Analysis of exonic region of *PCNT* gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects

Research project submitted to the Central University of Punjab

For the award of

Master of Science

Life Sciences with Specialization in Human Genetics

In

Department of Human Genetics and Molecular Medicine

By

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Central University of Punjab, Bathinda

Declaration

I declare that all changes suggested by examiner in research project entitled **"Analysis of exonic region of** PCNT **gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects"** submitted by me for the award of degree of **M.Sc. in Life Science with specialization in Human Genetics** in the **Department of Human Genetics and Molecular Medicine** has been incorporated in the research project.

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CERTIFICATE

I declare that the Research project entitled **"Analysis of exonic region of** PCNT **gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects"** has been prepared by me under the guidance of Dr. Preeti Khetarpal, Assistant Professor, and Department of Human Genetics and Molecular Medicine, School of Health Sciences, Central University of Punjab. No part of this project work has formed the basis for the award of any degree or fellowship previously.

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I certify that Neha Gupta has prepared her Research project entitled, **"Analysis of exonic region of** PCNT **gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects"** for the award of M.Sc., degree of the Central University of Punjab, under my guidance. She has carried out this work at the Department of Human Genetics and Molecular Medicine, School of Health Sciences, Central University of Punjab.

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ABSTRACT

Analysis of exonic region of PCNT **gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects**

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Key words: PCNT, MOPD type II, Mutations, structure, Primordial dwarfism, IUGR, PNGR, Variants

MOPD II is an autosomal recessive disorder. It is characterised by the presence of intra uterine growth retardation as well as post natal growth retardation. The adult height is not more than 100 cm. It has been found that mutation in PCNT gene is associated with MOPD II. The cytogenetic location of this gene is 21q22.3 and it contains 47 exons. It encodes for PCNT protein which is a very large coiled scaffold protein and helps in microtubule polymerisation ensuring proper cell division. Till date 74 mutations have been identified this includes deletion, stop, frame shift and non sense mutation. The present study was carried out to analyse the exonic region of PCNT gene in Microcephalic Osteodysplastic Primordial Dwarfism Type II subjects. As it is an autosomal rescessive disorder both male and female were equally affected. The study included three subjects diagnosed with MOPD II .The DNA was extracted from whole blood and was amplified using locus specific primers. The products were sequenced using Sanger sequencing and were analysed. Total 12 variants were detected and 2 of which were pathogenic and 2 were synonymous and remaining 8 were polymorphic variants. 3 were present in exon 44 and 1 in exon 31 .These 3 variants were found to be present in all four subjects while 1 was present in only one subject. Change in nucleotide sequence may produce deleterious affect which is needed to be explored along with the complete structure of PCNT protein.

Neha Gupta Dr. Preeti Khetarpal

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LIST OF ABBREVIATIONS

S.No.	Full Form	Abbreviation
1	Intrauterine Growth Retardation	IUGR
2	Postnatal Growth Retardation	PNGR
3	Primordial Dwarfism	PD
4	Silver-Russell Syndrome	SRS
5	Uniparental Disomy	UPD
6	Microcephalic Osteodysplastic Primordial Dwarfism	MOPD
7	Pericentriolar Material	PCM
8	Pericentrin	PCNT
9	Microtubule Organizing Centre	MTOC
10	Pericentrin AKAP450 C-terminal	PACT
11	Type II regulatory sub-unit	RII
12	Differentially methylated region	DMR
13	Protein Kinase A	PKA
14	Gamma tubulin ring complex	γ-TuRC

CHAPTER I

INTRODUCTION

1. Introduction

Growth is a regulated process which determines the size and shape of an individual. As soon as the embryogenesis begins the size of the individual is determined (Klingseisen and Jackson, 2011). It depends upon the proper cordination between cell division and apoptosis. Various environmental, genetic and metabolic factors are known to be associated with growth regulatory pathway. Disturbance in any of the pathway may result into wide variety of pre- natal and post- natal growth retardation. Intrauterine growth retardation results into structural as well as mental inabilities (Daniel *et al*., 2008 ; Khetarpal *et al*., 2016).

Primordial dwarfism is a combination of monogenic disorder in which affect the growth during early developmental stages (IUGR) as well as after birth (PNGR) (Klingseisen and Jackson *,* 2011). The final height of the individual is reduced to 1 m (Rauch *et al*., 2008). No effect of growth hormone therapy is seen on the individuals (Rauch 2011) and group of these disorders can also be called as hypocellular forms of dwarfism (Klingseisen and Jackson *,* 2011). On the basis of clinical features PD is categorised into five sub-types: Silver-Russell Syndrome (Silver *et al.,* 1953) (Russell, 1954), Meier-Gorlin syndrome, Seckel syndrome and Microcephalic Osteodysplastic Primordial Dwarfism (MOPD) types I and II (Hall et al. 2004). Silver Russel syndrome is diagnosed with relative macrocephaly ,clinodactyly, feeding difficulty, triangular face, and body assymetry. Epimutation in ICR region of chromosome 11 (Begemann *et al*., 2011) and maternal UPD of chromosome 7 (Eggermann *et al.,* 2010) is the main cause of SRS.

