Molecular Study of a Consanguineous Family with Autosomal Recessive Mental Retardation and Speech Disorder

Researcher

Syed Farhan Ahmad
MS Biotechnology
Reg. No. 6-FBAS/MSBT/S11

Supervisors

Dr. Jabar Zaman Khan Khattak
Assistant Professor
Department of Bioinformatics and Biotechnology

Co-Supervisor

Dr. Abdul Hameed
Principal Scientific Officer
Institute of Biomedical and Genetic Engineering
Islamabad.

Department of Bioinformatics and Biotechnology
Faculty of Basic and Applied Sciences
International Islamic University, Islamabad.

(2013)

Accession No. 10783

MB
362.3
AHM

-Mental Retardation
Mental Deficiency

DATA ENTERED
18/9/13

Molecular Study of a Consanguineous Family with Autosomal Recessive Mental Retardation and Speech Disorder

By

SYED FARHAN AHMAD

MS Biotechnology

Department of Biotechnology & Bioinformatics

Faculty of Basic and Applied Sciences

International Islamic University Islamabad

(2012-2013)

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيْمِ

Faculty of Basic and Applied Sciences

Department of Bioinformatics and Biotechnology

International Islamic University Islamabad

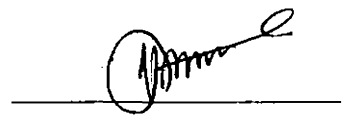
Dated: 06/02/2013

FINAL APPROVAL

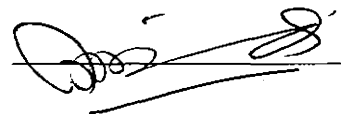
It is certified that we have read the thesis submitted by **Mr. Syed Farhan Ahmad** and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Biotechnology.

COMMITTEE

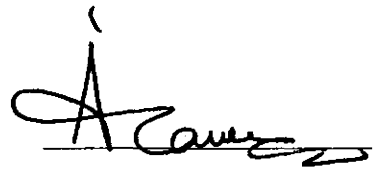
External Examiner
Dr. Muhammad Ismail



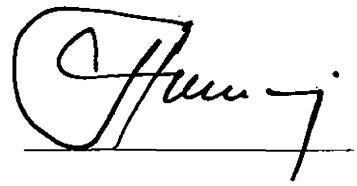
Internal Examiner
Dr. Arshad Malik



Supervisor
Dr. Jabar Zaman Khan Khattak
Assistant Professor
Department of Bioinformatics and Biotechnology
International Islamic University Islamabad



Co-Supervisor
Dr. Abdul Hameed
Principal Scientific Officer
Institute of Biomedical and Genetic Engineering
Islamabad.

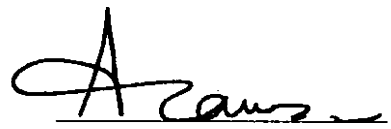


Faculty of Basic and Applied Sciences

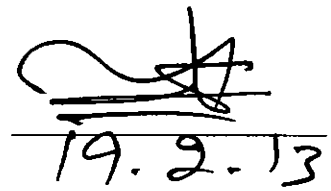
Department of Bioinformatics and Biotechnology

International Islamic University Islamabad

Chairman
Dr. Jabar Zaman Khan Khattak
Assistant Professor

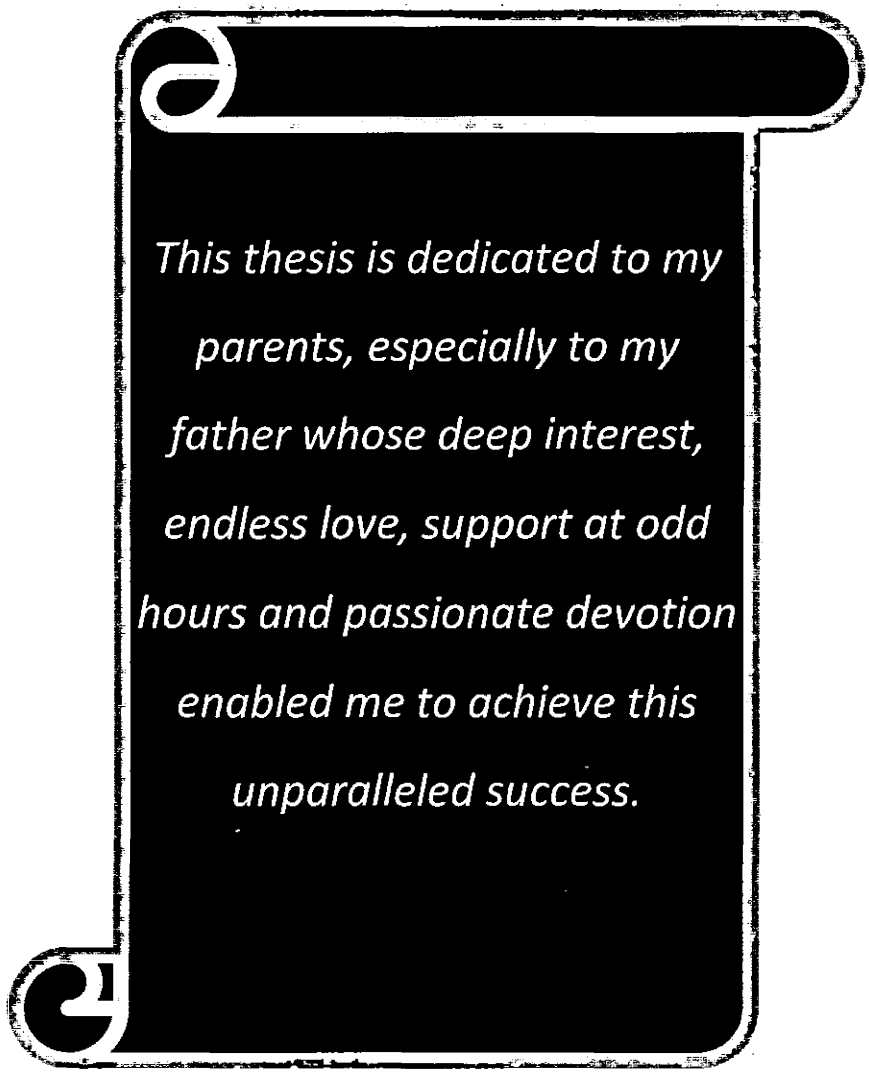


Dean, FBAS
Dr. Muhammad Sher
International Islamic University Islamabad



A thesis submitted to Department of Environmental Sciences,
International Islamic University, Islamabad as a partial
Fulfillment of requirement for the award of the
Degree of (MS BIOTECHNOLOGY)

DEDICATION


This thesis is dedicated to my parents, especially to my father whose deep interest, endless love, support at odd hours and passionate devotion enabled me to achieve this unparalleled success.

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, Except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 06|02|2013

Farhan

SYED FARHAN AHMAD

CONTENTS

ACKNOWLEDGEMENTS

All praise to Almighty Allah, who gave me the courage, ability and strength to complete this project and all respects are for his last prophet Muhammad (peace be upon him) whose teachings guided me in every sphere of my life.

I am thankful to the Chairman, Dr. Abdul Qadeer Khan Research Laboratories, Kahuta (KRL), for granting me the opportunity of working at the Institute of Biomedical and Genetic Engineering (IBGE), KRL, Islamabad.

I am highly grateful to my supervisor Dr.Jabar Zaman Khan Khattak, Assistant Professor and Chairman, Department of Biotechnology & Bioinformatics, International Islamic University, Islamabad for his constant guidance, encouragement, inspiration and advice he has provided throughout the year. Without his guidance and persistent help this thesis would not have been possible.

I offer my sincerest gratitude to Dean Faculty of basic and applied science (FBAS), for his endless support and encouragement throughout my study period.

I would like to express my deepest gratitude to Dr. Abdul Hameed, Principle Scientific Officer, IB&GE, KRL Islamabad for supervising the laboratory work presented in this thesis and for his guidance and support.

I feel great pleasure to place on record the physicians of KRL for their help in sorting out families suffering from inherited autosomal recessive mental retardation and providing me the detailed clinical descriptions of the affected family.

I am also thankful to Mr. Muhammad Ajmal, Scientific Officer and my other colleagues at IBGE, KRL for their valuable assistance at different occasions during this entire period of research. My special thanks to my class fellows and other friends for their suggestions and help in writing this thesis.

In last I would like to express my heartiest thank to my loving parents for their moral support and constant encouragement in my life. Allah bless on them.

Syed Farhan Ahmad

10th December, 2012

LIST OF ABBREVIATIONS

A	Adenine
AO	Antisense Oigonucleotides
ARNS-ID	Autosomal Recessive Non Syndromic Intellectual Disability
BLAST	Basic Local Alignment Search Tool
C	Cytosine
CC2D1A	Coiled-Coil and C2 Domain containing protein 1A
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CI	Cognitive Impairment
CNV	Copy Number Variants
CRBN	Cereblon
DNA	Deoxyribonucleic Acid
G	Guanine
GB	Giga base
GRIK2	Glutamate Receptor, Ionotropic Kainite 2
ID	Intellectual Disability
IQ	Intelligence Quotient
MBD	Methyl Binding Domain
MCPH1 or MECP2	Microcephaly 1, 2
MR or MRD	Mental Retardation
NCBI	National Centre for Biotechnology Information
NMD	Nonsense-Mediated Decay
NS-ADID	Non-Syndromic Autosomal Dominant Intellectual
NS-ARID	Non-Syndromic Autosomal Recessive Intellectual Disability
NS-ID	Non- Syndromic Intellectual Disability
NS-XLMR	Non-Syndromic X-linked Mental Retardation
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase Chain Reaction
PRSS12	Protease, Serine, 12
SID	Syndromic Intellectual Disability
SNP	Single Nucleotide Polymorphism
SSRs	Simple Sequence Repeats
STRs	Short Tandem Repeats
T	Thymine
TRAPPC9	Trafficking Protein Particle Complex Subunit 9
TUSC3	Tumor Suppressor Candidate 3

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Mental retardation is one of the most frequently found major genetic disorders around the world, affecting 1-3 % people in the general population. The recent advancement in molecular biology and cytogenetic study has allowed identifying new genes for a variety of genetic disorders including autosomal recessive mental retardation. The recessive genetic disorders are common in Pakistan due to high rate of cousin marriages. A central focus of the present study was to map and identify the disease causing gene in a mentally retarded consanguineous Pakistani family with speech disorder. The present study comprises of 20 individuals including 10 patients. Genetic analysis of Autosomal recessive mental retardation and speech disorders was done for eight known fundamental loci sorted out on the basis of clinical features. These loci including 8p22, 3p26.2, 11p15, 14q11.2-q12, 19p13.12, 7q22, 3p21.3 and 22p13 were screened by using Polymorphic microsatellite markers. To identify the disease locus, genomic DNA from each individual was genotyped for homozygosity analysis. Microsatellite markers were amplified using PCR. The study provided us valuable data to exclude linkage of the abovementioned loci. The absence of linkage of Pakistani family with autosomal recessive mental retardation to eight well known loci, confirms the genetic heterogeneity of mental retardation. There is need to verify these results and find the candidate gene by whole genome scan. It may help us in establishing the genotype-phenotype correlation of mental retardation, improved genetic counseling, carrier screening, DNA based prenatal diagnosis and the opportunity to develop appropriate animal models to test new forms of cell, protein or gene therapies.

Keywords: Mental retardation, Linkage analysis, Consanguineous Pakistani family.

CHAPTER 1
INTRODUCTION

CHAPTER 1

INTRODUCTION

Over the last few years, tremendous advances in the field of molecular biology have opened a new era of research for geneticists. Newly developed powerful techniques are being used to explore new facts of human genetics which have increased the pace of research.

Genetic research, spanning over a century, has affirmed that inherited traits are carried from parents to their progeny in the form of a chemical polymer called deoxyribonucleic acid (DNA). DNA is a linear sequence of deoxynucleotides containing four kinds of bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T). Almost all the DNA present in eukaryotic cells is packaged into the nucleus in organized structures known as the chromosomes. Human beings have 23 pairs of chromosomes containing approximately 3.2 GB (Giga bases). Only 1.1%-1.4% of total DNA sequence actually encodes proteins (Baltimore, 2001). The bulk of the human genome includes various brands of non-coding repetitive sequences. These sequences are vital because they are helpful in analysing chromosome composition, dynamics and are also used as genetic markers, which give informations for medical and population genetic studies. The protein-encoding sequences of DNA are called genes. According to a recent research the total number of genes in human beings lies between 25, 000-35,000 (Crollius *et al.,* 2000 and Ewing and Green, 2000). DNA is also found in the mitochondria, which are small organelle in all eukaryotic cells. Mitochondria are only inherited from the mother. The complete sequence of human mtDNA has been elucidated and found to be 16,569 bp long circular DNA (mtDNA) that is tiny as compared to nuclear DNA (Anderson *et al.,* 1981). Thus there are two types of DNA in eukaryotes, chromosomal and mitochondrial DNA. Both are involved in inherited disorders.

Inherited Disorders

Genetic research has confirmed the fact that genes control all body functions and a tiny change (mutation) in the sequence of DNA may alter the normal functioning of body, which may

lead to a particular inherited disorder. Such inherited disorders may run in the families from one generation to the next and will increase in frequency if the affected family continues to practise consanguineous marriages. Because it is established that consanguineous marriages result in an increase in inherited diseases due to the concentration of the same gene pool in a family (Kingston, 2002 and Bittles, 2001).

Frequency of consanguineous marriages is very high in Pakistan; therefore, unlike rest of the world large numbers of Pakistani families are suffering from variety of genetic diseases, particularly recessively inherited disorders. In Pakistan, the custom of consanguineous marriages has occurred for long history and, therefore, inherited diseases have been segregating in large multigenerational families. These affected families provide the scientists with a good biological material to study the inherited basis of these diseases including disorders of eye, nervous system, blood, heart, kidney, lungs, hearing loss, skin disorders and many more. Significant research work has been carried out in Pakistan so far to find the genetic causes and ways to treat these diseases, but there is more room to explore this area.

Mental Retardation; Definition and Prevalence

The aim of this study was to search out families suffering from inherited disorders, particularly mental retardation, and to find out defective genes responsible for the disorder.

A state in which an individual have intelligence quotient (IQ) level below normal i.e. 70) is often known as Mental Retardation, Intellectual disability or cognitive impairment. In many cases patients of mental retardation are lacking the onset of vital learning abilities such as communicating with people, understanding, inscription, self care etc before 18 year of age is lacking. (American Psychiatric Association, 2000).

With an occurrence of almost 2%, the disorder of mental retardation is familiar and frequently result severe disability all over the world, hence causing a big trouble to the suffered families as well as to the society (Ropers, 2006). In many countries cousin marriages are the

prior choice of the people due to cultural values; therefore the people of those countries including Pakistan are at higher risk to be affected by mental retardation and other congenital disorders. Both severe and mild mental retardation is considered to be more common in the offsprings of consanguineous marriages (Al-Ansari, 1993).

Mental retardation is found at higher rate in males as compared to women. The incidence of this disorder is higher in males (roughly 30%) than females (American Psychiatric Association, 2000; McLaren and Bryson, 1987). Though, with the reduction of IQ this prevalence decreases regardless of a greater percentage of males to females amongst non severe cases of MR. (American Psychiatric Association, 2000; McLaren and Bryson, 1987). According to several research studies, serious cases of the disease may be more widespread in females (Katusic *et al.,* 1996; Bradley *et al.*, 2002). Nevertheless, specific populations were taken in consideration for the research studies, and cannot principally be applicable to other areas.

