2O	1ul
Total volume per sample	10ul

This reaction was put on the thermocycler Geneamp PCR System 9700 (Applied Biosystems) using the indicated program below:

Step1: **94** ºC for 5 min.

Step2 (35 cycles): **94** ºC for 30 sec, **60** ºC for 40 sec, **72** ºC for 45 sec

Step3: **72** ºC for 7 min, **4**ºC for ∞

3.8.3 Second Cleaning Step

Multiplexed PCR products were cleaned using Antarctic Phosphatase enzyme (BioLabs, Cat#M0289L), using one enzyme only in order to get rid of any remained dNTPs, using the indicated PCR program below:

Step1: **37** ºC for 60 min.

Step 2: **80** ºC for 30 min.

Step 3: **4** ºC for ∞

3.9 Gene Scan Reaction

Items	Volume
HiDiFormamaide (Applied Biosystems, Cat # 4311320)	9ul
Liz 120 Size Standard (Applied Biosystems, Cat # 4322362)	0.5ul
Diluted multiplex PCR products	0.5ul
Total volume per sample	10ul

For Heat denaturing step, the samples were denatured at 95°C for 5 minutes then immediately placed on ice for 5 minutes. The Samples were then put on the capillary electrophoresis using the ABI 3130 Genetic Analyzer (Applied Biosystems, S/N: 20355-023).

3.10 Data Analysis

After each run, collected data was analyzed on the GeneMapper software according to the color and size of the developed peak.

3.11 Validation by Sanger Sequencing

Positive or homozygous mutant samples were validated by Sanger sequencing using approximately 10ng per 100bp of PCR DNA with 1ul 10mM primer and adding ddH_20 to a total of 16ul.

Items	volume
5X buffer (Applied Biosystems, CAT#4336697)	1.5ul
64X buffer (MCLAB, CAT#BDX-100)	0.75ul
Primer	1ul
Clean PCR product	2ul
ddH2O	10.5ul

The samples were sequenced using sequencing PCR program indicated below:

Step1: 96^{o}C for 3 min.

Step2 (30 cycles): 96^{0}C for 10 sec, 50^{o}C for 5 sec, 60^{o}C for 2 min .

Step3: 4^{o}C for ∞

3.12 Cleaning of Sequencing PCR Product Using EDTA/ Ethanol Precipitation Method and Capillary Electrophoresis

Each sequenced PCR reaction was precipitated and cleaned by adding 100 ul of Absolute Ethanol and 5 ul of 125mMEDTA (Amresco-Cat # 0720). This was followed by incubating the reactions at -20° for 30 minutes, and centrifugation for 30 minutes at 3800 RPM at 4°C. The supernatant was discarded and 60 ul of 70% Ethanol were added to each reaction, and centrifuged again for 20 minutes. Then the samples were put on tissue paper for 1 minute at 500 RPM. Drying the samples was performed at 95°C for 5 minutes. 16ul of Hi-DiFormamide (Applied Biosystems, Cat # 4311320) were then added, and the samples were dried again at 95°C for 2 minutes. At last, the reactions were put on ice for 5 minutes before proceeding to sequencing.

Finally Sanger sequencing was done by using the ABI 3130 Genetic Analyzer (Applied Biosystems, S/N: 20355-023).

CHAPTER 4

Results

4.1 Development of Snapshot Multiplex Assay

4.1.1 Panel One Development

As seen in figure 4.1.1, seven variants that are known to cause HL are calibrated in order to be pooled together in one reaction. The sample that was tested in this panel is homozygous for Otoancorin D356V A>T variant. A red peak (T) instead of green peak (A) appeared indicating homozygosity, while it appeared to be wildtype for the rest of the variants. This variant was validated by Sanger sequencing.