Meier Gorlin syndrome also known as ear patella syndrome. Mutation in prereplicative complex (ORC1, *ORC4*, *ORC6*, *CDT1*, and *CDC6*) causes various abnormalities like missing knee caps, microcephaly, small ear which are observed in Meier Gorlin syndrome (Bicknell *et al.,* 2011).

The characteristics features of Seckel syndrome is the presence of microcephaly present at the time of birth, skeleton abnormalities, mild or absent mental retardation, beak like protrusion of nose and micrognathia (Majewski and Goecke 1982). It is a heterogenous condition which is caused due to the mutation in ATR gene which is found to be associated with DNA damage signalling (O'Driscoll *et al*., 2003; Ogi *et al.,* 2012). Hypomorphic mutation had been reported in the ATR gene (Sckl1, 3q22.1-q23) (O'Driscoll *et al*., 2003; Griffith *et al*., 2008).MOPD-I is

characterised by the presence of sparse hair, decrease in subcutaneous fat, long clavicles, hip dislocation and bowed femora. Mutation in RNU4ATAC gene have been found associated with MOPD-I (He *et al*.,2011). MOPD (Microcephalic osteodysplastic primordial dwarfism) is a group of heterogenous and rare autosomal recessive disorder, which is characterised by the presence of pre and postnatal growth retardation, dental abnormalities, microcephaly. It possessed some phenotypes which were similar to Seckel syndrome but Majeswki proposed that they both were entirely different (Majewski and Goecke 1982). Initially it was classified into three types: MOPD-I, MOPD-II, MOPD-III (Majewski and Goecke 1982) but later it was found that MOPD –I and MOPD-III were same (Meinecke and Passarge, 1991; Winter et al., 1985).

MOPD I was firstly reported as cephaloskeletal dyspasia by (Taybi *et a.*, 1967) and later is was described as MOPD I/III by (Majewski *et al*.,1982). It a rare autosomal disorder which shows the presence of thinness of hair in scalp, eyebrow and eyelashes ,decreased sub-cutaneous fat, prominent superficial veins over chest, protruding eyes, prominent nose with a flat nasal bridge, small low set ears, dental abnormalities, small chin and short neck , short vertebrae, elongated clavicles, skeletal abnormality, corpus callosum agenesis and microcephaly. (Abolila et al., 2012 ; Shawky *et al*.,2017).The main cause of MOPD I is the homozygous or heterozygous mutation in RNU4ATAC gene. The cytogenetic location of the gene is 2q14.2. This gene encodes for a small nuclear RNA which is an important component of U12 dependent minor splicesome (Jafarifar *et al*., 2014).

MOPD II is one of the well characterised form of primordial dwarfism. There is the presence of IUGR, PNGR, microcephaly, micrognathia, teeth abnormalities and short stature in patients suffering from MOPD II. Some other symptoms include feeding difficulties, small iliac wings, coxa vara, scoliosis, abnormalities of limb, high squeaky voice, unusual facial features, epiphyseal delay, and sparse hair (Hall *et al*., 2004).. The adult height of patient is not more than 100 cm (Rauch *et al*, 2008).Mutation in PCNT gene is associated with MOPD II. . PCNT is found to be located on long arm of chromosome 21.Its cytogenetic location is 21q22.3 and it encodes PCNT protein. In the individual affected with MOPD II either there is truncation of protein or it is functionally impaired. PCNT protein maintains the centrosomal integrity of cell and has an important role in

spindle fibre organization but mutation causes loss in integrity and death of the cell which causes growth retardation related problems (Rauch *et al*., 2008).

Diagnosis of MOPD II is principally supported by clinical features of patients. However, variation in features is a huge challenge for its correct diagnosis. To administer the genetic counselling to the parents of the patient it's essential to characterize the patients genetically. As it has been found that mutation in PCNT gene causes MOPD II (Rauch *et al*., 2008) but there was no report of PCNT gene mutation among Indian population. Hence, present study was done with the following objective.

Objective:

- To analyse exonic region of *PCNT* gene in Microcephalic Osteodysplastic Primordial Dwarfism (MOPD) type II subjects.