In spite of identification of new contributing genes in a number of these disorders, the molecular origin of several disorders is still unidentified, and usually the task of the known disease-gene products remains an unanswered myth.

Types of Mental Retardation

There are five main categories of MR such as Mild, moderate, severe, profound and unable to classify (DSM IV). The basis of this classification is the IQ level which decrease from mild to severe form. Nevertheless, epidemiology research has revealed simple taxonomy, and stating that the persons in the range of IQ 50-70 suffer from mild disorders while those having IQ less than 50 are severely affected (Ropers and Hamel, 2005). Although the occurrence of severe cases of this disease is comparatively constant, the incidence of mild form is changeable. The most important cause of the inherited low IQ level is also due to peripheral environmental factors which include the skills of maternal training, availability of special education, and access to special healthcare units (Leonard and Wen, 2002, Drews *et al.,* 1995 and Roeleveld *et al.,* 1997). In addition to environmental factors, the approach of study, the sample population and

different techniques applied for the study significantly contribute to variability seem across mild cases of mental retardation (Leonard and Wen, 2002, Drews *et al.,* 1995 and Roeleveld *et al.,* 1997).

Apart from the aforementioned classification by IQ level, mental retardation may be categorized into syndromic mental retardation (SMR or SID) and non syndromic mental retardation (NSMR or NSID). In SID, person suffers from several types of symptoms and combined clinical manifestations or co-morbidities besides ID. Syndromic form of mental retardation is understandable and has a clear-cut description; however, there is difference of opinion about the classification of NS-ID.

Usually, the presence of a single clinical aspect acts as a useful characterization for NS-ID. Nevertheless, the exclusion of the incidence regarding several delicate brain and psychiatric anomalies is extremely cumbersome in these patients because of non apparent signs, and diagnosis is especially difficult because of mental damage. Furthermore, it is possible that most of the syndromes might consist of such complicated symptoms that they are exceptionally difficult to analyze unless the characteristics are particularly compared with the background of a recognized inherited deficiency formerly related to these signs (Ropers, 2006). In short we can say that the difference between syndromic mental retardation and non syndromic mental retardation is often not clearly understood.

Causes of Mental Retardation

Both genetic and environmental factors are responsible for causing MR. But, in almost 60% of the cases, the actual cause could not be identified precisely (Rauch *et al.,* 2006). Many environmental contacts to specific viruses, teratogens, or radiation can lead to MR. Severe shock or cranium injury is a major cause to resist oxygen supply to the central nervous system. It shed light on some cases of NSMR but it is vital to analyze the genetic etiology as well.

Almost 25–50% of cases of mental retardation are thought to be caused from genetic mutations, although this figure varies according to the severity of the disease (McLaren and Bryson, 1987). In most cases of MR, the major cause was the variations in chromosomes number and structure (Rauch *et al.,* 2006). Numerical aberrations such as increase in autosomal and sex chromosomes number nearly always effect in a number of syndromic mentally retarded patients, as demonstrated by numerical chromosomal aberrations such as Down Syndrome (21 pairs of chromosomes), which is extremely common inherited type of MR (Rauch *et al.,* 2006). Furthermore, disease caused due to structural variation in genome has been responsible for mental retardation in a huge number of research studies. The discovery of many ID causing genes will be helpful to study ID in coming decades (Ropers and Hamel, 2005 and Zahir and Friedman, 2007).

Several monogenic causes of NSID have been discovered during the last fifteen years. Various NSMR genes might correspondingly cause SMR, autism and additional abnormal phenotypes of neurological development. It has confirmed that several inherited changers or environmental factors might be engaged in disease etiology. The significance of careful genotype/phenotype relationships is also revealed, that are frequently complicated to expose. Multigenic cases of NSMR are also thought to be at high risk, involving several mutated genes in an individual. However this concept has not been well understood. Autosomal abnormalities are most prominent than X chromosomal variations in ID (Chelly *et al.,* 2006).

Classification and Types

Non-Syndromic X-linked Intellectual Disability (NS-XLID)

Due to high frequency of males affected with MR in general population X chromosome is highly considered to be the part of most studies. Almost 40 genes have been discovered that are responsible to cause NS-ID, and more than 80% of these genes are located on the X chromosome. Among these genes some can cause both S-ID and NSID depending on the

mutation or might even differ within families, probably due to extra features. The similar genetic variation in a similar family in which all family members affected with mental retardation and had representative phenotypic abnormalities, excluding one patient who had non syndromic disorder in ATRX (MIM: 300032) (Guerrini *et al.,* 2000). This DNA segment also launched many other MR diseases. Numerous alterations have been known for this candidate gene (Yntema *et al.,* 2002, Gibbons *et al.,* 2003 and Howard *et al.,* 2004). A number of additional genes that typically form the basis of syndromes can also cause NS-XLMR. The MECP2 (MIM: 300005), which is responsible to cause Rett syndrome, has been detected in a number of NSMR cases (Orrico *et al.,* 2000, Couvert *et al.,* 2001 and Dotti *et al.,* 2002). As the ratio of ID is high in males, an MECP2 missense mutation was found to cause severe intellectual disability in males as compared to females that concern a much milder phenotype (Dotti *et al.,* 2002). These findings suggest that there may be a quantifiable genotype/phenotype association for definite mutations. Especially, in Rett syndrome, a number of studies have demonstrated a genotype/phenotype correlation in terms of severity, as well as for specific phenotypic measures (Bebbington *et al.,* 2008 and Ham *et al.,* 2005).

Non-Syndromic Autosomal Dominant Intellectual Disability (NS-ADID)

The general mechanism of NS-ID is autosomal inheritance, and it is much needed to be studied. Unlike XLMR genes, which contain relatively frequent missense and nonsense mutations, the NS autosomal dominant and recessive genes identified so far appear to have chromosomal abnormalities. The defected genes involved in NS-ADID are few in number. MIM uses the acronym MRD for loci for "mental retardation, autosomal dominant". For MRD1 (MIM 156200), the methyl binding domain 5 gene, SNP microarray analysis revealed MBD5 (MIM 611472) on 2q23.1, signifying a 200Kb de novo deletion, removing at least 6 exons of the gene in a female proband with sandal-toe and epilepsy but no facial dysmorphic features (Wagenstaller *et al.,* 2007). The gene for MRD2, on 9p24, dedicator of cytokinesis 8 (DOCK8; MIM 611432) was identified in 2 unrelated patients, by mapping breakpoints of a deletion and translocation respectively (Griggs *et al.,* 2008).

Non-Syndromic Autosomal Recessive Intellectual Disability (NS-ARID):

MIM uses the short form MRT for "mental retardation, autosomal recessive. Just 6 MRT genes have been made known till now. Only two genes have been detected in more than one family. TUSC3 was the earliest of these to be studied (MIM: 601385), for MRT7, which translate a protein that is expected to be involved in catalyzing the transfer of a 14-sugar oligosaccharide from dolichol to nascent protein, an essential step in N-linked protein glycosylation (Molinari *et al.,* 2008 and Garshasbi *et al.,* 2008). TUSC3 is required for Mg2+ regulation, and knockdown of this gene causes decreased total and free intracellular Mg2+ in human cell lines, as well as growth arrest or abnormal development in zebrafish embryos (Zhou and Clapham, 2009). The next NS-ARID gene identified in more than one family is TRAPPC9 (MIM: 611966), for MRT13, which encodes a protein called NIBP. TRAPPC9 mutations have been studied in 4 dissimilar families from various parts of the world (Mir *et al.,* 2009; Philippe *et al.,* 2009 and Mochida *et al.,* 2009). Direct interaction of NIBP with NIK and IKKβ leads to the activation of NF-κB pathway (Hu *et al.,* 2005). It resulted in axonal outgrowth in vitro, and may be a causative factor in neuronal cell survival (Hu *et al.,* 2005). Same result was found in two families from two different counties with defective genes (Mir *et al.,* 2009; Mochida *et al.,* 2009). It shows that a significant variation has passed down through several generations, and more research to find various alterations in candidate genes in future.

Genetics of Autosomal recessive mental retardation:

The symptom of inability to learn efficiently and mal adjustment with normal social life along with delayed intellectual function due to inherited neurodevelopmental disorder is called mental retardation. MR is supposed to have effect on about 2-3% of the general population that comprises of high number of affected males than females (Leonard *et al.,* 2002). Genetic variations such as structural aberrations of chromosomes, hidden genomic recombination, and monogenic mutations are the leading cause of MR (Stevenson and Schwartz, 2009; Ropers, 2010). Most genes that are considered to be connected with syndromic and non-syndromic MR

are multifunctional; Moreover, a large number of MR linked genetic mutations are uncommon, mutations, representing that the genetic etiology of MR encompasses a diversity of greatly heterogeneous genetic defects. In spite of the several identified genes having a large association with MR, more MR genes are unknown so far (Stevenson and Schwartz, 2009; Ropers, 2010; Rauchet *et al.,* 2006). Translocations in chromosomes may be responsible to clinical phenotypes via direct gene disruption, formation of chimera genes, or modification of the expression of genes near the breakpoint via the position effect (Abeysinghe *et al.,* 2004; Bache *et al.,* 2006; Kleinjan *et al.,* 1998). By mapping of the breakpoints of chromosomal translocations many genes linked with MR have been explored, such as the dedicator of cytokinesis 8 gene (DOCK8) at 9p24 (Griggs *et al.,* 2008).

Normally it is considered that autosomal recessive mental retardation comprises of ~25% of genetic Intellectual disability (ID) patients (Bartley and Hall, 1978). Till now six known genes that are localized on 30 different loci have been founded to be responsible for causing autosomal recessive NS-ID (ARNS-ID) (OMIM database). These include PRSS12 (Protease, Serine, 12 or Neurotrypsin; MIM# 606709) (Molinari *et al.,* 2002), CRBN (Cereblon; MIM# 609262) (Higgins *et al.,* 2004), CC2D1A (Coiled-coil and C2 domain having protein 1A; MIM# 610055) (Basel-Vanagaite *et al.,* 2006), GRIK2 (Glutamate receptor, ionotropic, kainite 2; MIM#138244) (Motazacker *et al.,* 2007), TUSC3 (Tumor suppressor candidate 3; MIM# 601385) (Garshasbi *et al.,* 2008 and Molinari *et al.,* 2008), TRAPPC9 (Trafficking protein particle complex subunit 9; MIM# 611966) (Mir *et al.,* 2009, Mochida *et al.,* 2009 and Philippe *et al.,* 2009). Chromosome 8 is considered as an average chromosome with respect to size (146.364 Mb), number of genes (1198), repeat content and degree of segmental duplication (Nusbaum *et al.,* 2006). But its p arm showed high degree of sequence variations, particularly within its distal-most ~15 megabase region. This region is believed to be of prime importance in the human genome because of the high expression pattern of nervous system related genes, and has recently been touted as a "hub" for neuropsychiatric developmental disorders (Tabarés-Seisdedos *et al.,* 2009). Many genomic imbalances on 8p locus, such as duplication of 8p23.1-8p22.2, are associated with learning

disability (Glancy *et al.*, 2009). Also one gene for microcephaly (MCPH1) and one gene for NS-ID, namely TUSC3, have been identified on 8p.

Genetic diagnosis of MR patients:

After excluding any non genetic cause, patients with mental retardation should be referred to a human geneticist or to a pediatrician specializing in medical genetics. A clinical genetic examination will be performed by physician to facilitate the accurate diagnosis of the known syndromic types of MR, if one of these is present. After matching the clinical signs and symptoms to an exacting syndrome the familiar patients are selected and are then subjected to different tests, in next step molecular analysis isperformed using several molecular genetics techniques such as chromosomal examination, hybridization test to detect copy number variation, linkage analysis by STRs or microarray, depending upon the availability of the tests. Chromosomal analysis analysis is required to diagnose non-syndromic mental retardation in all patients. Test for fragile X syndrome is preferably done in males irrespective of any distinctive clinical features. Those families that have high number of affected male individuals should be advised for the tests which are mandatory to detect or exclude all known X-chromosomal genetic defects; for financial reasons, set up of the international research projects that are currently in progress must be encouraged so that the researchers can give advance information. Despite all of the available diagnostic tools are useful, only about 50% of patients with severe ID are detected with an appropriate cause; while this percentage further lowers dealing with mild ID (Chelly *et al.*, 2006). The recognition of an inherited defect assists parents to understand the illness and keenly compete with it, e.g., by participating in self-help groups. The people affected with MR are poor and for their accurate diagnosis research centers should be established in the country.

Possible Treatments

NS-MR which is caused by mutations in single gene, therefore it can be treated by gene therapy. Some of trials have shown fruitful results for MR-linked phenotypes in mouse models. Experiments have shown that using genetically modified mouse models one can effectively insert functional MECP2 (the gene that is responsible in causing Rett syndrome and some cases of NS-MR) into MECP2 lacking mice. The mice having no MECP2 gene were generated by insertion of transgenes. Therefore, the expression of this gene may be helpful in increasing the efficiency of pharmacological therapies (Luikenhuis *et al.*, 2004, Giacometti *et al.*, 2007, Guy *et al.*, 2007 and Jugloff *et al.*, 2008). Most of the neurological and behavioral phenotypes of these mice were saved with introduction of the transgene, which shows that the damage done by lack of MECP2 is not entirely unalterable, and that ID phenotypes can be rescued to some level (Luikenhuis *et al.*, 2004, Giacometti *et al.*, 2007, Guy *et al.*, 2007 and Jugloff *et al.*, 2008). Mice deficient with MECP2 have a Rett-like phenotype, although most probably, similar phenotype rescue methods could be employed to mark NS-ID genes. Using this approach in humans has been ineffective so far due to the difficulty in delivering the functional copies of the gene to applicable tissues and cells. There is also a high risk of over-expression of the incorporated gene, which itself may cause unfavorable effects.

Exon skipping is another technique that is applied to study neuromuscular disorders and can be applied to NS-ID. In this method antisense oligonucleotides (AO) or siRNA is used (Arnett *et al.*, 2009). This procedure involves the splicing of non-coding sequences containing the truncating mutation so that the rest of the gene may synthesis mRNA (Arnett *et al.*, 2009).

Viral vectors are useful in delivering the AOs. The limited application of this method includes provoking of immune response against the strange virus and inserted gene (Arnett *et al.*, 2009). Additionally, this method has only been assessed for effectiveness in muscle tissues. One of the main issues here would be to identify a vector that could transverse the blood-brain barrier. However, if this could be overcome, it could be an effective way to overcome nonsense-

mediated RNA decay of ID genes, and as a consequence restore some function to NS-ID proteins.

The most pragmatic treatment that is compatible with humans is aminoglycoside mediated suppression of nonsense mutations. Aminoglycosides, commonly used as antibiotics, can prevent the alterations caused during translational step of central dogma hence producing potentially functional proteins. Administration of aminoglycosides is recommended and relatively more successful for prevention of numerous diseases, including those found in MECP2 that cause Rett syndrome (Brendel *et al.,* 2009).