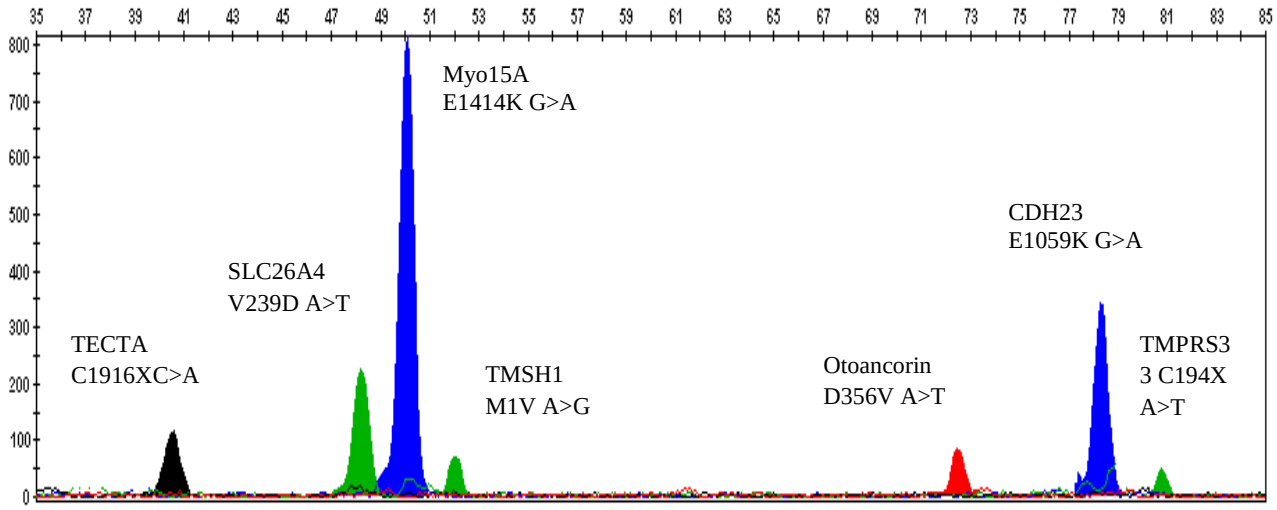

Figure 4.1.1: Seven variants shown in this figure with different sizes; TECTA C1916X (C>A):40.5, SLC26A4 V239D (A>T):48.2, Myo15A E1414K (G>A):50.1, TMSH1 M1V (A>G):52.0, Otoancorin D356V (A>T): 72.4, CDH23 E1059K (G>A):78.2, TMPRS33 C194X (A>T):80.7. X-axis indicates the size and Y-axis indicates the relative fluorescent units (RFUs).

4.1.2 Panel Two Development

As seen in figure 4.1.2, six variants that are known to cause HL are calibrated in order to be pooled together in one reaction. The sample that was tested in this panel is homozygous for GPR98 G3476R G>A variant. A green peak (A) instead of black peak (G) appeared indicating homozygosity, while it appeared to be wildtype for the rest of the variants. This variant was validated by Sanger sequencing.

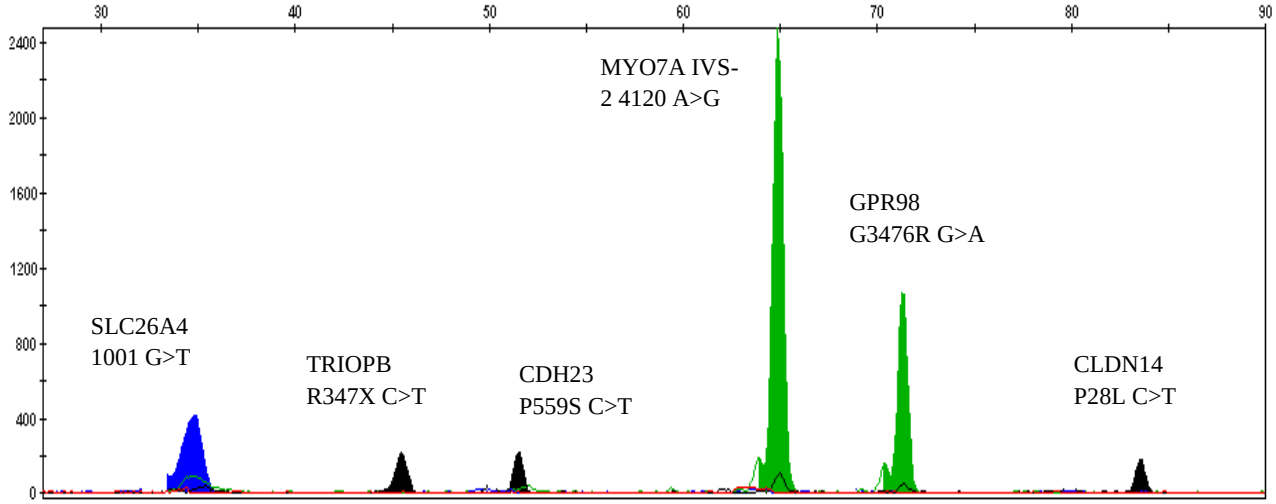

Figure 4.1.2: Six variants shown in this figure with different sizes; SLC26A4 1001 G>T: 34.8, TRIOPB R347X (C>T):45.4, CDH23 P559S (C>T):51.4, MYO7A IVS-2 4120 (A>G):64.86, GPR98 G3476R (G>A):71.3, CLDN14 P28L (C>T):83.5. X-axis indicates the size and Y-axis indicates the relative fluorescent units (RFUs).