CHAPTER II
REVIEW OF LITERATURE

2. Review of Literature

MOPD II is a rare autosomal disorder and is a subtype of primordial dwarfism which is characterised by intrauterine as well as post natal growth retardation. It was first described by Majewski *et al* in 1982.The cause of MOPD II is the biallelic null mutation (nonsense or frameshift) in *PCNT* gene which is located on long arm of chromosome 21 (21q22.3).The pattern of inheritance may be homozygous or heterozygous .The major characteristic features of MOPD II are short stature, microcephaly, bone dysplasia, squeaky voice, abnormal dentition, mild or absent mental retardation, far sightedness, scoliosis, ear abnormalities, unusual pigmentation, coxa vara, relatively short arms , precocius puberty, feeding difficulties and diabetes type 2 (Piane *et al*.,2009;Rauch *et al*., 2008).

2.1 *PCNT* gene

PCNT gene is located on chromosome 21.Its cytogenetic location is 21q22.3.It is a long gene having 47 exons (Rauch *et al*., 2008). It encodes for Pericentrin protein.

It is an important element of centrosomal assembly and plays an important role in organizing spindle fibre and in separation of chromosome at the time of cell division. It shows interaction with gamma tubulin which is an important component of microtubule nucleation, cell division, cell cycle progression and various other function of cell. It is a highly conserved protein. There is high expression of Pericentrin in muscles, testis and heart. Various splice variants of PCNT are found in different tissue and each splice variant is known to perform different function (Flory and Davis., 2003).

Pericentrin B and Percentrin S are two isoforms which are result of alternatively spliced *PCNT*. Expression of Pericentrin B takes place in all developmental stages while Pericentrin S is expressed during late fetal stages and in adult skeletal and heart muscles (Miyoshi *et al*., 2006 ; Zebrowski *et al.,* 20015). Pericentrin B is the largest isoform having a molecular weight 380 kDa in humans and 360 kDa in mice. It serves as scaffold for various proteins and protein complexes. The important function of PCNT B is in centrosomal integrity.

Mutation in PCNT gene is tissue and cell specific (Muhlhans and Giebl*.,* 2012). Heterogeneous diseases with varied clinical manifestations has been found to be

associated with mutation in *PCNT* gene (Delaval and Doxsey*.,* 2010).Various mutation such as non-sense, duplication, transversion, splicesite and insertion are associated with mutation in *PCNT* gene mutation which results in truncated as well as functionally impaired PCNT protein

2.2 Structure of PCNT Protein and its Function

Pericentrin (kendrin) is a very large (molecular weight about 360kDa) coiled-coil protein encoded by *PCNT* gene (Rauch *et al*.,2008).It contains about 3336 amino acid (Li *et al.,* 2001).

This protein contains various domains which are functionally diverse. Some functions performed by different domains of the protein are: maintaining genomic stability, regulation and serves as a scaffold for various other protein complexes (Diviani *et al*., 2000; Gillingham and Munro, 2000; Flory *et al*., 2000).

RII (Type II regulatory sub-unit) is a highly conserved domain of AKAPs which shares about 100 amino acid ranging from 220 to 320 amino acid in PCNT protein. This region is rich in leucine and valine (hydrophobic amino acid).Hence, it has been found that there is a hydrophobic interaction between AKAPs and RII. It recruits PKA holoenzymes (Protein kinases) to particular intracellular locations (Diviani *et al*., 2000).

Microtubules in cells are usually organised at the Microtubule Organising Centre (MTOC). MTOC contain a pair of centrioles and known as centrosome. One of the known protein of the MTOC is gamma tubulin which helps in the nucleation of microtubule formation. Gamma tubulin ring complex (γ-TuRC)is a protein complex which contains γ-tubulin and various other proteins and helps in microtubule polymerisation. γ-tubulin is recruited by the N- terminal of PCNT and helps in polymerisation of microtubule. Disruption in this mechanism leads to improper mitosis and apoptosis (Flory *et al*., 2000; Zimmerman *et al*., 2004).

PCNT and AKAP450 both share binding site at C terminus which recruits both the protein to the centrosome. This particular site is known as PACT domain (Pericentrin AKAP450 Centrosomal Targeting). Calmodulin binds at this site and recruits dynein and PKA which helps in centrosomal assembly (Gillingham *et al*., 2000; Flory *et al*., 2000).

CDK5RAP2, Ninein and Centriolin, associates with PCNT and forms a complex. Ninein and centriolin is anchored by CDK5RAP2 when the carboxy terminal of PCNT and CDK5RAP2 associates (Luo and Pelletier, 2014).The complex is driven to the centrosome by dynein dependent pathway (Purohit et al., 1999).

PCNT is found in association with Cep 215 in Pericentriolar matrix (PCM).PCNT is set free when Cep 215 is cleaved by separase which results in the separation of centrioles leading to centrosome disentanglement.This whole process leads to mitotic exit (Pagan *et al.,* 2015; Kim *et al.,* 2015).