Mutant types of MECP2 gene that are involved in causing common Rett syndrome was inserted in cancerous Hela cell line, read-through after treatment with aminoglycosides was from 10% to 21% depending on the mutant gene (Brendel *et al.,* 2009). Same results have been found for other defective genes. In a research of cystic fibrosis (CF) in humans, administering of aminoglycoside (gentamicin) drops through nasal pathway of the persons having nonsense mutations in the CFTR gene was successful and confirmed some restored function of CFTR in 90% of patients (Wilschanski *et al.,* 2003). Although this method has had relatively encouraging results, there are some issues with it. Facts exist to recommend that natural stop codons cause proficient termination due to their framework, whereas premature stop codons may be more at risk to read-through because of their position (Kerem, 2004). This concept is additionally held up by the evidence that the efficiency of certain mutations response to aminoglycosides is specific (Kerem, 2004). Nevertheless, it may be due the interactions of pseudogenes or other genes with nonsense mutations that did not express a phenotype. "Junk" DNA is thought to be making copies of mRNA and then these products may compete for active sites with normal, functional proteins. Moreover, proof has been found in CFTR nonsense mutation cell lines that the level of nonsense-mediated decay (NMD) has an effect on the efficiency of aminogylcosides (Linde *et al.,* 2007). This makes sense, as NMD would result in there being less available transcript to read through. Other potential issues include nephrotoxicity and ototoxicity that occurs with aminoglycoside usage (Nagai and Takano, 2004). However, some cases of NS-MR involving

single genetic mutation cannot be treated by this therapy. Generally, the evidence that, for some therapies, there is a certain enhancement in the condition of mice with features of ID suggests that damage to the nervous system may be reversible in some types of ID. This has offered an important expectation for possible therapy of MR and developing functional behaviors in mentally retarded people. Extraction more information of the candidate genes and biological pathways involved in ID is to be encouraged, in order to step forward the objectives of medicinal cure of human MR.

Genetic Counseling and the hazards of recurrence

The parents of a child suffering from mental retardation are often in doubt that the disease may reappear in their future children. These problems can usually be coped with identification of syndromes. In case of a known genetic defect, prenatal diagnosis is performed. For children with non-syndromic ID as well, prenatal genetic testing is enabled by the expression of a gene mutation that gives an accurate statement of the possibility of the disease to be repeated in next generation. In most cases, the gene mutations are not detectable. If no positive family history persists then the risk of mental retardation in each future child is ca. 8%, as long as the patient specificity for the type of MR is not understood (Basel-Vanagaite *et al.,* 2006). This risk is increased correspondence to the baseline risk level of 2% and parents are usually advised not to give birth to any more children. A more accurate evaluation of the risk will be promising only after more MR genes have been studied and more comprehensive tests have been extended to detect or exclude defects within them.

Clinical Features and Characteristics

The common presenting symptoms in people with mental retardation are impairment in adaptive functioning, rather than low IQ. Adaptive functioning refers to the ability of effective person who can normally tackle with the activities concerning his general life. It means how effectively individuals can perform various tasks such as personal independence expected of someone in their particular age group, sociocultural background and community setting.

Adaptive functioning may be manipulated by different factors such as education, inspiration, behavior characteristics, societal and professional prospects and the mental disorders and general medical conditions that can coexist with mental retardation.

It is valuable to assess discrepancies in adaptive functioning via information collected from one or more different independent sources (e.g., teacher assessment, and learning, developmental and medical histories). Numerous interview scales have been planed to determine adaptive functioning or behavior (e.g., Vineland Adaptive Behavior Scales and the American Association on Mental Retardation Adaptive Behavior Scale). As in the evaluation of intellectual functioning, the appropriateness of the mechanism to the person's sociocultural background, education, associated handicaps, motivation and cooperation should be focused. A part from that, several activities that would normally thought to be maladaptive (e.g., dependency, passivity) can provide proof of good adaptation in the perspective of the particular life setting of a person with mental retardation.

Mild Mental Retardation

Mild mental retardation can be initially difficult to analyze during the childhood. The reason may be due to normal communications and social dealing of the patient during the ages of 0-5 years. There is minimal brain impairment at earlier age and hence the patient suffering from mild MR may not be differentiated from normal individuals until a later age. As the patients enter in their teen age, they are faced to the problems of self care and disability of performing social activities. During their adult years, they usually attain social and vocational skills adequate for minimum self-support, but may call for supervision, direction and help, especially when under unusual social or economic stress. With suitable supports, patients of mild mental retardation can frequently survive a successful life in the society, either independently or in supervised settings.

Moderate Mental Retardation

In this group of MR, most people attain fundamental communication skills during the early childhood. They are benefited from vocational training and, with moderate supervision, can train themselves how to take care. However in spite of being trained they are a little passive in social and occupational skills and are unlikely to improve beyond the second-grade level in academics. They may become skilled at traveling alone in memorable places. During teenage years their difficulties in distinguishing social gatherings may hinder with peer relationships. In their adult years, the majority are able to perform unskilled or semiskilled work after being supervised in sheltered workshops or in the general work force. They adapt well to life in the community, often after proper guidance.

Severe Mental Retardation

As a group, people suffering from severe MR obtain little or no communicative speech during the early childhood years. During the school-age period, they may be trained to have a discussion and can learn basic self-care skills. Their skills to proceed instruction in pre-academic subjects are limited. They can recognize the alphabet and simple counting, and can master skills such as learning sight reading of some 'survival' words. In the adult years the performance and ability of simple tasks are prepared under strongly supervised settings. Many adjust well to life in the society, in supervised group homes or with their families, unless they have an associated handicap that requires specialized nursing or other care.

Profound Mental Retardation

As a group, people with profound mental retardation have an already determined neurological condition that is responsible for causing the mental retardation. During the early childhood years they have substantial abnormaity in sensorimotor functioning. Optimal development may occur in a highly structured environment with regular assistance and

supervision and a personal relationship with a caregiver. Proper training can recover the abnormal behavioral features of MR. Some can carry out simple tasks in personally supervised and covered settings.

Figure 1.1. Clinical Features of mental retardation with speech disorder

A Pakistani family collected from Swabi, Khyber Pakhtoon Khuwa Pakistan, for the present study. Patient with typical characteristics of Mental retardation: A) Bulging eyes. B) No Articulation in Tongue. C) Speech and Language Impairments. D) Gait abnormality. E) Mild microcephaly. F) Muscle weakness or myasthenia

Gene Hunting

A number of different techniques and parameters are currently being used to search for disease causing genes. An understanding of the molecular aetiology of inherited diseases requires that we find out everything about the gene(s) involved, their chromosomal locations, DNA sequence, expression control and finally the function of the product. For this, two approaches are possible. If the biochemical pathway and the lesion leading to disease are known, then mutations can be examined in a gene or its product. For example, mutation in the haemoglobin molecule can be studied in haemoglobinopathies, or insulin in diabetes etc. This is often referred to as "forward" genetics. However, for many diseases such as cystic fibrosis or Duchenne muscular dystrophy etc., the underlying genetic defect was not known. In these instances the chromosomal location of the disease locus and positional cloning proved successful. This process is known as "reverse" genetics (Davies and Read, 1992). In reverse genetics different approaches like candidate gene approach or positional cloning approach are used however, in the hunting of disease gene genetic linkage analysis is often the first critical step. Linkage analysis is a PCR based technique which is used to compare, within a family, the inheritance of a disease gene with specific DNA segments termed markers (Khaliq *et al.*, 2000).

Linkage Analysis

The basis of linkage analysis is that during gamete formation DNA recombination takes place in parental chromosomes. If two loci are close to each other they will co-segregate in a pedigree. Co-inheritance of a locus (e.g. disease locus) with a specific DNA marker suggests that they are physically close i.e. linked on a specific chromosome.

To find out genetic loci through linkage analysis different genetic markers are used which include;

a) Microsatellite and minisatellite markers. Both of these markers are simple sequence Repeats (SSRs). Microsatellite markers having short repeats unit (n=1-13 bases), also called Short

Tandem repeats (STRs). Minisatellite markers are longer repeat units (n= 14-500 bases) (Toth *et al.,* 2000). Genetic markers based on SSRs have been the workhorse of most human disease-mapping studies due to their high degree of length polymorphisms in humans (Broman *et al.*, 1998 and Dib *et al.*, 1996).

b) Single nucleotide polymorphisms (SNPs) – single base differences between genome sequences, are the most common source of variations between humans (International human genome sequencing consortium, 2001). More than 1.4 million SNPs have been assembled into a genome wide-map (Sachidanandam *et al.*, 2001). These variations also act as a sensitive indicator for medical genetic studies. In contrast to microsatellite markers, which are more mutable, SNPs have a low rate of recurrent mutations, making them stable indicator for mapping disease genes (Horikawa *et al.*, 2000) and for probing population history (Kidd *et al.*, 2000). By comparing patterns and frequencies of SNPs in patients and controls, researchers can identify which SNPs is associated with the disease (Guigo *et al.*, 2000 and Stormo, 2000).

By using the microsatellite, minisatellite, or SNP markers, specific DNA segments can be amplified and analysed for the presence or absence of linkage between marker and the disease locus. However, Linkage analysis provides an approximate position of the disease gene within the genome.

If a particular marker is found to be linked to the disease under consideration, statistical analysis is done to evaluate whether two loci are linked or not. Powerful statistical computer programmes like LIPED (Ott, 1974) and MLINK (Lathrop and Lalouel, 1984) are used to shows the extent to which a particular marker is linked to the disease gene.

Gene Identification and Mutation Screening

A gene may be isolated by either a candidate gene approach or positional cloning approach. In the candidate approach, the disease gene is searched on the basis of the function of protein involved, its amino acid sequence, or the underlying biochemical defect. Unfortunately, for most skin disorders, there is little information about the disease gene or its function. One

therefore has to rely on the laborious positional cloning strategy, which requires no information about gene function. A third approach that is gaining increasing popularity because it can expedite gene discovery is the positional candidate gene approach. This relies on a combination of the above two strategies, i.e., a gene is mapped to a chromosomal region by linkage analysis and then the attractive candidates in the area are surveyed. For example, when autosomal dominant retinitis pigmentosa was mapped to 3q21-24, the rhodopsin gene was already known to be in this area. The gene was examined and within the rhodopsin gene, mutations were found that cause retinitis pigmentosa. When the detailed and accurate sequencing of the human genome and the location of all the human genes becomes known, the positional candidate strategy is likely to predominate (Collins, 1995).

Once the candidate gene is identified then next step is mutation screening of the gene. Mutation screening of different exons of the candidate gene give us `clear and in-depth picture about the molecular basis of the inherited disorder.

Besides these techniques a combination of different bioinformatics tools and web resources also help us in localization, identification and mutation screening of candidate genes. Commonly used Internet resources include the NCBI (http://www.ncbi.nlm.nih.gov /genome/guide) and Ensemble (http://www.ensembl.Org), which are widely used for gene identification, analysis and large-scale gene annotation. OMIM (McKusick, 1998) (http://www.ncbi.nlm.nih.gov/Omim/) provides valuable information about diseases and genes involved. To date more than 10,000 genes have been catalogued in this web site. BLAST (http://www.ncbi.nlm.nih.gov: 80/BLAST) is a very useful net-based resource for comparison of gene and protein sequences etc. (Altschul *et al.*, 1990).

By using these different approaches we can find out the molecular basis of different inherited disorders prevailing in Pakistan. Although different treatments and supplements are used to partially treat the symptoms in few inherited disorders like diabetes, hemophilia and some skin disorders etc however, to date no proper treatment available to completely cure the

inherited disorders. To find a permanent solution of such inherited disorder required extensive research in the field of molecular biology. The main objective of the study is to collect genetic data of Pakistani families suffering from mental retardation which will not only help us in the future treatment of genetic disorders by techniques such as gene therapy and stem cells therapy but also enable us to provide genetic counseling to the family to minimize further transfer of the disease in the coming generation. This will also support genetic research in Pakistan, encourage the scientists and doctors to find the genetic risk factors in our population and public awareness about genetic diseases. In number of developed countries including UK and USA, genetic counseling is in common practice to grow Public interest in genetic information and genetic testing. All such efforts will collectively helps in understanding the genetic and biochemical basis of inherited diseases and lead to accurate disease diagnosis and development of novel therapies to treat such diseases.

CHAPTER 2
REVIEW
OF
LITERATURE

CHAPTER 2

LITERATURE REVIEW

The central theme of this research was to evaluate the genetic exploration of mental retardation. Literature review exposed that one of the most alarming issue to be considered amongst the researchers across the entire world was the molecular analysis of genetic diseases. The impact of consanguineous marriages on heritable mutation is at the high frequency in Pakistani society. A review of the results of the most related studies is expressed in this chapter. These research studies have been performed in several countries and present the importance of exactly the same study performed in Pakistan.

Madhavan and Narayan (1991) recommended that marriages among the people having same blood relations often results in genetic disorders in their children especially the occurrence of mental retardation is possible. Madhavan and Narayan conducted this study to observe the influence of cousin marriages on mental retardation where the contributing cause is not conventional. They analyzed a total of 517 mentally retarded persons and their families out of which 160 were born of consanguineous marriage and 357 were of non-consanguineous marriage. Their conclusion showed that the frequency of MR was high in the families where cousin marriages were common. In general population the risk of occurrence of MR increases (x^2=11.52; P<0.001) by the consanguineous marriages among the parents. The family relationship of uncle-niece is thought to be affecting more generations at higher risk.

Keeping in view concerns of the above mentioned research the present research was based on analogous background. The consequences of this project can deliver a guideline to the researchers how to analytically investigate the influence of cousin marriages on the genetic mutations of mental retardation.

A study was conducted by Khan *et al.,* (2011) based on a new deletion alteration in the TUSC3 gene in a Pakistani family with autosomal recessive nonsyndromic mental retardation due to consanguineous marriages. They reported that mental retardation (MR) is a severe abnormal condition of the central nervous system with a occurrence of 1- 3% in overall population. In the previous years, the study emphasis has been largely on X-linked MR (68 loci and 19 genes for non syndromic X linked ID) while for autosomal recessive nonsyndromic ID (NSID) only 30 loci and 6 genes have been described so far. Complete genome homozygosity mapping with 500 K Nsp1 array (Affymetrix), copy number variation analysis, PCR based breakpoint mapping and DNA sequencing was done to determine the genetic basis of autosomal recessive nonsyndromic ID in a large Pakistani family. The result indicated linkage at 8p23 locus with common homozygous region between SNPs rs6989820 and rs2237834; covering a region of 12.494 Mb. Homozygous deletions of 170.673 Kb was exposed through the subsequent copy number variation (CNV) study of the data which involved the TUSC3 gene. After the discovery of the primary gene known as TRAPPC9 that has been participating in causing autosomal recessive NSID in many families, a novel deletion mutation in TUSC3 gene was described as the second gene responsible for the same genetic disorder. This research work contributed in understanding the molecular pathway of consideration.

A review comprising the knowledge of the molecular analysis of non-syndromic intellectual disability by Kaufman *et al.,* (2010) concluded that Intellectual disability (ID), also known as mental retardation (MR), and is commonly due to the cause of participation of genetic alterations. In case of syndromic mental retardation the combined clinical manifestations provide enough information for diagnosis and enable the physician to detect a syndrome easily, which may then extract the proof of identity of the causal defect. Conversely in absence of multiple clinical symptoms simultaneously in a single disorder (nonsyndromic) one can use different sophisticated techniques of molecular genetics to narrow down a specific gene. An organized assessment of inherited causes of nonsyndromic mental retardation was presented in this study. The researcher made an effort to summarize the shared aims of the genes and the molecular pathways of their encoded proteins. As MR is a mutual charecteristic of autism, and on the other

hand autistic features are normally present in individuals with MR, the researchers also observed the probable overlaps in genetic etiology with non-syndromic ID.