4.1.3 Panel Three Development

As seen in figure 4.1.3, eight variants that are known to cause HL are calibrated in order to be pooled together in one reaction. The sample that was tested in this panel is heterozygous for MYO7A G2163S G>A variant. A green peak (A) and blue peak (G) with the same size appeared, indicating heterozygosity, while it appeared to be wildtype for the rest of the variants. This variant was validated by Sanger sequencing.

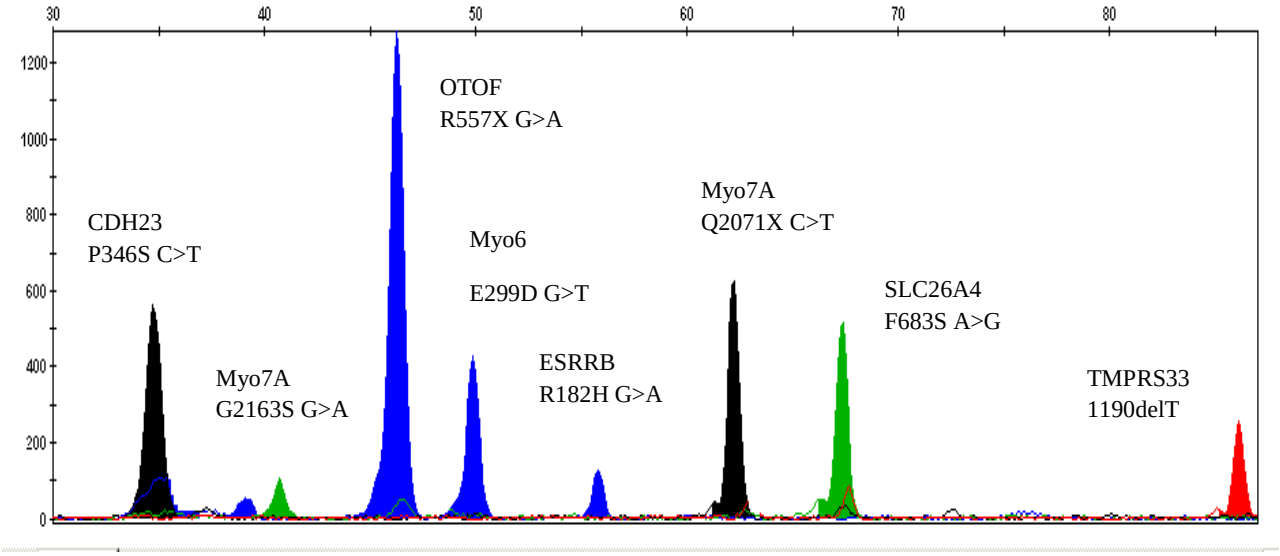

Figure 4.1.3: Eight variants shown in this figure with different sizes; CDH23P346SC>T: 34.7, MYO7AG2163S (G>A):40.2, OTOFR557X (G>A):46.2, MYO6 E299D (G>T):49.8, ESRRB R182H (G>A):55.7, MYO7A Q2071X (C>T):62.2, SLC26A4 F683S (A>G):67.3, TMPRS33 1190del T: 86.1. X-axis indicates the size and Y-axis indicates the relative fluorescent units (RFUs).

4.1.4 Panel Four Development

As seen in figure 4.1.4, eight variants that are known to cause HL are calibrated in order to be pooled together in one reaction. The sample that was tested in this panel is homozygous for GPR98 E299E G>A variant. A green peak (A) instead of black peak (G) appeared indicating homozygosity, while it appeared to be wildtype for the rest of the variants. This variant was validated by Sanger sequencing.