At the G2 phase of cell cycle NEK2 phosphorylates PCNT which triggers segregation of centrosome. This complete process lead to the duplication of chromosome along with the proper chromosomal segregation during cell cycle (Matsuo *et al.,* 2010).

2.3 Mutation Spectrum in the PCNT gene as reported world wide

Rauch *et al*.,2008 was the first who detected 21 novel mutations among 25 patients in *PCNT* gene .Mutations included were deletion, stop, splicesite, insertion and non sense which resulted in premature PCNT protein truncation (Rauch *et al.,* 2008).Many more mutations have been identified which are enlisted in table 2.1.

Table.2.1: Compilation of *PCNT* mutations from Literature Search.

S. No	Mutation Type	Patient Mutations	Amino Acid Change	Inheritance Pattern	Exon/ Intron No.
1	Splicesite And Deletion +	IVS12-1G>C and C.8846delg	Splicesite And p.G2949fsx2954	Heterozygous	Exon 13 And Exon 39
2	Insertion +	C.841_842insg	p.R281fsx327	Homozygous	Exon 5
3	N/A -	C.6329G>A	p.W2110X	Homozygous	Exon 30
4	N/A ^	C.1388C>G	p.S463X	Homozygous	Exon 9
5	Deletion(Frameshift) *	486-BP Deletion IVS30 At Nucleotide 84 To IVS31 At Nucleotide 569	p.IVS30-84_IVS31-569del	Homozygous	Exon 31
6	Stop *	C.3109G>T	p.E1037X	Homozygous	Exon 15
7	Stop *	C.5767C>T	p.R1923X	Homozygous	Exon 28
8	Stop *	C.5992C>T And C.9316C>T	p.Q1998X And p.Q3106X	Heterozygous	Exon 28 andExon 42
9	Stop *	C.7813C>T	p.Q1605X	Homozygous	Exon 26
10	Splicesite (Frameshift) *	IVS35+1G>A	Splicesite	Homozygous	Exon 35
11	Splicesite(Framshift) And Stop *	IVS10-1G>C And C.5365C>T	Splicesite And p.Q1789X	Heterozygous	Exon 11 And Exon

					28
12	Stop *	C.1336C>T	p.Q446X	Homozygous	Exon 8
13	Stop And Splicesite(Frameshift) *	C.886C>T And IVS9+5G>A	p.Q296X And Splicesite	Heterozygous	Exon 5
14	Deletion(Frameshift) *	C.366_369delcagt andC.4542delc	p.V122fsx251 and p.P1514fsx1536	Heterozygous	Exon 3 And Exon 23
15	Frameshift :Splicesite And Insertion *	IVS8-1G>A And C.2346insc	Splicesite And p.Q789fsx796	Heterozygous	Exon 9 And Exon 14
16	Deletion(Frameshift) *	C.2980_2992delgagcagacttt	p.R994fsx1053	Homozygous	Exon 15
17	Stop *	C.8752C>T	p.R2918X	Homozygous	Exon 39
18	Stop *	C.1042G>T	p.E348X	Homozygous	Exon 7
19	Deletion(Frameshift) *	C.4974_4978delaaaag	p.L1658fsx1666	Homozygous	Exon 27
20	Deletion(Frameshift) *	C.6711delg	p.E2237fsx2244	Homozygous	Exon 30
21	Deletion(Frameshift) *	C.605dela And C.9078delg	p.D202fsx252 And p.L3026fsx3049	Heterozygous	Exon 3 And Exon 40
22	Deletion(Frameshift) *	C.234_235delat	p.T2128fsx2129	Homozygous	Exon 30
23	Deletion(Frameshift) *	C.3015_3016delct	p.1005dfsx1055	Homozygous	Exon 15
24	Stop *	C.8752C>T	p.R2918X	Homozygous	Exon 39
25	Frameshift(Insertion) <	(1527_1528insa)	Thr510fs	Homozygous	Exon 10
26	Nonsense >	C.3382C/G	p.Gln1128X	Homozygous	Exon 17