Janneke *et al.*, (2011) narrated that Autosomal recessive mental retardation (AR-MR) may comprise of almost 25% of inherited mental retardation (MR). Till now, 6 nonsyndromic genes have been mapped for AR-MR in families with common cousin marriages, whereas more than 2000 genes might contribute to AR-MR. They proposed to use families that produced offspring outside a particular family with multiple affected members for AR-ID gene detection. Homozygosity mapping in ten outbred families with affected brother–sister pairs using a 250 K single nucleotide polymorphism array revealed on average 57 homozygous regions over 1Mb in size per affected individual (range 20–74). Of these, 21 homozygous regions were shared between siblings on average (range 8–36). None of the shared regions of homozygosity (SROHs) overlapped with the nonsyndromic genes. A total of 13 SROHs had an overlap with previously reported loci for AR-MR, namely with MRT8, MRT9, MRT10 and MRT11. Among these was the longest observed SROH of 11.0Mb in family ARMR1 on chromosome 19q13, which had 2.9Mb (98 genes) in common with the 5.4Mb MRT11 locus (195 genes). These data support that homozygosity mapping in outbred families may contribute to identification of novel AR-MR genes.

Rafiq *et al.*, (2010) began to evaluate the Mapping of 3 different new locations on chromosomes for non-syndromic autosomal recessive mental retardation (NS-ARMR) having high frequency of multigenerational cousin marriages in Pakistani families. They summarized that till now, 13 loci with linkage to non-syndromic autosomal recessive mental retardation (NS-ARMR), only six genes have been recognized with linked mutations. They presented their research on to non-syndromic autosomal recessive mental retardation (NS-ARMR) among the Pakistani population, where people are bound traditionally in cousin marriages or in the broader tribe. In a remarkable and extensive genetic survey fifty consanguineous families have been collected more than revealing clinical features/phenotypes of NS-ARMR. In the initial phase,

nine families (MR2-9 and MR11) with many persons who were suffering from mental retardation were nominated for molecular screening. Linkage was found in the already reported loci for NS-ARMR of two others families (MR3, MR4). Genotyping was performed in fifteen patients and 10 normal individuals from six (MR2, MR6, MR7, MR8, MR9 and MR11) families by using Affymetrix 5.0 or 6.0 single-nucleotide polymorphism (SNP) microarrays. SNP microarray data was visually examined by dChip and genome-wide homozygosity analysis was completed by Homozygosity Mapper. Further mapping was done (to eliminate false-positive regions of homozygosity called by Homozygosity Mapper and dChip) on all accessible patients and normal members in seven NS-ARMR families, using microsatellite markers. In this way three novel loci were enabled to map in seven different families originating from several regions of Pakistan. Linkage was found in two families having mutations in MR2, MR5 genes on chromosome 2p25.3-p25.2. Three families (MR7, MR8, and MR9) that have been collected from the similar community and belong to the similar tribe were mapped on chromosome 9q34.3. MR11 maps to a locus on 9p23-p13.3. Study of MR6 exhibited two positive loci, on chromosome 1q23.2-q23.3 and 8q24.21-q24.23. Genotyping in additional family members has been restricted till now, but not omitted the 1q locus. In short, through this study three new loci for NS-ARMR have been recognized, namely MRT14, 15 and 16.

Nolan *et al.*, (2008) revealed the adequate mapping of a chromosomal position for nonsyndromic mental retardation on chromosome 19p13. According to their research Mental retardation (MR) occurs in approximately 3% of the population and therefore considerably influences community health. In spite of this comparatively great incidence, the exact causes of MR stay unidentified in many cases, while together genetic and environmental aspects are recognized to contribute. Researchers described a family that suffered from with autosomal recessive (AR) nonsyndromic MR (NSMR) and cousin marriages were common. Since the consanguinity of this family is quit complicated, they discovered alternative approaches for creating precise estimations of the confirmation for linkage in this family, and determined indication for linkage to chromosome 19p13 (lod score ranging from 1.2 to 3.5, depending on suppositions of allele incidences). Crucial region of 3.6 Mb was mapped finely, which overlays

with a lastly described gene (CC2D1A) for MR. Nevertheless; no alterations in the coding region of this gene were found in the family. These outcomes recommended that another gene causing autosomal recessive nonsyndromic MR (ARNSMR) is positioned within this genomic region.

Fisher *et al.,* (1998) concluded that from 2 to 5% of children face critical problems in attaining normal communications skills and speaking. These children are the victims of learning disability having poor intelligence and social interactions. Although a wide research on twin specify an important part for genetic causes in progressive disorders of speaking and language, most of the families segregating such disorders indicates a multifaceted arrangement of heritage, and are thus not responsive for conventional linkage study. An infrequent exclusion is the KE family, a large three-generation pedigree in which almost half of the members were patients of a severe speech and language disorder which seems to be transmitted as an autosomal dominant monogenic trait. This family has been extensively exposed as suffering principally from a disease in the use of linguistic suffixation rules, thus apparently supporting the presence of genes specific to language rules. The phenotype, conversely, is wide-ranging in nature, with almost every single feature of grammar and of language affected. Moreover, affected members have a severe orofacial dyspraxia, and their dialogue is principally unconceivable to the common audience member. A genome-wide search was initiated for linkage in this family and a region on chromosome 7 was identified on chromosome 7 which co-segregates with the speech and language disorder (maximum lod score = 6.62 at theta = 0.0), confirming autosomal dominant inheritance with full penetrance. Additional study of microsatellites from within the region allowed us to fine map the responsible locus (labeled SPCH1) to a 5.6-cM interval in 7q31, thus providing an significant period towards its determination. Segregation of SPCH1 might propose the initial vision into the molecular genetics of the developmental process that culminates in speech and language. Mental retardation (MR) has numerous variety of inherited defects associated with genetic causes. Alterations in the structure and number of Chromosomes are one of the most common causes of MR. the causes of chromosomal abnormality due to consanguinity that have been reported in families are sparse. A research study was organized to detect chromosomal aberrations rate in idiopathic mental retardation from frequent cousin

marriages, overall 322 Iranian families with MR in their mutigenerational pedigrees were explored in the Genetics Research Center.

FarkhondehIn *et al.*, (2011) showed that no specific chromosomal aberrations which cause syndromic disorders and non disjunction like Down syndrome were found most of the families having MR. All the patients were screened by the specific cytogenetic techniques using high resolution GTG banding. The overall chromosome abnormality rate contributing to mental retardation was 1.24% (4 cases). They concluded that although autosomal recessive is the most probable genetic cause of intellectual disability in patients bound by cousin marriages, the evidence that 1.24% of MR patients had chromosomal abnormalities highlights the significance of cytogenetic exploration as the paramount laboratory genetic tests for all MR patients. Researchers claimed that according to their knowledge, this is the first report on the rate of chromosome abnormality among patients with idiopathic mental retardation from consanguineous marriages.

Mir *et al.*, 2009 mentioned in their research that Mental retardation/intellectual disability is an overwhelming neurodevelopmental illness with severe influence on affected individuals and their family members, as well as on their health and their social lives. It arises with an occurrence of ~2%, is an etiologically heterogeneous disorder, and is commonly the result of genetic variations. Although the most common type of mental retardation is Autosomal-recessive nonsyndromic MR (NS-ARMR), still there are just five genes that have been recognized. The candidate gene for NS-ARMR in a large Pakistani observed family was mapped on the basis of homozygosity. Using Affymetrix 5.0 single nucleotide polymorphism (SNP) microarrays, we identified a 3.2 Mb region on 8q24 with a continuous run of 606 homozygous SNPs shared among all affected members of the family. Additional genotype data from microsatellite markers verified this, allowing us to calculate a two-point LOD score of 5.18. Within this region, we identified a truncating homozygous mutation, R475X, in exon 7 of the gene TRAPPC9. A 4 base pairs deleted mutation in the exon 14 of TRAPPC9 was identified in a second large non-

syndromic mentally retarded family, formerly linked to 8q24 in a study of Iranian families, also segregating with the phenotype and truncating the protein. This gene encodes NIK- and IKK-b-binding protein (NIBP), which is involved in the NF-kB signaling pathway and directly interacts with IKK-b and MAP3K14. Brain (MRI) magnetic resonance imaging of the patients designates the existence of minor cerebral white matter hypoplasia. Some of the individuals were affected with microcephaly but others patients did not show any symptoms of microcephaly. Therefore, according to the knowledge of researchers, this was the sixth gene for NS-ARMR to be revealed.

Taking into consideration the aforementioned listed studies it is summarized that various researchers have come within reach of the views in several ways, replicating different concepts about the genetic analysis of mental retardation. The widespread research completed by various researchers all over the world has unlocked numerous of significant disciplines of investigations for upcoming studies. The researcher had nominated the variables for personal study after reviewing this enormous literature.

CHAPTER 3
MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

Clinical Examination:

A Pakistani family affected with an autosomal recessive trait was examined by KRL Hospital Islamabad, Pakistan. 10 living members of the family were found to be affected with Mental retardation. Detailed clinical examination was performed on the available family including a total of twenty one members out of which ten were affected, eleven were normal individuals and twenty were available for study. The affected individuals aged between 2-23 years have shown typical sign and symptoms of autosomal mental retardation including speech delay, abnormal gait and oral salivation. Diagnosis was performed on the basis of the following parameters.

Family history:

There was no patient of mental retardation or any other type of inherited disorders recorded in earlier generations.

Consanguinity:

Cousin marriages were common, starting form fore grandfather and fore grandmother up to grandsons and granddaughters.

Disease condition:

Total 10 persons were affected, severity of disease was variable from individual to individual.

APGAR score:

(1) There was normal cry of baby at birth
(2) Weight of the baby at birth was normal
(3) Normal urination and meconium

(4) Vaccination had not been done

(5) Baby colour was normal

(6) Most of the babies borne at hospitals

Milestones:

(1)Suckling ability of the babies was normal but due to low production of milk of mother most children were fed by feeder.

(2) Eyes were found to be budged out but movement in any direction was normal.

(3) Sitting was normal, crawling, standing and walking was abnormal in some patients during childhood.

(4) Patients spoke 1st word (naa, haaa) at the age of 10 years.

(5) Mental ability was found abnormal

(6) Limbs structure was normal but seems weak.

(7) No hypertelorism was detected

(8) Patients had less physical power and were normal statured.

(9) Palbarel fissury was detected in some patients.

(10) Gait was abnormal and understanding of language started at the age of 4 years.

(11) Speaking power improved with the age.

(12) Special common features were

Incisors extended out

Mild microcephaly

Dribbling of saliva starting from the age of crawling and decrease with the passage of time

Phonic was found negative i.e. vocal card was functional but tongue articulation was positive which cause speech delay

Eating and drinking ability was not normal.

Unaffected parents and sibling revealed no clinical signs and symptoms upon detailed examination to rule out presence of autosomal recessive mental retardation.

Pedigree Drawing:

Family history was taken during interviews with different family members and family tree or pedigree was drawn to clarify the genetic relationship. Pedigree drawing was performed using the Cyrillic (v2.10) program. This program also produce graphical output for illustrational purposes, and also generates both individual pedigree (mlink.pre) and relevant polymorphic marker (mlink.dat) data files suitable for input into linkage analysis software packages such as M-LINK.

Squares and circles in the pedigree represent male and female members of the family respectively. Normal individuals are symbolized with unfilled squares and circles while the affected ones with black-coloured filled square and circles. Crossed squares and circles represent the deceased individuals. Consanguineous marriages are shown with double lines between the partners.

Sample Collection:

Blood samples from patients and other clinically normal family members, including siblings and parents were collected with informed consent. About 5-10 ml of blood sample was collected in 10ml vacationer tubes (Becton Dickinson, Mountain View, CA.) containing acid citrate dextrose (ACD) or heparin from each indiviual.

Genomic DNA Extraction:

Genomic DNA was extracted from peripheral blood by the standard phenol–chloroform DNA extraction procedure (Sambrook *et al.*, 1989). This method is known as organic method of DNA extraction that involve digestion of cells with proteinase K in the presence of EDTA (ethylenediaminetetraacetic acid) and a detergent such as SDS (sodium dodecyl sulfate, Sodium lauryl sulphate) followed by extraction with phenol.

Equipment and Materials:

1) Refrigerated centrifuges
2) 15 and 50 ml Centrifuge tubes
3) Water baths

Chemicals Preparation:

A) 1M Tris, pH 8.0	**1L**
Trizma Base	121.1g

Tris was dissolved in approximately 800 ml $d.H_2O$ (deionized water), pH adjusted to 8.0 with concentrated HCl. Volume was q.s. (quantum sufficiat or satis) to 1L by adding $d.H_2O$ and filtered through 0.4µm filter paper. Stored at room temperature (0.1M and 10mM Tris were prepared from this stock).

B) Cell Lysis Buffer: I	**1L**
Ammonium Chloride	8.29g
Postassium Bicarbonate	1 g
0.5 M EDTA, pH 8.0	200µl

Ammonium Chloride and Potassium Bicarbonate were dissolved in dH_2O. 200µl of 0.5 M EDTA was added. pH was adjusted to 7.4 by adding 1M HCl or 1M NaOH and stored at room temperature.

C) Cell Lysis Buffer: II	**400ml**
Sucrose	43.8 g

1M Tris	4ml
5.0 mM $MgCl_2$	0.406 g
1% Triton X 100	4ml

Sucrose and $MgCl_2$ were dissolved in approximately 200 ml $d.H_2O$. 4ml each of 1M Tris and Triton X 100 were added and volume was q.s to 400ml with $d.H_2O$. Store at 4°C (Always use freshly prepared lysis buffer).

D) 0.5 M EDTA, pH 8.0	**1L**
EDTA	186.15g

EDTA was dissolved in ~700ml $d.H_2O$. pH was adjusted to 8.0 with 4N NaOH and Volume raised to 1L with $d.H_2O$

E) STE (Saline Tris EDTA) pH 8.0	**1L**	**Final Conc.**
3M NaCl	33.3ml	100mM
1M Tris, pH 8.0	50ml	50mM
0.5 M EDTA, pH 8.0	2.0ml	1mM

Measured reagents were mixed in $d.H_2O$ and volume raised to 1L with $d.H_2O$. Stored at room Temperature

F) 10% SDS (Laryl Sulphate)	**100ml**
SDS	10g

Dissolved in $d.H_2O$, then filtered and stored at room temperature.

G) Proteinase K (20mg/ml)	**1ml**
Proteinase K	20mg

Dissolved in 1ml of autoclaved dH_2O and stored at -20°C

H) RNase (10mg/ml)	**1ml**
RNase A	10mg
10mM Tris, pH 7.5	1ml

Dissolved in 1ml of 10mM Tris and stored at -20°C

I) Phenol Equilibration

Phenol	1KG
1.0M Tris, pH 8.0	2L
0.1M Tris, pH 8.0	2L
8-hydroxy quinoline	1g
ß-mercaptoethanol	2ml

8-hydroxy quinoline was added to melted distilled phenol. Phenol extraction was done with equal volume of 1.0M Tris, pH 8.0, and then with an equal volume of 0.1M Tris, pH 8.0 until pH of aqueous layer became 8.0. Finally 100ml of 0.1M Tris pH 8.0 containing 0.2% ß-mercaptoethanol was added to equilibrated phenol.