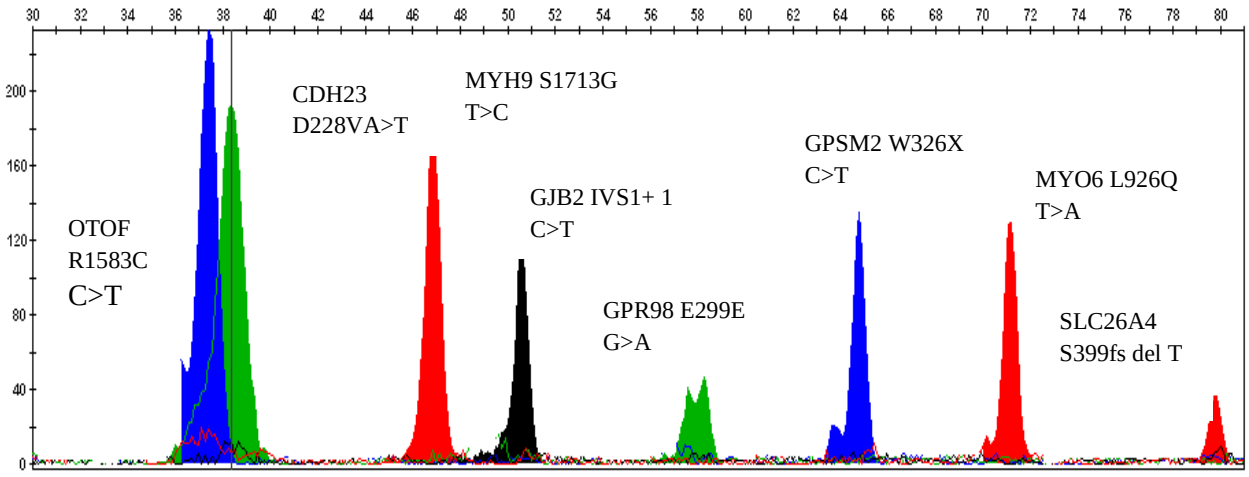

Figure 4.1.4: Eight variants shown in the figure with different sizes; OTOF R1583C C>T: 37.3, CDH23 D228V (A>T):38.3, MYH9 S1713G (T>C):46.8, GJB2 IVS1+ 1 (C>T):50.5, GPR98 E299E (G>A): 58.2, GPSM2 W326X (C>T):64.7, MYO6 L926Q (T>A): 71.2, SLC26A4 S399fs (del T):79.7. X-axis indicates the size and Y-axis indicates the relative fluorescent units (RFUs).

4.2 Validation by Sanger Sequencing

All positive samples for the following variants Otoancorin D356V (A>T), GPR98 G3476R (G>A), MYO7A G2163S (G>A) and GPR98 E299E (G>A) were validated by using Sanger sequencing. These variants sequences are shown in the figures below.

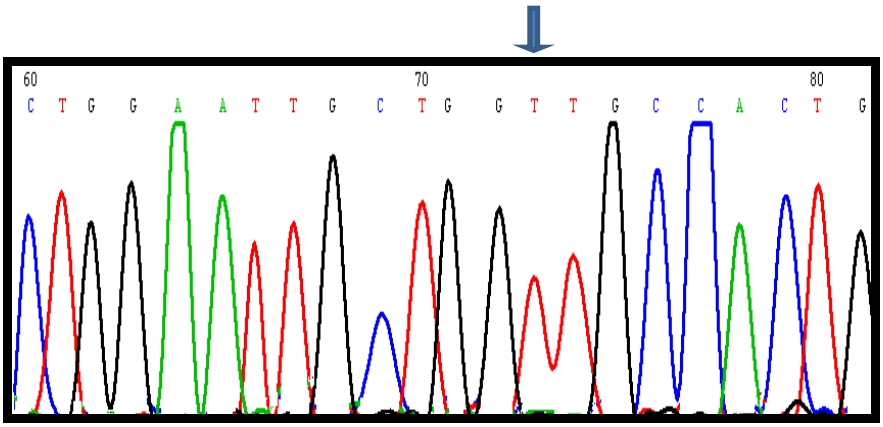

Figure 4.2.1: DNA sequencing chromatogram shows panel one homozygous sample for the Otoancorin D356V (A>T) variant.