27	Splicesite >	C.9099+2T/C	Splice Site	Homozygous	Intron 40
28	Deletion(Frameshift) >	C.6316_6325delgtttggagagca	p.Leu2106Alafs18 C3	Homozygous	Exon 30
29	Deletion(Frameshift) >	C.2326_2327delga	p.Glu776Lysfs3C1	Homozygous	Exon 14
30	Splicesite >	C.3608-2A/G	Splice Site	Homozygous	Intron 18
31	Nonsense >	C.5578G/T	p.Glu1860X	Heterozygous	Exon 28
32	Nonsense >	C.7338C/A	p.Cys2446X	Homozygous	Exon 34
33	Nonsense and Deletion/	C.5767C>T And C.9460_9462delaag	(p.Pro1923X) And Lys3154del	Heterozygous	Exon 28 And Exon 43
34	Nonsense /	3460G>T	p.Glu1154X	Homozygous	Exon 17
35	N/A “	C.1468C>T + unknown	p.Q490X And no protein	Homozygous	N/A
36	Duplication and Deletion “	[C.6162_6163delAG]+ [C.9273+1G>C]	p. K2054fsx57 And	Heterozygous	N/A
37	N/A “	[C.1937-1G>C] + [C.8048_8049insg]	p.A2682X60	Heterozygous	N/A
38	N/A “	[C.5993A>C]+ [C.7179+1G>A]	p.Q1998P (Splicing)	Heterozygous	N/A
39	N/A “	[C.2347dupc]+ [C.1336-1G>A]	p.Q783fsx7	Heterozygous	N/A
40	N/A “	[C.1843C>T]+ [C.7852C>T]	p. Q615X And p.Q2618X	Heterozygous	N/A
41	Deletion “	[C.2981_2991delgagcagacttt	p.R994fsx60	Homozygous	N/A

		]			
42	Deletion “	[C.2473G>T]+ [C.2168delt]	p.E825X And p.L723fsx9	Heterozygous	N/A
43	N/A “	[C.2731C>T]+ [C.8917C>T]	p.Q911X And p.R2973X	Heterozygous	N/A
44	N/A “	[C.2731C>T]+ [C.8917C>T]	p.Q911X And p.R2973X	Heterozygous	N/A
45	N/A “	[C.5365C>T]+ [C.5667C>T]	p.Q1789X And p.R1923X	Heterozygous	N/A
46	N/A “	[C.5993A>C]+ [C.7179+1G>A]	p.Q1998P (Splicing)	Heterozygous	N/A
47	Deletion “	[C.3015_3016 delct]	p.1005dfsx50	Homozygous	N/A
48	N/A “	[C.8917C>T]	p.R2973X	Homozygous	N/A
49	Deletion “	[C.2980_2992 delgagcagacttt]	p.R994fsx59	Homozygous	N/A
50	Duplication and Deletion “	[C.2188G>T]+ [C.4917_4918 delac]	p.E730X And p.Q1640fsx27	Heterozygous	N/A
51	Deletion “	[C.2543_2547 delacggg)	p.D848fsx26	Homozygous	N/A
52	N/A “	[C.6329G>A]	p.W2110X	Homozygous	N/A
53	Deletion “	[C.3514delac]+ [C.4938delag]	p.T1172fsx20 And p.R1646fsx21	Heterozygous	N/A
54	Duplication And Deletion “	[C.2347dupc]+ [C.4976_4980 delaagaa]	p.Q783fsx7 And p.K1659fsx7	Heterozygous	N/A
55	N/A “	[C.5992C>T]+ [C.9316C>T]	p.Q1998X And , p.Q3106X	Heterozygous	N/A
56	N/A “	[C.307C>T]+ [C.6814C>T]	p.Q103X And p.Q2272X	Heterozygous	N/A
57	N/A“	[C.1042G>T]	p.E348X	Homozygous	N/A
58	Deletion “	[C.6899dela]	p.H2300fsx25	Homozygous	N/A

59	Nonsense %	C.1753C.T And C.5320G.T	(p.R585X) And (p.E1774X)	Heterozygous	N/A
60	Splicesite #	1937-2 A>G	Splicesite	Homozygous C.	Splice Site
61	Splicesite #	1937-2 A>G	Splicesite	Homozygous C.	Splice Site
62	N/A #	2037 A>T	p. K678X	Homozygous C.	Exon 13
63	N/A #	3109 G>T	p. E1037X	Homozygous C.	Exon 15
64	N/A #	3109 G>T	p. E1037X	Homozygous C.	Exon 15
65	N/A #	C.3121 G>T	p. E1041X	Homozygous C.	Exon 15
66	Nonsense !	C. 1468C>T	p.Q490X	Homozygous	Exon 10
67	Deletion And Deletion @	(6619Del-C) And (9366-9381Del-16)	N/A	Heterozygous	Exon 30 And Exon 41
68	Transversion @	(9842A>C) (Unaffected Parent)	(p. 3245Arg>Ser)	Heterozygous Carrier	Exon 45

Symbols alloted	References
+	Majewski and Goecke.,(1998)
-	Young *et al*., (2004)
^	Brancati *et al*., (2005)
*	Rauch *et al*., (2008)
<	Piane *et al*., (2009)
>	Wiliems *et al*., (2009)
/	Kantaputra *et al*.,(2011)
“	Bober *et al*., (2012)
%	Muller *et al*., (2012)
#	Unal *et al*., (2014)
!	Pachajao *et al*.,(2014)
@	Li *et al*.,(2015)