J) Chloroform: Isoamyl Alcohol (24:1)	**500ml**
Chloroform	480ml
Isoamyl alcohol	20ml

Two solutions were mixed in glass cylinder and stored at 4°C.

K) Isopropanol (extra pure)

Stored at 4°C

L) TE (Tris-EDTA)	**500ml**	**Final Conc.**
1M Tris	5m	10mM
0.5 M EDTA, pH 8.0	1ml	1mM

Mixed and vol. was raised to 500ml with dH_2O. Stored at room temperature.

M) 10 M Ammonium Acetate	**500ml**
Ammonium acetate	385.4g

Weighed ammonium acetate mixed in $d.H_2O$ (less than 100ml) on magnetic stirrer. Volume was q.s. to 500ml and stored the salt solution at room temperature.

Procedure for DNA Extraction:

Day 1

- 5 ml of each blood samples was transferred into labelled 50 ml Falcon tubes (Becton Dickinson).
- To the each blood sample three times (the volume of blood), cell lysis buffer was added. Shaken gently and incubate for 30 min (minutes) on ice.
- Centrifuged at 1200 rpm (revolution per minute) for 10 minutes at 4o C in refrigerated centrifuge (IEC Centra-8R centrifuge, USA).
- Supernatant was discarded (blood waste) and pellet was re-suspended. (If the pellet is still reddish, centrifugation step can be repeated with 10ml of lysis buffer at 1200 rpm for 10min at 4o C,).
- 4.75 ml of STE was added to the re-suspended pellet.
- 250µl of 10% SDS was added drop wise while vortexing the tube.
- 10µl of proteinase K (20mg/ml) was added and samples tubes were placed in shaking water bath at 55°C for overnight (LAB LINE, Orbit Shaker Bath, USA).

Day 2

- Equal volume (5ml) of equilibrated phenol, pH 8.0, was added to each sample and mixed for 10 minutes and then kept on ice for 10 minutes.
- Samples were centrifuged at 3200 rpm for 30min at 4°C.
- Aqueous layer was removed with cut tip into separate 15ml labelled Falcon tube (Becton Dickinson). 15ml
- 5 ml of chilled chloroform-isoamylalcohol (24:1) was added to each sample and mixed for 10 min and then kept on ice for 10 min.
- Centrifuged at 3200 rpm for 30 min at 4°C.
- Aqueous layer was removed with cut tip into separate 15ml labelled Falcon tube.
- To this aqueous layer 10µl of RNase (10mg/ml) was added and incubated in shaking water bath at 37°C for 2 hours to degrade RNA in the sample.
- 250µl of 10% SDS was added drop wise without vortex.
- 10µl of proteinase-K (20mg/ml) was added and tubes were incubated in shaking water bath at 55°C for 1 hour.
- **2nd extraction**: Equal volume (5ml) of equilibrated phenol, pH 8.0, was added to each sample and mixed for 10 minutes and then kept on ice for 10 minutes.

- Samples were centrifuged at 3200 rpm for 30min at 4°C.
- Aqueous layer was removed with cut tip into separate labelled Falcon tube (15ml).
- 5 ml of chilled chloroform-isoamylalcohol (24:1) was added to each sample and mixed for 10 min and then kept on ice for 10 min.
- Centrifuged at 3200 rpm for 30 min at 4°C.
- Aqueous layer was removed with cut tip into separate 15ml labelled Falcon tube.
- After extraction 500µl ($1/10^{th}$ vol. of aqueous solution) of 10M ammonium acetate and 5 ml of chilled isopropanol (or 10 ml of chilled absolute ethanol) was added and samples were shaken until DNA precipitates become visible as white threads.
- Samples were placed overnight at -20°C (or for 15 min at -70°C).

Day 3

- Samples were centrifuged for 60min at 4°C.
- Supernatant was discarded and pellet was re-suspended.
- Washing of each sample was carried out with 5ml of chilled 70% ethanol at 3200 rpm for 40 min at 4°C.
- Supernatant was discarded and pellets were air dried (we can vacuum dry also).
- Dried pellet was re-suspended in 10mM Tris-HCl (pH 8.0, volume of Tris was added according to the size of pellet).
- Optical densities (O.D) of the samples were taken at 260 nm and 280 nm by using spectrophotometer. (U-3210 spectrophotometer, Hitachi).

(Ideally 260/280 ratio=1.7-2.0; Ratio>2.0 phenol contamination; Ratio<1.7s protein contamination).

DNA concentration was calculated by the following formula;

Abs 260nm X 50 =DNA concentration (µg/ml)

(Where 50 is correction factor)

Samples were transferred into the labelled Eppendorf tubes and stored at 4°C.

Microsatellite and Linkage Analysis:

To identify the disease locus, genomic DNA from each individual was genotyped using microsatellite markers for homozygosity analysis. Polymorphic microsatellite markers (Human Mapping Set, ver. 8: Research Genetics, Inchinnan, Scotland, UK) were amplified using Polymerase chain reaction (PCR). Each reaction was carried out in 10 µl volume containing 1.5mM $MgCl_2$, 0.6µM of each primer, 0.2mM dNTPs, 1U *Taq* DNA polymerase and PCR buffer {16mM $(NH_4)_2SO_4$, 67mMTris-HCl (pH 8.8), and 0.01% of the non-ionic detergent Tween-20} (Bio-line, London, UK). Amplification was performed under the following conditions;

PCR Conditions

1 cycle of	95°C x 4 minutes
35 cycles of	95°C x 45 seconds
	55°C x 45 seconds
	72°C x 45 seconds
1 cycle of	72°C x 10 minutes

The PCR products were separated on 8% non-denaturing polyacrylamide gels (Protogel; National Diagnostics, Edinburgh, Scotland, UK).

Non-denaturing Polyacrylamide Gel Electrophoresis:

This technique was employed to distinguish the different alleles of the polymorphic microsatellite markers used in linkage / homozygosity analysis. The standard apparatus used (Bio-Rad) measured 49cm by 68cm and utilised 1.5 mm thick spacers.

Equipment and Materials:

1) Gel Casting System (Biorad SequiGen system)
2) Power supply

3) Gel documentation system

Chemicals Preparation

A) 10X TBE	**1L**	**Final Conc.**
Trizma base	108 g	0.89M
Boric acid	55g	0.88M
EDTA	9.04 g	0.02M

pH 8-8.2

Filtered through Millipore filter paper (0.45 μm).

B) 40% Acrylamide solution	**1L**
Acrylamide	389.6g/L
N,N'-methylene bis-acrylamide	10.4g/L

Stored in dark bottles at 4°C

C) Gel loading dye

7.50% Ficoll

0.01% bromophenol blue

0.01% xylene cyanol

D) 25% APS (Ammonium persulphate, freshly prepared)

E) Siliconization solution (Sigmacote, Sigma)

F) 70% ethanol

G) TEMED

H) 10% ethidium bromide

Polyacrylamide Gel Electrophoresis:

- The gel plates were first washed and ethanol wiped. They were then assembled with according to the manufacturer's instructions with 0.75mm spacer between plates and placed in a gel casting tray. The back plate (Integral plate chamber, IPC) was siliconized prior to assembly with Sigmacote (Sigma) according to the manufacturer's instructions and outer plate was wiped with 70% ethanol.
- The concentration of acrylamide required for maximum resolution depends upon the size of the DNA fragments being resolved. In microsatellite analysis, the PCR products are usually in the range of 80 to 400bp and thus 8% gels were routinely used.
- 8% polyacrylamide gel solution was prepared by adding 50 ml of 40% acrylamide solution and 25 ml of 10X TBE. Volume was q. s. to 250 ml with d.H_2O.
- 50 ml from the 250 ml of 8% acrylamide solution was taken in a cylinder and 300 µl of 25% APS and 300 µl of TEMED each were added. Solution was poured into the base of Biorad SequiGen system and allowed the gel to polymerize for 2-3 minutes.
- To the remaining 200 ml of 8% acrylamide solution, 850 µl 25%APS solution and 150 µl of TEMED was added and poured between assembled gel plates. 0.75mm 68 well comb (square) was inserted and plates were clamped. The plates were then laid almost horizontally and allowed polymerization for at least ~ 2 hours.
- After polymerization gel plates were removed from the casting tray and placed in a buffer tank. After filling the tank and reservoir with 1X TBE (2L) the gels were pre-run for 10-15 minutes at 100 watts constant power.
- The comb was removed carefully to avoid breakage of wells and wells rinsed out with 1X TBE buffer prior to loading the samples.
- Amplified products were suspended in 5 µl of 6X gel loading dye. Loading dye containing 0.1% bromophenol blue and 0.1% xylene cyanol. On an 8% acrylamide gel, bromophenol blue and xylene cyanol co-migrate with DNA fragments of around 45bp and 160bp respectively. These dyes were used to assess the location of DNA migrating within the gel in order to determine the ideal time to stop the electrophoresis.

- 10 µl of each suspended sample was loaded using a pipetteman with an elongated tip. DNA molecular weight marker VIII was also loaded in the first lane of the gel.
- Gel was run at 100 watts for 4-5 hours. (Depending up on the size of PCR products).
- Following electrophoresis the plates were separated and the gel divided into sections. Each section was stained in 0.5µg/ml ethidium bromide for five minutes before being photographed under UV illumination.

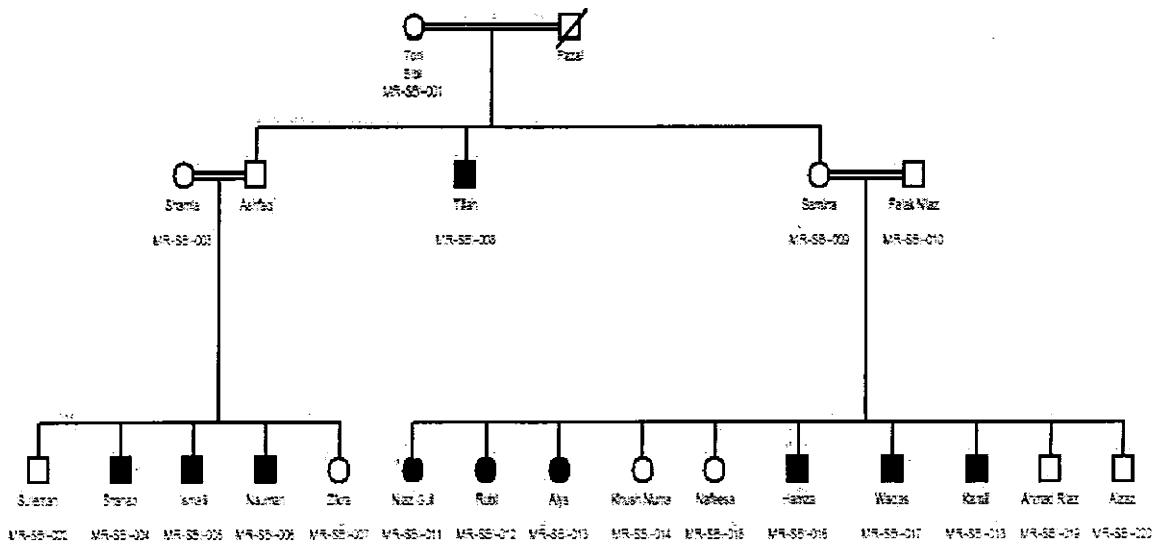


Figure 2.1. Pedigree of Pakistani family suffering from Mental Retardation.

Key:

- Normal male
- Normal female
- Affected male
- Affected
- Deceased, normal male
- Deceased, affected male
- Deceased, normal female
- Deceased, affected female
- Proband

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

Detailed clinical examination by physicians confirmed Pakistani family was suffering from rare autosomal recessive mental retardation (MR) and speech disorder. After samples collection and DNA preparation through organic method, as described in materials and methods, quality of all the DNA samples was checked by taking their optical densities (ODs) (that were found to be around 1.8) and by PCR amplification and subsequent running polyacrylamide gel electrophoresis.

Linkage Analysis

After DNA extraction, linkage analysis studies were carried out. Different sets of microsatellite markers were used for linkage analysis. The aim was to identify shared homozygous microsatellite DNA markers in the affected individuals.

The family under examination consisted of ten affected individuals from two different extended branches and who suffer from speech impairment with mental retardation. The clinical information of the affected individuals (on the basis of questionnaire) is presented in Table 3.1.

Different reported loci for autosomal recessive mental retardation were screened using STRs markers. Those loci were selected for linkage analysis on the basis of closely related clinical symptoms of the patients. To check for each locus these clinical features were compared with the work of researchers on each locus. PAGE was preformed for each available markers of the respective locus. Different STRs marker i.e. D19S586, D19S1034, D3S2387, D3S1304, GATA164B08, D11S2362, D11S1997, D11S1981, D14S742, D14S1280, D14S608, D14599, D8S1130, D8S1106, D8S1145 , D8S136, D22S685, D22S283, D22S423, D22S274, D3S1766, D3S4542, D3S1285, D3S2406 , D7S1799, D7S3061,D7S1804, D7S2487 and D7S530 were run for finding the defected gene in loci 8p22, 3p26.2, 11p15, 14q11.2-q12, 19p13.12, 7q22, 3p21.3 and 22p13. Some of the markers

showed high polymorphism which gave the information about the variation among the individuals. Some were not amplified and none of the markers was linked to any locus.

TABLE 4.1 Clinical details of the mentally retarded Pakistani family

Clinical Findings	MR-SBI-008	MR-SBI-004	MR-SBI-005	MR-SBI-006	MR-SBI-011	MR-SBI-012	MR-SBI-013	MR-SBI-016	MR-SBI-017	MR-SBI-018
Sex	male	male	male	male	female	female	female	male	male	male
Age on assessment	22 years	7 years	8 years	5 years	13 years	17 years	4 years	9 years	19 years	3 years
Developmental delay	+	+	+	+	+	+	+	+	+	+
Dysmorphic feature	+	–	+	–	–	–	+	+	+	–
Skeletal Problem	–	–	–	–	–	–	–	–	–	–
Epilepsy	–	–	–	–	–	–	–	–	–	–
Speech disorders	+	+	+	+	+	+	+	+	+	+
Mental retardation	severe	severe	severe	severe	severe	severe	severe	severe	severe	severe
Growth	normal	weak	weak	normal	weak	weak	weak	weak	weak	normal
Schooling	–	–	–	–	–	–	–	–	–	–
Learning Disability	–	–	–	–	–	–	–	–	–	–
Self biting	–	–	–	–	–	–	–	–	–	–

Each column indicates the data of an individual and row presents the category of clinical symptoms, (- indicates absence, + indicates presence).