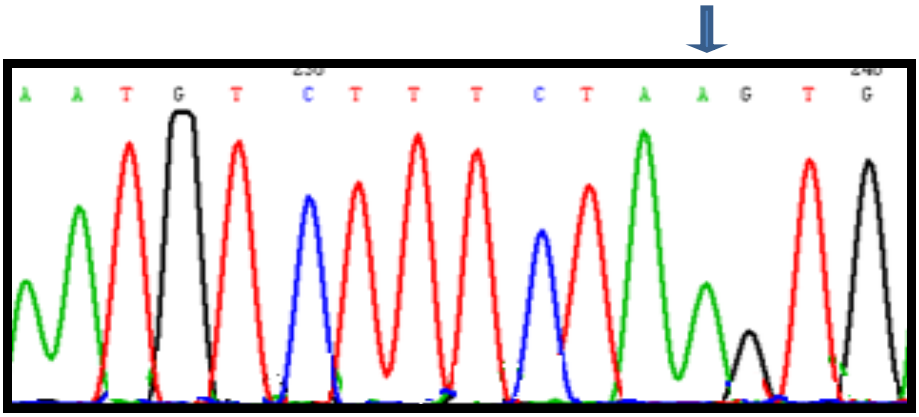

Figure 4.2.2: DNA sequencing chromatogram shows panel two homozygous sample for the GPR98 G3476R (G>A) variant.

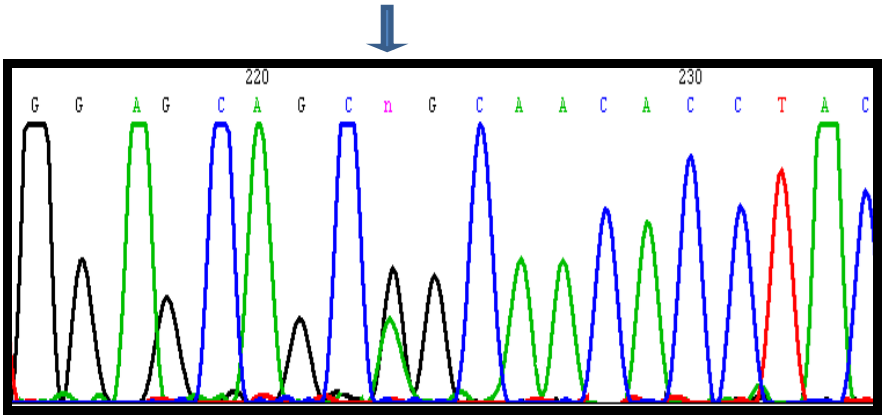

Figure 4.2.3: DNA sequencing chromatogram shows panel three heterozygous sample for the MYO7A G2163S G>A variant.

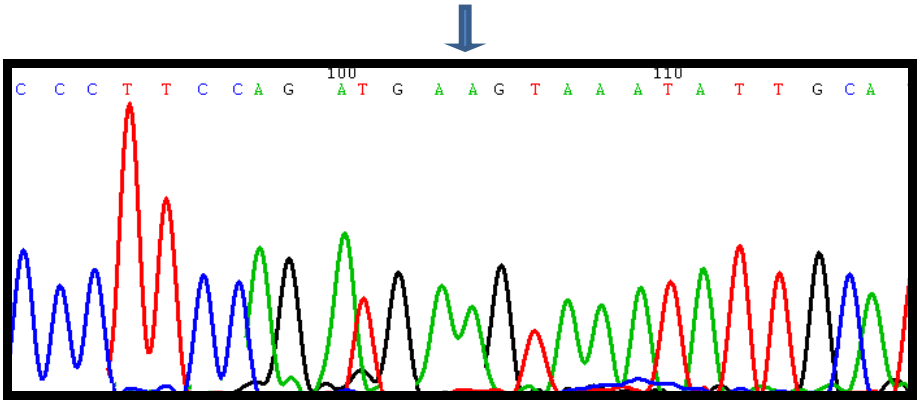

Figure 4.2.4: DNA sequencing chromatogram shows panel four homozygous sample for the GPR98 E299E G>A variant

In summary we have calibrated four snapshot panels; each panel contains couples of variants in order to be tested together. Homozygous, heterozygous and wildtype samples resulted from these panels as seen in the result figures. Optimal snap primer concentrations used were 2pmol/ul and 1.5pmol/ul. PCR Product sizes ranged from 190-220 bp.

Some peaks' intensities are not high compared to others in the same panel and this will be discussed later. However, the results of the Snapshot technique are based on the existence of the peak or not. It is also based on the size of the peak for discrimination but not the intensity.

Note: many reasons can lead to a low signal or intensity of the peak and this will be discussed in details in the discussion section.