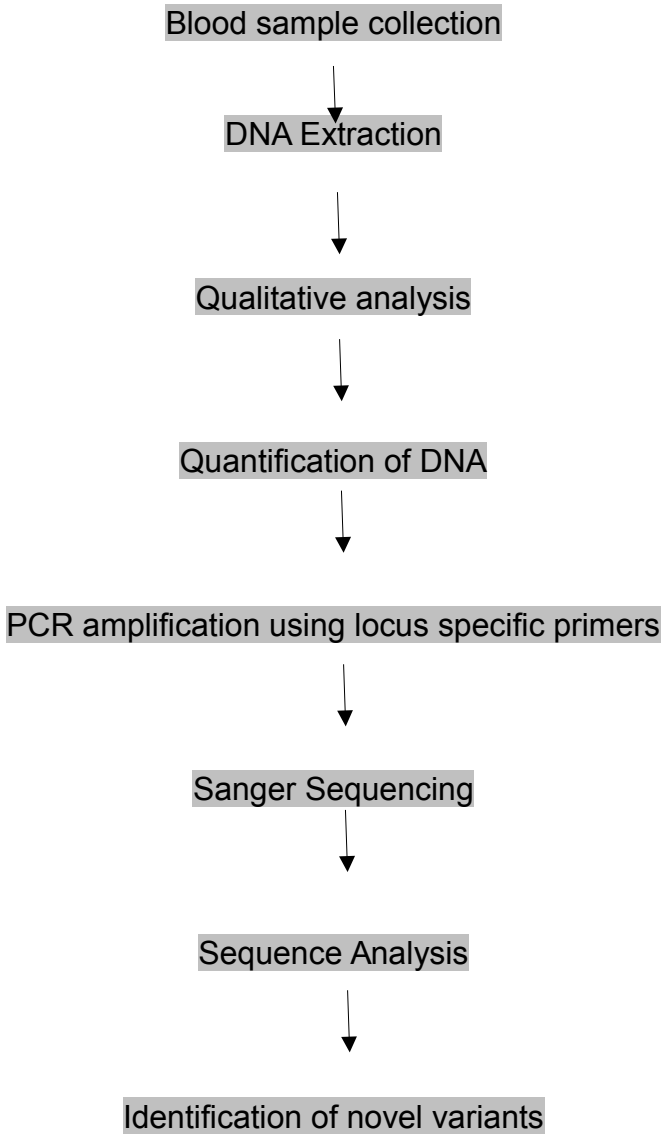
CHAPTER III
MATERIAL AND METHOD

3. Materials and Methods

3.1 Subjects

Three subjects were recruited for the study who were clinically identified as MOPD –II by clinician. Ethical clearance had been obtained for the study from Institutional ethics committee (IEC), Central University of Punjab, Bathinda vide reference number CUPB/CC/14/IEC/4471, dated: 15/12/2014. Parents of the subjects were also recruited. Blood sample was collected from the subjects as well as from the parents.

Overall methodology has been provided in figure 3.1



3.2 Blood Sample collection

Approximately 3 ml blood was taken by a technician from each subject .It was taken into vacutainers coated with sodium EDTA.

3.3 DNA Extraction

DNA was isolated from frozen blood by organic method (phenol chloroform method) (Sambrook and Russell, 2001) with slight changes. The method involved the following steps:

a) In 1 ml of whole blood 3 ml of Lysis buffer – I was added in a 15 ml centrifuge tube which was kept in ice for 15 minutes,
b) The tube was centrifuge at 3000 rpm for 15 minutes. Supernatant was discarded,
c) Step (a) and (b) were repeated 3-4 times until a white pellet is obtained,
d) 1ml Lysis buffer-II , 59 µl of 10% SDS and 25 µl of proteinase K were added to the tube containing white pellet.It was kept in water bath for incubation at 60 ° C for 3 hours
e) After 3 hours the sample was removed from water bath and was allowed to cool down until its temperature becomes equal to room temperature, then PCA in the ratio (25:24:1) was added for deproteinisation.It was gently mixed for 15 to 20 minutes. It led to the formation of emulsion.
f) It was centrifuged at 2500 rpm for 15 minutes and then supernatant was transferred to a other clean 15 ml centrifuge tube.
g) CA in equal volume was added to it and it was again mixed gently followed by centrifuge at 2500 rpm for 15 minutes, supernatant was taken into other 15 ml centrifuge tube.
h) Step (g) was repeated one more time
i) Addition of sodium acetate measuring 1/30th volume and 2.5 ml chilled was done for the precipitation of DNA
j) It was kept in -20 ° C for complete precipitation
k) DNA along with 500 µl absolute alcohol was transferred into microcentrifuge tube after complete precipitation
l) It was centrifuged at 10,000 rpm for 12 minutes and removal of alcohol was done. Washing was done using 70% ethanol for two times and it was air dried to remove alcohol
m) Dissolution of DNA was done in 50 µl TE at 37 °C for overnight.