TABLE 4.2 list of different searched loci for autosomal recessive mental retardation

S.No	Chromosome locus	Gene	Markers	Available Markers
1	8p22	TUSC3 ,MRT7	SNP1, rs113990-rs1537587	**D8S1130,D8S1106,D8S1145,D8S136,**
2	3p26.2	MRT2	D3S3525 and D3S1560 , D3S630 and D3S1304	**D3S2387, D3S1304, GATA164B08**
3	11p15	MRT17	SNPs rs10769544 and rs11040272	**D11S2362**, D11S1997, **D11S1981**
4	14q11.2-q12	MRT26 MRT9	SNPs rs1998463 and rs243286	**D14S742, D14S1280,D14S608,D14599**
5	19p13.12	MRT3	D19S840, D19S547 and D19S1165, D19S564 and D19S547,	**D19S586, D19S1034**
6	7q22	AUTS3	D7S1799, D7S3061, D7S1804, D7S2487, D7S530	**D7S1799, D7S3061, D7S1804,** D7S2487, D7S530
7	3p21.3	AMT	D3S1766, D3S4542, D3S1285, D3S2406	**D3S1766,** D3S2406, **D3S4542**
8	22p13	PRODH	D22S685, D22S283, D22S423, D22S274	D22S685, D22S283, D22S423, D22S274

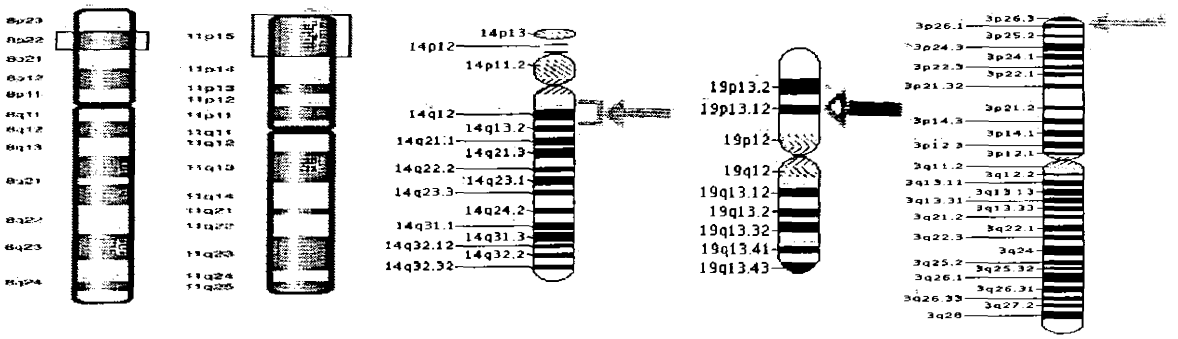


Figure 3.1. Images of different loci for autosomal recessive mental retardation.

TABLE 4.3 list of markers used for linkage analysis in current research

Linkage analysis of MR-SWBi-1						
S.No	**Marker**	**Probe**	**Position**	**Range**	**Date(PCR)**	**Remarks**
1	D7S1799	GATA23F05	115.6	175-187	28-5-12	Not amplified
2	D7S3061	GGAA6D03	132.2	114-154	28-5-12	Not linked (Hints)
3	D7S1804	GATA43C11	139.0	238-302	28-5-12	Not amplified
4	D7S2487	AFMb294yd1	128.5	237-249	4-6-12	Not linked (Hints)
5	D7S530	AFM249xf9	135.2	106-118	4-6-12	Not linked (Hints)
6	**D3S1766**	GATA6F06	66.9	208-236	5-6-12	Not linked (Hints)
7	**D3S4542**	GATA148E04	80.4	236-260	5-6-12	Not amplified
8	**D3S1285**	AFM191yg5	81.1	232-242	5-6-12	Not linked
9	D3S2406	GGAT2G03	91.2	306-350	5-6-12	Not linked
10	D22S685	GATA6F05	42.2	172-208	09-07-12	Not linked
11	D22S283	AFM262vh5	43.6	128-152	09-07-12	Not linked
12	D22S423	AFM261xd9	44.2	215-235	09-07-12	Not linked
13	D22S274	AFM164th8	45.1	202-214	09-07-12	Not linked
14	**D8S1130**	GATA25C10	9.9	132-156	09-07-12	Not linked informative
15	**D8S1106**	GATA23D06	12.2	127-159	09-07-12	Not linked informative
16	**D8S1145**	GATA72C10	21.8	257-293	09-07-12	Not linked informative
17	**D8S136**	cos140D4	25.5	74- 80	09-07-12	Not linked informative
18	**D3S2387**	GATA22G12	0.3	167-215	12-07-12	Not linked informative
19	**D3S1304**	AFM234tf4	2.4	253-269	12-07-12	Not linked
20	**D11S2362**	ATA33B03	2.5	209-230	16-07-12	Not linked
21	[redacted]	ATA19D12	12.6	124-145	18-07-12	Not linked

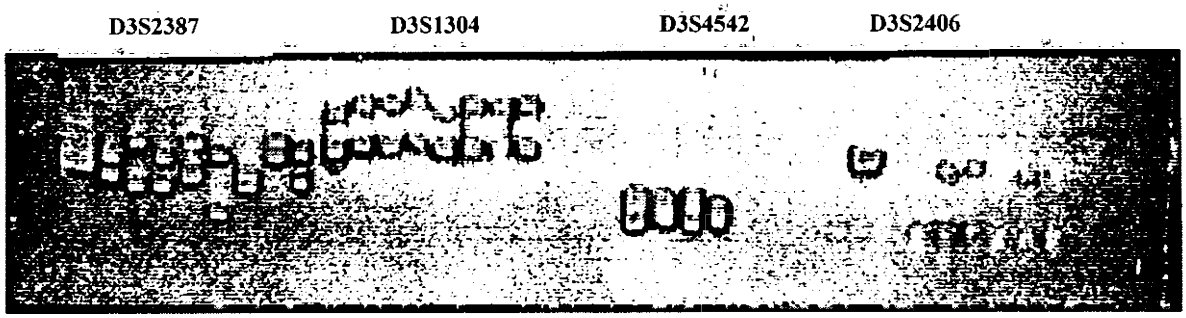


Figure 3.2. Polyacrylamide Gel Electrophoresis of 3p26.2 and 3p21.3 for detecting linkage in MRT2 and AMT.

A multigenerational consanguineous Pakistani family in which mental retardation is segregating as an autosomal recessive trait. The affected individuals are homozygous for the mutant allele while the parents and their normal sibs are heterozygous (carriers) having a normal allele. The marker **D3S2387** was **highly** polymorphic and was informative to some extent but was not linked. The remaining markers such as **D3S1304, D3S4542, D3S2406** showed no linkage at all.

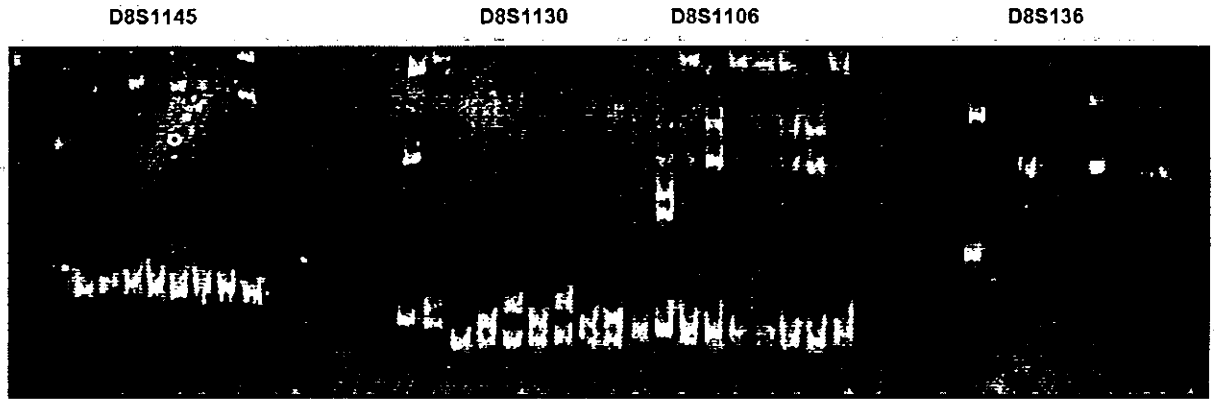


Figure 3.3.Polyacrylamide Gel Electrophoresis of 8p22 for detecting linkage in TUSC3 gene.

The marker D8S1145 results was satisfactory in seven patients and two normal persons because of same bands results, the bands of the remaining patients and normal were not matched so linkage was not found. At the same locus three other markers D8S1130, D1106 and D8S136 were run for the confirmation of linkage. No linkage was detected.

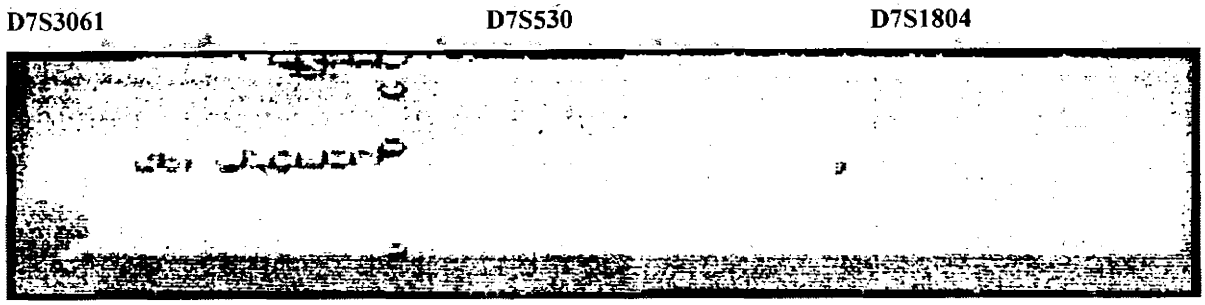


Figure 3.4.Polyacrylamide Gel Electrophoresis of 7q22 for detecting linkage in AUTS3.

Three markers **DS73061, D7S530** and **D7S1804** showed no polymorphism in any patient and hence linkage was not determined.

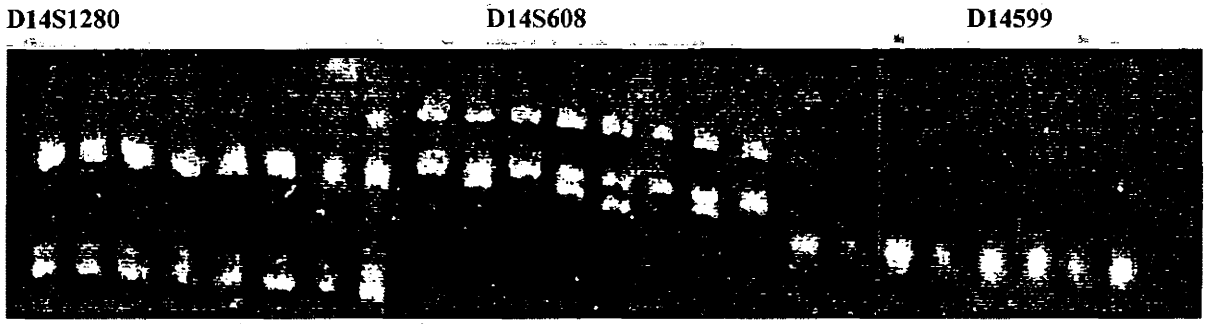


Figure 3.5.Polyacrylamide Gel Electrophoresis of 14q11.2-q12 for detecting linkage in MRT26 and MRT9 gene.

Each of the eight samples was by three markers D14S1280, D14S608 and D14599 and no marker was linked.

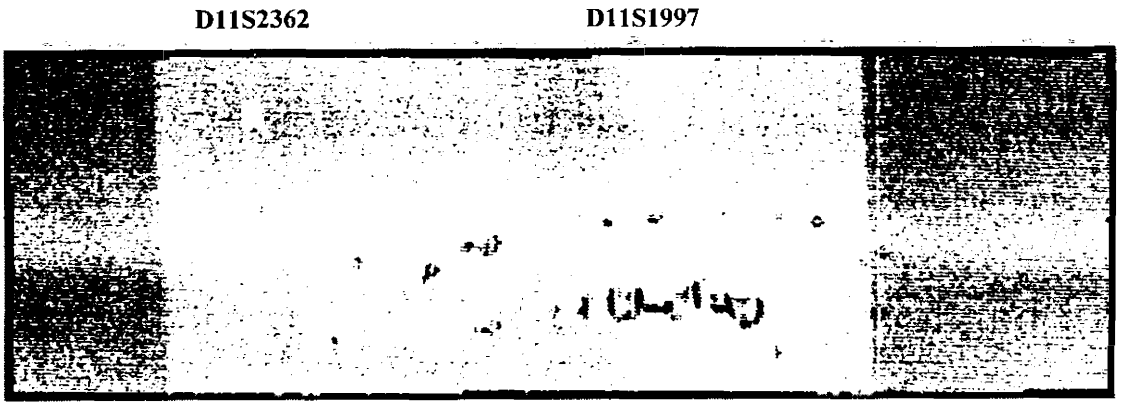


Figure 3.6.Polyacrylamide Gel Electrophoresis of 11p15 for detecting linkage in MRT17 Gene.

The picture of bands of D11S2362 and D11S1997 markers indicating no linkage in seven samples.

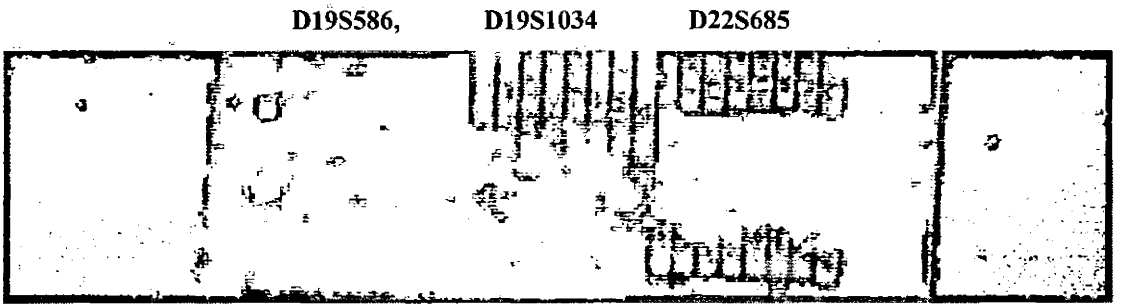


Figure 3.7.Polyacrylamide Gel Electrophoresis of 19p13.12 and 22p13 for detecting linkage in MRT3 **and PRODH Gene.**

Three markers D19S586, D19S1034 and DS22685 were run to identify the linkage in defected genes of MRT3 and PRODH Gene. The results showed no linkage in for any marker.

CHAPTER 5
DISCUSSION

CHAPTER 5

DISCUSSION

The involvement of mutated genes has become a serious issue in advanced countries and health has been more affected. These causative genes are the have turned out to be more noticeable as nonhereditary causes of early death as compared to infectious disease or nutritional deficiency. Online Mendelian Inheritance in Man (OMIM), an influential database of genetic human conditions, determines more than 4000 human inherited defects and the number is growing rapidly as researchers study more about our DNA. Most of these disorders are severe and even life threatening which have made them one of the big challenges for scientists and doctors all around the world. There is no proper treatment available to completely cure the inherited disorders. Therefore, to find out the disease causing genetic factors and procedures to cure them in future, several hundred research projects based on disease genetics are underway in different universities and research Institutes of the world.