CHAPTER 5

Discussion

In this study we are capable to optimize and calibrate four Snapshot panels for doing rapid genetic screening for patients that are suffering from hearing loss in the Palestinian population especially newborns. The main advantage of Snapshot technique is overcoming the difficulty of testing a genetic disease that is highly heterogeneous such as hearing loss.

The first development of the Snapshot technique was in 1990s by Smith et al (Smith et al., 1998). In 1990s Snapshot technique was based on multiplex PCR reaction followed by two-dimensional PCR. Recently, Snapshot technique is developed and is now based on multiplex PCR followed by single nucleotide primer extension with fluorescently labeled ddNTPs.

By using Snapshot technique we could test many variants in the same panel. In addition we can put the most frequent variants among the population in the first panel, thus reducing the time and the cost of the test. As previously mentioned, Snapshot technique is a cost effective technique, since snapshot probes are unlabeled probes compared to other techniques like pyrosequencing. Also, Snapshot technique is very flexible technique, since we could change the target loci simply by designing PCR and snap primers and add another variant to the panel, so any novel mutation discovered later could be easily added to the panel for testing (Wu et al., 2009). Mutational screening by Snapshot leads to mitigation of deafness patients in the Palestinian population and compared to other techniques it has an economic efficiency and is highly sensitive, since it allows for screening for multiple variants in the same reaction (Sagong et al., 2013). Snapshot technique is more sensitive than Sanger sequencing when we deal with cancer samples, since it could detect 10% tumor sample (see figure 2.2) (Farina

Sarasqueta et al., 2011). Efforts like this will accelerate the usage of genetic information for better diagnosis and treatment.

Snapshot technique was used to identify mitochondrial variants, for phylogenetic analysis, and for forensic identification (Paneto et al., 2011, Grignani et al., 2006). Many studies used this technique in heterogeneous diseases other than hearing loss. Snapshot technique was used to test KRAS and BRAF variants in colon cancer patients (Magnin et al., 2011) and it helped in targeted therapy in Non-small Cell lung cancer with mutations in KRAS, BRAF and EGFR and many other genes (Su et al., 2011)

Although snapshot reaction panels could have up to ten variants, the four panels that are previously discussed contain 6-8 variants. We went through different trials and error reactions and concentrations of the annealed primers in order to optimize these variants, and we concluded that there is a chance that the specificity decreases due to cross reactivity between probes during multiplex reaction (Kotoula et al., 2009) and this is the reason that the panels don't have 10 variants pooled together. Also, the signal or the intensity of some of the peaks as seen in the results figures is low. The low signal of the peaks could be due to different reasons. First, the LIZ intensity eclipsed the intensity of the peak or makes it lower. Second, insufficient concentration of the annealed primers is another issue that needs optimization. Third, injection time could be insufficient. Finally, the DNA input is imprecise. Slight shifts in the sizes were also observed due to the mobility of the fluorophores that were used for the detection. These shifts did not affect the results or the mutation detection and we can discriminate between the peaks easily based on the size (Hurst et al., 2009).

CHAPTER 6

6.1 Conclusion

Snapshot screening provides a robust, reliable, fast, simple and cost effective genotyping method for screening of hearing loss variants among Palestinian patients. This technique can help implement hearing loss genetic screening in clinical practice before therapy. Routine screening by this technique coupled with genetic counseling leads to lowering the incidence of hearing loss especially in population with heterogeneous ethnic backgrounds. Transmission of the mutated gene from both parents to their offspring can be prevented by either doing prenatal testing by testing the fetus for the mutation during pregnancy (Chorionic Villus Sampling (CVS) or doing Preimplantation genetic diagnosis (PGD) used to identify genetic defects in embryos created through in vitro fertilization (IVF) prior to their implantation.

Snapshot technique could be considered as first pass screening tool prior to next generation sequencing (NGS). And mutation carriers should be aware of their genetic status before stepping into marriage especially consanguineous marriages.

6.2 Limitations and recommendations

In order to do multigene genetic screening using Snapshot technique, we should have previous knowledge about the genes and the variants that lead to a particular disease like hearing loss. So we cannot detect novel or new mutation by this technique.

My recommendation that any newborn patient that fail the OAE and ABR hearing tests and diagnosed to have hearing loss should be tested by this technique as a first pass screening

genetic test before stepping to other clinical tests or treatment, thus reducing the cost and achieving essential management of hearing loss patients.

CHAPTER 7

References

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