3.4 Qualitative analysis of DNA

Qualitative analysis of DNA was done by check run using gel electrophoresis

(0.8%) It involves the following steps:

a) In a flask 200 mg of agarose and 25 ml of TAE buffer was taken
b) It was heated in oven until the agarose completely dissolves
c) After the complete dissolution of agarose it is removed from oven and 2 µl of ethidium bromide was added
d) It was poured into the gel casting tray along with comb and was allowed to set.
e) Comb was removed as the gel cooled down and 5 µl of DNA along with 2 µl dye were loaded into the wells
f) Electrophoresis was carried out at 80 V for 60 minutes.

3.5 Quantification of DNA

DNA was quantified by Qubit fluorometer. It is also used for the quantification of RNA and protein. Certain fluorescent dyes are used for determining the concentration of DNA and then spectrometer measures the absorbance of UV at 280 nm for DNA.

a) Caliberation of Qubit is done using solution Standard 1 and Standard 2 provided in the kit.
b) For the quantification of DNA a mixture containing 199 µl buffer and 1 µl dye was prepared.
c) 199 µl of the above mixture was added to 1 µl of DNA and the tube was centrifuged.
d) Quantification was done by clicking on Read button.

3.6 PCR amplification using locus specific primer

Samples were amplified using locus specific primer (Rauch *et al*., 2008). Details of primers, reaction mixture and PCR condition are given in the table 3.1, 3.2 and 3.3 and analysis of the PCR amplified products was done using 1.5% agarose gel at 100 V for 1 hour.

Table.3.1: Sequence of primers used

	PCR	Sequence		Annealing
		Forward Primer	Reverse Primer	

EXON	product			Temperature(°C) (a)
S3	size 599	acaggcctgcagatagagga	gctctctatgcttccagtttcc	56
4	398	caaccattcatccctcagga	ccacacacagaacgtggaaa	51
5	589	gtggcatctcagtggcatc	ttgttcacccctctcatggt	52
6	400	gatcgtcctgtgtgtctgga	gaacgcaatacactttcaacca	51
9	498	cagacctggaagttcctgaga	cctcaaccttcaggatgtcaa	51
10	498	Ctcatccctctccggttct	aaatctggaccataaaatgtgttt	52
12	426	tggtctcatgaacctagtgagg	agaagaggtcggagcactca	52
13	492	gcacagagcttgaaaatcca	ggactgtcgcccttacaaat	50
14	700	tgctgtgtgcagcgtaatg	cagacctttcgcccagtg	54
17	484	cgaagtgcctgctcctttt	tcttcccggtgcttacaatc	52
18	380	ccgaggtgtgcaaactggt	agtgcggaaactagcaggaa	54
20	400	ggtggtgacggcctgaag	tcccatgttggctttgttt	50
23	354	gggcagttgcacttgtacg	ctcctcacctctccatccag	54
24	243	gacagccaggtgagtcagtg	agcccaaacaagagcaagaa	51
25	470	gtctccctttgggtctaggg	gtgtttctggtgcagacgtg	52
26	400	aggtccacctgctctgctt	acagtggtcacaagcccatc	54
30a	565	atgtgcagggctcattgtg	gctggtcggtgaagaaagag	54
30b	600	tcaaaaatcaggccatagacg	gtgtgtgctgtcaggaggtg	52
31	394	ctggggtctatgtgggaaga	aggcgtgttttcagaagtcaa	52
32	396	cttgacgtgcgtctttctca	gcacatgtgaagctgtgagg	53
33	391	agaggtgggcttgaaaacct	gccattaccacaagctcctt	53
34	497	tgatggagacagcgagtgag	gcgtcaacacctgggtaagt	54
35	400	gcggagctggttttgagg	gcttgactagatgatgccctgt	56

36	483	attgagcatcctggttccac	ccaccagaggttctgtctcc	54
37	497	gggagacagaacctctggtg	cagccctatcctgctacgtc	54
38a	600	catgtggctaatagggtgcat	tcagtgtggcacttacctcct	56
40	374	cacacacaggctcctcaga a	ccacccactcacccagata g	54
42	391	ctgaggggtgctgaagtt	actgctacacccggcaga c	54
44	296	ccattctgcttgtttggtca	cggattttcttaccgtgctt	52
45	384	aaaattgccatacaggctctt	ctggagagtttcctgccttg	49
47	293	ccactgtataattcatatttttac caa	gtgtcaccgcacatgaaga g	52