Like rest of the world research on genetics has a great potential in Pakistan. In Pakistan the practice of consanguineous marriages has occurred for centuries which resulted in various inherited disorders segregating in large multigenerational families. Such families demand attention of molecular biologist to find out the molecular basis and ways to treat these inherited disorders. Therefore, in the view of facts we collected a consanguineous Pakistani family, in which mental retardation and speech disorder is segregating as an autosomal recessive inherited disorder. Mental retardation (MR) is a devastating neurodevelopmental defect that has a bad effect on the health and social life of the patient as well as healthy individuals of his family. The overall prevalence of metal retardation is ~1%–3% within the population, (Roeleveld *et al.,* 1997 and Leonard *et al.,* 2002) and is mainly caused due to genetic. Most of the persons from the family under current project also suffered from severe mental retardation due to high frequency of cousin marriages in many generations. MR may involve a single clinical symptom referred as non-syndromic mental retardation, or patients may suffer from multiple clinical or dysmorphological features known as syndromic-mental retardation. Normally, the genetic cause of mental retardation is

found out easily because the collection of many genes enable assist a medical geneticist to confine the doubted cause to a mutation in a short list of genes, or perhaps in a single gene. Nevertheless, for persons with NS-MR, no secondary indications support the molecular diagnosis. MR is more prevalent in males as compared to females, and~25% of severe cases had been supposed due to defect in X chromosome; however, according to a latest review X-linked variations are responsible to cause MR in less than 10% of cases, (Ropers and Hamel 2005) and thus an increase in the frequency of autosomal aberrations is predictable for both dominant and recessive mental retardation. Conversely, because of the great amount of genetic heterogeneity, mutations in merely seven genes have been described to cause NS-ARMR, and all of these have been recognized on the basis of mapping regions of autozygosity or homozygosity-by-descent (HBD) in multiplex consanguineous families (Kaufman et al., 2010). Same is the case with the patients of current study in which male were more affected than female however their parents were not patients and did not show any phenotypic symptoms of mental retardation. This proved that the disease was autosomal recessive. The patients suffer from multiple symptoms such as tongue inarticaulation, abnormal gait, muscular weakness and mild microcephaly. Thus we can conclude that the disease was syndromic and multigenic.

PCR-based linkage analysis was carried out to map the reported loci for autosomal mental retardation and to find out any potential pathogenic mutation in Pakistani MR Family. This task was quit cumbersome because many loci had been reported for autosomal recessive mental retardation. As more than 2000 genes might contribute to AR-MR and the mutation frequency of each single gene is presumably below 0.1%, each family in this study will most probably have a unique genetic defect, giving rise to the MR. Therefore, the overlap of several families with part of the MRT7, MRT8 and MRT10 loci, is unlikely to contain the causative genetic defect in all families (Lencz *et al.*, 2007).

A consanguineous Iranian family (M100) was reported by Najmabadi *et al.*, (2007) having 4 individuals of NSID. MRT7 gene having lod score of 3.3 was analysed a candidate locus on chromosome 8p by Linkage analysis. Haplotype analysis defined a 6.5-Mb candidate region between SNPs rs1113990 and rs1534587. Higgins et al. (2000) recognized

a candidate disease locus, named MRT2A, on chromosome 3p25-pter. Multipoint linkage study pointed out the critical region to a 6.71-cM interval edged by the markers D3S3525 and D3S1560. Applying the technique of autozygosity mapping and microarray SNP technology in a Pakistani family suffering from autosomal recessive NSID, Rehman *et al.*, (2011) found a mutation in homozygous 6-Mb telomeric region on chromosome 11p15 between SNPsrs10769544 and rs11040272. The results of Linkage analysis displayed a maximum lod score of 3.31 was found. The locus was named as MRT17. *Amra et al.*, (2011) analysed a Syrian family with frequent cousin marriages leading to nonsyndromic mental retardation. Clinical Indications comprised of serious motor delay, loss of ambulation, shrinking in size of a cell, tissue, organ, or part of the body (atrophy) and spasticity of the lower limbs, severe intellectual disability with no communication, normal or mild microcephaly, and growth retardation. By homozygosity mapping of a consanguineous Syrian family with mental retardation, Jamra et al. (2011) found linkage to a 9.1-Mb region on proximal chromosome 14q between SNPs rs10132585 and rs1278951 (lod score of 3.85). Jamra *et al.*, (2011) referred to this locus as 'MRT26. Basel-Vanagaite *et al.*, (2003) studied in 4 consanguineous families of Israeli-Arab origin with 10 patients and 24 healthy' members. All families belonged from the similar small village and had the same family designation. 5 further families with nonsyndromic mental retardation from the same village were reported by Basel-Vanagaite *et al.*, (2006) comprising of overall 16 patients with the same family name. The early medical feature in all nonsyndromic mental retardation patients was psychomotor developmental delay in early childhood. All the affected individuals had no ability to speak only single word and were thoroughly mentally retarded; autistic features or seizures were not detected, and there were no dysmorphic symptoms. four consanguineous Israeli-Arab families firstly reported by Basel-Vanagaite *et al.*, (2003) and 5 further families with nonsyndromic mental retardation from the same village and with the same family name were considered under molecular investigation, Basel-Vanagaite *et al.*, (2006) identified a mutual homozygous disease-bearing haplotype for the polymorphic markers RFX1 and D19S840 that defined a critical 0.9-Mb region between D19S564 and D19S547 on chromosome 19p13.12. Ritvo *et al.*, (1988) described the corresponding incidence of autism and retinoblastoma in a patient with a deletion that extended from 13q12 to 13q14. Steele *et al.*, (2001) described a case of

autism with a de novo deletion of 13q14-q22. Bradford *et al,*. (2001) analysed the effect of combined language information and parental structural language phenotypes into the genome screening for autism. Their results exposed 2 separate peaks of linkage on 13q.

The same loci were screened in the present study but there was no defect found in the corresponding genes of consanguineous Pakistani family. The results were probably controversial due to the presence of multiple clinical features in the family of mental retardation under current analysis. The manifestations were found to be in different combinations unlike the other project studies. Some of the symptoms were common for the the patients of this family as compared to others but still the mutation in any gene was not found. The results of the current study showed deviation from the results of the previous works due variation in biochemical techniques as well such as SNPs and microarray was not accessible in the present research. Up to our knowledge no any study has been conducted which has cover all the symptoms as found in the disease so far.

CONCLUSION AND FUTURE WORK

Most of the closely related reported loci for autosomal recessive mental retardation were searched during the present study and no linkage was found. Up to our knowledge there may be a novel mutation involved to cause the disease. Hence whole genome scan should be performed to point out the defected gene. Due to limited period of the current research project and non availability of techniques like microarray we were not able to perform the whole genome search. The most likely genetic cause of mental retardation in patients with consanguineous parents is autosomal recessive conditions. Recent advancements in science and technology can uncover numbers of hidden facts and provide valuable information regarding genetic mutations involved in causing mental retardation, protein functions and their versatile role in the body. The identification of the novel homozygous changes in Pakistani family with mental retardation may help us in further understanding the multi-functional role of protein in human brain development and establishing genotype– phenotype correlation. For MR patients, the genetic findings permit

more precise diagnosis, enhanced genetic analysis and carrier screening, the practicability of DNA based prenatal identification and the opportunity to develop suitable animal models to test new forms of cell, protein or gene therapies. Further characterization of the cytology, consequently, is assessed to provide attractive new insight into its part in health and disease.

CHAPTER 6

REFERENCES

CHAPTER 6
REFERENCES

Abeysinghe S.S., Stenson P.D., Krawczak M And Cooper D.N., (2004) Gross Rearrangement Breakpoint Database (GRaBD), Hum Mutat, 23(3), p. 219-221

Al-Ansari A., (1993) Etiology of mild mental retardation among Bahraini children: a community-based case control study, Ment Retard, 31, p. 140–143

Altschul S.F., Gish W., Miller W., Myers E.W And Lipman D.J., (1990) Basic local alignment search tool, J Mol Biol, 215, p. 403-410

American Psychiatric Association., (2000) Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association Washington, 4Bartley J.A And Hall B.D., (1978) Mental retardation and multiple congenital anomalies of unknown etiology: frequency of occurrence in similar affected sibs of the proband, Birth Defects Orig Artic Ser, 14, p. 127-137

ATP-dependent Lon protease gene in kindred with mild mental Retardation, Neurology, 63, p. 1927-1931

Bache I., Hjorth M., Bugge M., Holstebroe S., Hilden J., Schmidt L., Brondum- Nielsen K., Bruun-Petersen G., Jensen PK And Lundsteen C et al., (2006) Systematic reexamination of carriers of balanced reciprocal translocations: a strategy to search for candidate regions for common and complex diseases, Eur J Hum Genet, 14(4), p. 410-417

Basel-Vanagaite L., Alkelai A., Straussberg R., Magal N., Inbar D., Mahajna M And Shohat M., (2003) Mapping of a new locus for autosomal recessive non-syndromic mental retardation in the chromosomal region 19p13.12-p13.2: further genetic heterogeneity, J Med Genet, 40, p. 729-732

Basel-Vanagaite L., Attia R And Yahav M et al., (2006) The CC2D1A, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation, J Med Genet, 43, p. 203–210

Basel-Vanagaite L., Attia R., Yahav M., Ferland R.J., Anteki L., Walsh C.A., Olender T., Straussberg R., Magal N., Taub E., Drasinover V., Alkelai A., Bercovich D., Rechavi G., Simon AJ And Shohat M., (2006) The CC2D1A, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation, J Med Genet, 43, p. 203-210

Basel-Vanagaite L., Attia R., Yahav M., Ferland R.J., Anteki L., Walsh C.A., Olender T., Straussberg R., Magal N., Taub E., Drasinover V., Alkelai A., Bercovich D., Rechavi G., Simon A.J And Shohat M., (2006) The CC2D1A, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation, J Med Genet, 43, p. 203-210

Bebbington A., Anderson A And Ravine D et al., (2008) Investigating genotypephenotype relationships in Rett Syndrome using an international data set, Neurology, 70, p. 868–875

Behjati F., Firouzabadi S.G., Kahrizi K., Kariminejad R., Bagherizadeh I., Ansari J., Fallah M., Mojtahedi F., Darvish H., Monajemi G.B., Abedini S.S., Jamali P., Mojahedi F., Zadeh-Vakili A And Najmabadi H., (2011) Chromosome abnormality rate among Iranian patients with idiopathic mental retardation from consanguineous marriages, Arch Med Sci, 7, p. 321-325

Bradford Y., Haines J., Hutcheson H., Gardiner M., Braun T., Sheffield V., Cassavant T., Huang W., Wang K., Vieland V., Folstein S., Santangelo S And Piven J., (2001) Incorporating language phenotypes strengthens evidence of linkage to autism, Am J Med Genet, 105, p. 539-547

Brendel C., Klahold E., Gartner J And Huppke P., (2009) Suppression of nonsense mutations in Rett Syndrome by aminoglycoside antibiotics, Pediatr Res, 65, p. 520–603

Broman K.W., Murray J.C., Sheffield V.C., White R.L And Weber J.L., (1998) Comprehensive human genetic map: individual and sex-specific variation in recombination, Am J Hum Genet, 63, p. 861-869

Chelly J., Khelfaoui M And Francis F et al., (2006) Genetics and pathophysiology of mental retardation, Eur J Hum Genet, 14, p. 701–713

Chelly J., Khelfaoui M., Francis F., Cherif B And Bienvenu T., (2006) Genetics and pathophysiology of mental retardation, Eur J Hum Genet, 14, p. 701–713

Collins F.S., (1995) Positional cloning moves from perditional to traditional, Nature Genet, 9, p. 347-350

Couvert P., Bienvenu T And Aquaviva C et al., (2001) MECP2 is highly mutated in X-linked mental retardation, Hum Mol Genet, 10, p. 941–1006

Davies K.E And Read A.P., (1992) Molecular basis of inherited diseases, 2nd edition, IRL Press, Oxford.

Dotti M.T., Orrico A., De Stefano N., Battisti C., Sicurelli F And Severi S et al., (2002) A Rett Syndrome MECP2 mutation that causes mental retardation in men, Neurology, 58, p. 226–230

Drews C.D., Yeargin-Allsopp M., Decoufle P And Murphy C.C., (1995) Variation in the influence of selected sociodemographic risk factors for mental retardation, Am J Public Health, 85, p. 329–334

Fisher S.E., Vargha-Khadem F., Watkins K.E., Monaco A.P And Pembrey., (1998) MELocalisation of a gene implicated in a severe speech and language disorder, 18, p. 168-170

Garshasbi M., Hadavi V., Habibi H., Kahrizi K., Kariminejad R And Behjati F et al., (2008) A defect in the TUSC3 gene is associated with autosomal recessive mental retardation, Am J Hum Genet, 82, p. 1158–1164

Garshasbi M., Hadavi V., Habibi H., Kahrizi K., Kariminejad R., Behjati F., Tzschach A., Najmabdi H., Ropers H.H And Kuss A.W., (2008) A defect in the TUSC3 gene is associated with autosomal recessive mental retardation, Am J Hum Genet, 82, p. 1158-1164

Giacometti E., Luikenhuis S., Beard C And Jaenisch R., (2007) Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2, Proc Natl Acad Sci USA, 104, p. 1931–2006

Gibbons R.J., Pellagatti A., Garrick D., Wood W.G., Malik N And Ayyub H et al., (2003) Identification of acquired somatic mutations in the gene encoding chromatin-remodeling factor ATRX in the Alpha- Thalassemia Myelodysplasia Syndrome (ATMDS), Nat Genet, 34, p. 446–509

Glancy M., Barnicoat A., Vijeratnam R., de Souza S., Gilmore J., Huang S., Maloney V.K., Thomas N.S., Bunyan D.J., Jackson A And Barber J.C., (2009) Transmitted duplication of 8p23.1-8p23.2 associated with speech delay, autism and learning difficulties, Eur J Hum Genet, 14, p. 37-43

Griggs B.L., Ladd S., Saul R.A., DuPont BR And Srivastava A.K., (2008) Dedicator of cytokinesis 8 is disrupted in two patients with mental retardation and developmental disabilities, Genomics, 91(2), p. 195-202

Guerrini R., Shanahan J.L., Carrozzo R., Bonanni P., Higgs D.R And Gibbons R.J., (2000) A nonsense mutation of the ATRX gene causing mild mental retardation and epilepsy, Ann Neurol, 47, p. 117–121

Guigo R., Agarwal P., Abril J.F., Burset M And Fickett J.W., (2000) An assessment of gene prediction accuracy in large DNA sequences, Genome Res, 10, p. 1631-1642

Guy J., Gan J., Selfridge J., Cobb S And Bird A., (2007) Reversal of neurological defects in a mouse model of Rett Syndrome. Science, 315, p. 1143–1207

Ham A.L., Kumar A., Deeter R And Schanen N.C., (2005) Does genotype predict phenotype in Rett Syndrome?, J Child Neurol, 20, p. 768–778

Higgins J.J., Pucilowska J., Lombardi R.Q And Rooney J.P., (2004) Candidate genes for recessive non-syndromic mental retardation on chromosome 3p (MRT2A), Clin Genet, 65, p. 496-500

Horikawa Y., Oda N., Cox N.J., Li X., Orho-Melander M., Hara M., Hinokio Y., Lindner T.H., Mashima H., Schwarz P.E., del Bosque-Plata L., Horikawa Y., Oda Y., Yoshiuchi I., Colilla S., Polonsky K.S., Wei S., Concannon P., Iwasaki N., Schulze J., Baier L.J., Bogardus C., Groop L., Boerwinkle E., Hanis C.L And Bell G.I., (2000) Genetic variability in the gene encoding calpain-10 is associated with type 2 diabetes mellitus, Nature Genet, 26, p. 163-175

Hu W.H., Pendergast J.S And Mo X.M et al., (2005) NIBP, a novel NIK and IKK (beta)-binding protein that enhances NF-(kappa)B activation, J Biol Chem, 280, p. 29233–41

Jamra R.A., Wohlfart S., Zweier M., Uebe S., Priebe L., Ekici A., Giesebrecht S., Abboud A., Al Khateeb M.A., Fakher M., Hamdan S., Ismael A., Muhammad S., Nothen M.M., Schumacher J And Reis A., (2011) Homozygosity mapping in 64 Syrian consanguineous families with non-specific intellectual disability reveals 11 novel loci and high heterogeneity, Europ J Hum Genet, 19, p. 1161-1166

Janneke H.M., Schuurs-Hoeijmakers C, Hehir-Kwa J.Y., Pfundt R., Van Bon B.W.M., de Leeuw N., Kleefstra T., Willemsen M.A., Van Kessel A.G., Brunner H.G., Veltman J.A., Van Bokhoven H., De Brouwer A.M.P And de Vries B.B.A., (2011) Homozygosity mapping in outbred families with mental retardation, European Journal of Human Genetics, 19, p. 597–660

Jugloff D.G., Vandamme K., Logan R., Visanji N.P., Brotchie J.M And Eubanks J.H., (2008) Targeted delivery of an Mecp2 transgene to forebrain neurons improves the behavior of female Mecp2-deficient mice, Hum Mol Genet, 17, p. 1386–1396

Katusic S.K., Colligan R.C., Beard C.M., O'Fallon W.M., Bergstralh E.J And Jacobsen S.J et al., (1996) Mental retardation in a birth cohort, 1976–1980, Rochester, Minnesota, Am J Ment Retard, 100, p. 335–344

Kaufman L., Ayub M and Vincent J.B., (2010) The genetic basis of non-syndromic intellectual disability: A review, J Neurodev Disord, 2, p. 182–209

Kaufman L., Ayub M And. Vincent J.B., (2010) The genetic basis of non-syndromic intellectual disability, J Neurodevelop Disord, 2, p. 182–209

Kerem E., (2004) Pharmacologic therapy for stop mutations: how much CFTR activity is enough, Curr Opin Pulm Med, 10, p. 547–552

Khaliq S., Hameed A., Ismail M., Mehdi S.Q., Bessant D.A.R., Payne A.M And Bhattacharya S.S., (2000) A novel locus for autosomal recessive cone-rod dystrophy (CORD8) mapping to chromosome 1q12 –q24, Investig Ophthal Vis Sci, 41, p. 3709-3712

Khan M.A., Rafiq M.A., Noor A., Ali N., Ali G., Vincent J.B And Ansar M., (2011) A novel deletion mutation in the TUSC3 gene in a consanguineous Pakistani family with autosomal recessive nonsyndromic intellectual disability BMC Medical Genetics, 12, p. 56

Kidd J.R., Pakstis A.J., Zhao H., Lu R.B., Okonofua F.E., Odunsi A And Grigorenko E et al., (2000) Haplotypes and linkage disequilibrium at the phenylalanine hydroxylase locus PAH, in a global representation of populations, Am J Hum Genet, 63, p. 1882-1899

Kleinjan D.J And van Heyningen V., (1998) Position effect in human genetic disease. Hum Mol Genet, 7(10), p. 1611-1618

Lathrop G.M And Lalouel J.M., (1984) Easy calculation of LOD scores and genetic risks on small computers, Am J Hum Genet, 36, p. 460-565

Lencz T., Lambert C And DeRosse P et al., (2007) Runs of homozygosity reveal highly penetrant recessice loci in schizophrenia, Proc Natl Acad Sci USA, 104. p. 19942–19947

Leonard H., and Wen X., (2002) The epidemiology of mental retardation: Challenges and opportunities in the new millennium, Ment Retard Dev Disabil Res Rev, 8, p. 117–134

Linde L., Boelz S And Nissim-Rafinia M et al., (2007) Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin, J Clin Invest, 117,p. 683–692

Luikenhuis S., Giacometti E., Beard C.F And Jaenisch R., (2004) Expression of MeCP2 in postmitotic neurons rescues rett syndrome in mice, Proc Natl Acad Sci USA, 101, p. 6033–6108

Luikenhuis S., Giacometti E., Beard C.F And Jaenisch R., (2004) Expression of MeCP2 in postmitotic neurons rescues rett syndrome in mice, Proc Natl Acad Sci USA, 101, p. 6033–6108 Arnett A.L., Chamberlain J.R And Chamberlain J.S., (2009) Therapy for neuromuscular Disorders, Curr Opin Genet Dev, 19, p. 290–307

Madavan T And Narayan J., (1991) Consanguinity and mental retardation, Intellectual Disability Research, 35, p. 133–139

McKusick V.A., (1998) Mendelian Inheritance in Man. Catalogue of Human Genes and Genetic Disorders, John Hopkins Univ Press, Baltimore.

McLaren J And Bryson S.E., (1987) Review of recent epidemiological studies of mental retardation: prevalence, associated disorders, and etiology, Am J Ment Retard., 92, p. 243–254

McLaren J And Bryson S.E., (1987) Review of recent epidemiological studies of mental retardation: prevalence, associated disorders, and etiology, Am J Ment Retard, 92, p. 243–254

Menzel U., Mihalev A.H., Minoshima S., Murayama Y., Naylor J.W., Nicol R., Nguyen C., O'Leary S.B., O'Neill K., Parker S.C., Polley A., Raymond C.K., Reichwald K., Rodriguez J., Sasaki T., Schilhabel M., Siddiqui R., Smith C.L., Sneddon T.P., Talamas J.A., Tenzin P., Topham K., Venkataraman V., Wen G., Yamazaki S., Young S.K., Zeng

Q., Zimmer A.R., Rosenthal A., Birren B.W., Platzer M., Shimizu N And Lander E.S., (2006) DNA sequence and analysis of human chromosome 8, Nature, 439, p. 331-335

Mir A., Kaufman L., Noor A., Motazacker M.M., Jamil T., Azam M., Kahrizi K., Rafiq MA., Weksberg R., Nasr T., Naeem F., Tzschach A., Kuss A.W., Ishak G.E., Doherty D., Ropers H.H., Barkovich A.J., Najmabadi H., Ayub M And Vincent J.B., (2009) Identification of mutations in TRAPPC9, which encodes the NIK- and IKKbeta- binding protein, in nonsyndromic autosomal-recessive mental retardation, Am J Hum Genet, 85, p. 909-915

Mir A., Kaufman L., Noor A., Motazacker M.M., Jamil T., Azam M., Kahrizi K., Rafiq M.A., Weksberg R., Nasr T., Naeem F., Tzschach A., Kuss A.W., Ishak G.E., Doherty D., Ropers H.H., Barkovich A.J., Najmabadi H., Ayub M And Vincent J.B., (2009) Identification of Mutations in TRAPPC9, which Encodes the NIK- and IKK-b-Binding Protein, in Nonsyndromic Autosomal-Recessive Mental Retardation, Am J Hum Genet, 85, p. 909–915

Mochida G.H., Mahajnah M., Hill A.D., Basel-Vanagaite L., Gleason D And Hill R.S et al., (2009) A truncating mutation of TRAPPC9 is associated with autosomal-recessive intellectual disability and postnatal microcephaly, Am J Hum Genet, 85, p. 897–902

Mochida G.H., Mahajnah M., Hill AD., Basel-Vanagaite L., Gleason D., Hill R.S., Bodell A., Crosier M., Straussberg R And Walsh C.A., (2009) A truncating mutation of TRAPPC9 is associated with autosomal-recessive intellectual disability and postnatal microcephaly, Am J Hum Genet, 85, p. 897-902

Molinari F., Foulquier F And Tarpey P.S et al., (2008) Oligosaccharyltransferasesubunit mutations in nonsyndromic mental retardation, Am J Hum Genet, 82, p. 1150–1207

Molinari F., Foulquier F., Tarpey P.S., Morelle W., Boissel S., Teague J., Edkins S., Futreal P.A., Stratton M.R., Turner G., Matthijs G., Gecz J., Munnich A And Colleaux L., (2008) Oligosaccharyltransferase-subunit mutations in nonsyndromic mental Retardation, Am J Hum Genet, 82, p. 1150-1157

Molinari F., Rio M., Meskenaite V., Encha-Razavi F., Auge J., Bacq D., Briault S., Vekemans M., Munnich A., Attie-Bitach T., Sonderegger P And Colleaux L., (2002) Truncating neurotrypsin mutation in autosomal recessive nonsyndromic mental retardation, Science, 298, p. 1779-1781

Motazacker M.M., Rost B.R., Hucho T., Garshasbi M., Kahrizi K., Ullmann B., Abedini S.S., Nieh S.E., Amini S.H., Goswami C., Tzschach A., Jensen L.R., Schmitz D., Ropers H.H., Najmabadi H And Kuss A.W., (2007) A defect in the ionotropic glutamate receptor 6 gene (GRIK2) is associated with autosomal recessive mental retardation, Am J Hum Genet, 81, p. 792-798

Nagai J and Takano M., (2004) Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity, Drug Metab Pharmacokinet, 19, p. 159–170

Najmabadi H., Motazacker M.M., Garshasbi M., Kahrizi K., Tzschach A., Chen W., Behjati F., Hadavi V., Nieh S.E., Abedini S.S., Vazifehmand R., Firouzabadi S.G., and 9 others., (2007) Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci, Hum Genet, 121, p. 43-48

Nusbaum C., Mikkelsen T.S., Zody M.C., Asakawa S., Taudien S., Garber M., Kodira C.D., Schueler M.G., Shimizu A., Whittaker C.A., Chang J.L., Cuomo C.A., Dewar K., FitzGerald MG., Yang X., Allen N.R., Anderson S., Asakawa T., Blechschmidt K., Bloom T., Borowsky M.L., Butler J., Cook A., Corum B., DeArellano K., DeCaprio D., Dooley K.T., Dorris L., Engels R., Glöckner G., Hafez N., Hagopian D.S., Hall JL., Ishikawa S.K., Jaffe D.B., Kamat A., Kudoh J., Lehmann R.., Lokitsang T., Macdonald P., Major J.E., Matthews C.D., Mauceli E.,

OMIM database. [http://www.ncbi.nlm.nih.gov/omim/].

Orrico A., Lam C., Galli L., Dotti M.T., Hayek G And Tong S.F et al., (2000) MECP2 mutation in male patients with non-specific X-linked mental retardation, FEBS Lett, 481, p. 285–308

Philippe O., Rio M And Carioux A et al., (2009) Combination of linkage mapping and microarray-expression analysis identifies NF-kappaB signaling defect as a cause of autosomal-recessive mental retardation, Am J Hum Genet, 85, p. 903–1008

Philippe O., Rio M, Carioux A., Plaza J.M., Guigue P., Molinari F., Boddaert N., Bole-Feysot C., Nitschke P., Smahi A., Munnich A And Colleaux L., (2009) Combination of linkage mapping and microarray-expression analysis identifies NFkappa-B signaling defect as a cause of autosomal-recessive mental Retardation, Am J Hum Genet, 85, p. 903-908

Rauch A., Hoyer J And Guth S et al., (2006) Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation, Am J Med Genet A, 140, p. 2063–2074

Rehman S., Baig S.M., Eiberg H., Rehman S., Ahmad I., Malik N.A., Tommerup N And Hansen L., (2011) Autozygosity mapping of a large consanguineous Pakistani family reveals a novel non-syndromic autosomal recessive mental retardation locus on 11p15-tel, Neurogenetics, 12, p. 247-251

Ritvo E.R., Mason-Brothers A., Menkes J.H And Sparkes R.S., (1988) Association of autism, retinoblastoma, and reduced esterase D activity, Arch Gen Psychiat, 45, p. 600 only

Roeleveld N., Zielhuis G.A And Gabreels F., (1997) The prevalence of mental retardation: a critical review of recent literature, Dev Med Child Neurol, 39, p. 125–132

Roeleveld N., Zielhuis G.A., and Gabreels F., (1997) The prevalence of mental retardation: a critical review of recent literature, Dev Med Child Neurol, 39, p. 125–132

Ropers H.H And Hamel B.C., (2005) X-linked mental retardation, Nat Rev Genet, 6, p. 46–57

Ropers H.H And Hamel B.C., (2005) X-linked mental retardation, Nat Rev Genet,

Ropers H.H., (2006) X-linked mental retardation: many genes for a complex disorder, Curr Opin Genet Dev, 16, p. 260–269

Ropers H.H., (2010) Genetics of early onset cognitive impairment, Annu Rev Genomics Hum Genet, 11, p. 161-187

Ropers H.H., and Hamel B.C., (2005) X-linked mental retardation, Nat Rev Genet, 6, p. 46–57

Sachidanandam R., Weissman D., Schmidt S.C., Kakol J.M., Stein L.D., Marth G., Sherry S., Mullikin J.C., Mortimore B.J., Willey D.L., Hunt S.E., Cole C.G., Coggill P.C., Rice C.M., Ning Z., Rogers J., Bentley D.R., Kwok P.Y., Mardis E.R., Yeh R.T., Schultz B., Cook L., Davenport R., Dante M., Fulton L., Hillier L., Waterston R.H., McPherson J.D., Gilman B., Schaffner S., Van Etten W.J., Reich D., Higgins J., Daly M.J., Blumenstiel B., Baldwin J., Stange-Thomann N., Zody M.C., Linton L., Lander E.S And Altshuler D., (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms, Nature, 409(6822), p. 928-933

Steele M.M., Al-Adeimi M., Siu V.M., Fan Y.S., (2001) Brief report: a case of autism with interstitial deletion of chromosome 13, J Autism Dev Disord, 31, p. 231-234

Stevenson R.E And Schwartz C.E., (2009) X-linked intellectual disability: unique vulnerability of the male genome, Dev Disabil Res Rev, 15(4), p. 361-368

Tabarés-Seisdedos R and Rubenstein J.L., (2009) Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. Mol Psychiatry, 14, p. 563-589

Toth G., Gaspari Z And Jurka J., (2000) Microsatellites in different eukaryotic genomes: Survey and analysis, Genome Res, 10, p. 967-981

Wagenstaller J., Spranger S And Lorenz-Depiereux B et al., (2007) Copy-number variations measured by single-nucleotide-polymorphism oligonucleotide arrays in patients with mental retardation, Am J Hum Genet, 81, p. 768–779

Wilschanski M., Yahav Y And Yaacov Y et al., (2003) Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations, N Engl J Med., 349, p. 1433–1441

Yntema H.G., Poppelaars F.A., Derksen E., Oudakker AR., van Roosmalen T And Jacobs A et al., (2002) Expanding phenotype of XNP mutations: mild to moderate mental retardation, Am J Med Genet, 110, p. 243–307

Zahir F And Friedman J.M., (2007) The impact of array genomic hybridization on mental retardation research: a review of current technologies and their clinical utility, Clin Genet, 72, p. 271–287

Zhou H And Clapham D.E., (2009) Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development, Proc Natl Acad Sci USA, 106, p. 15750–15805