Table.3.2: PCR reaction mixture

S.No	Reagent	Conc.	1X	20X
1	Tris buffer with $MgCl_2$	-	1 µl	2 µl
2	dNTPs	-	0.75 µl	1.5 µl
3	Forward primer	10x	0.5 µl	1 µl
4	Reverse primer	10x	0.5 µl	1 µl
5	Distilled water	-	5.75 µl	11.5 µl
6	Taq DNA polymerase	-	1 µl	2 µl
7	DNA template	-	0.5 µl	1 µl

Table.3.3: Polymerase chain reaction conditions

S.No.	PCR condition	Time	Temperature
1	Pre heating	5 min	95 °C
2	Denaturation	30 sec	95 °C
3	Annealing	30 sec	a °C
4	Extension	30 sec	72 °C
5	Final Extension	7 min	72 °C

PCR cycles = 32

3.7 Sequencing of DNA

Sequencing of amplified DNA samples was done by chain-termination method also known as Sanger sequencing which was given by Sanger et al., 1977. The samples were outsourced to Central instrumentation Facility, University of Delhi South Campus. Clean up was done using Exo-SAP (Exonuclease I and Shrimp Alkaline Phosphate) digestion method and sequenced by ABI 3730 DNA analyser.The format of the sequenced result were given in three formats – abi format, SEQ format and 1 format for each sample.

3.8 Sequence analysis

3.8.1 Retrieval of Sequence Data of *PCNT* gene (Wild type)

The *PCNT* gene sequence in FASTA file format had been downloaded from Ensembl genome server 92 online database server (http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000160299;r=21:46324122-46445769).

Procedure:

1. Go to Ensembl genome server 8 Database at (http://www.ensembl.org/index.html) and in search tab type *Homo sapiens* in species for PCNT.
2. Click on PCNT (Homo Gene) after that on the left side choose export data
3. A new window will pop up there select the strand and check on the check box for coding sequence (cds).
4. Click to the next and choose the output format for the data.
5. Save the text in the notepad to the desktop.

3.8.2 Alignment of contigs in Seqman Pro:

1. Click on Seqman Pro software and run the program,a window will open.
2. Select and drag the sequenced data along with their chromatograms (ab1), reference sequence (for which amplification was done) and the coding sequence downloaded from Ensembl genome server 8 Database in SEQ file format.
3. Click on assemble. All the data will be aligned.

4. Check each and every nucleotide highlighted in red which indicate the change from reference sequence.
5. Note the change, exon number and position where the change has occurred.

3.8.3 Identification of novel variants

With the help of NCBI databases, dbVar, dbSNP these nucleotide changes were checked to determine whether these changes were polymorphism (SNP) or novel pathogenic variants.

Procedure

1. Go to NCBI databases by using URL http://www.ncbi.nlm.nih.gov.
2. Click on all databases and type the chromosome number along with the genomic position of the identified variants (eg; 21:46,346,225) in the search bar.
3. If no result appears it means it is a novel variant and if results appear it indicates that it is known polymorphism.

3.8.4 Translation of gene sequence in amino acid sequence:

Wild type nucleotide sequences were translated into amino. As well as the changes were also incorporated and then the sequence was translated using ExPASy Bioinformatics Resource Portal.

Procedure:

1. Go to ExPASy translate online tool at (http://web.expasy.org/translate/).
2. DNA sequence of interest was pasted and was translated by clicking on Translate sequence.
3. It was compared with the wild type amino acid sequence.

CHAPTER IV
RESULTS

4. Results

The present study was carried out on 3 Indian subjects (2 males and 1 female) possessing MOPD II phenotype. They aged between 0-10 years and their diagnosis accomplished somewhat three of the five key criteria; IUGR, PNGR, teeth abnormalities, relative microcephaly, height less than 100 cm.

4.1 Demographic features of the MOPD II subjects under study

Demographic details of the subjects have been given in Table 4.1. Minimum age of the subjects under study is one year and maximum age is 10 years. Weight of the subjects at the time of sample collection varied from 6.4 Kg to 14.2 Kg which gives an average of 10.3 Kg .As it is an autosomal recessive disorder so the males and female both are equally affected. On the basis of WHO guidelines growth chart of the subject was plotted using easy calculation (https://www.easycalculation.com) (Figure 4.1 and Figure 4.2).

4.2 Clinical features of MOPD II subjects under study

Three subjects had IUGR and PNGR. One subject had microcephaly and facial dysmorphism. Micrognathia was observed in two subjects. Speech difficulties, delayed bone age and enlarged ear were found in one subject and protruding eyes in other. Skeletal abnormalities, clinodactyly, syndactylyl and delayed psychomotor development were found in none of the subjects. Clinical features of all the three subjects are given in table 4.2.

4.3 Amplification of coding regions of PCNT *gene*

Quantification of DNA sample was done using Qubit fluorometer (Figure 4.3) and was qualitatively analysed by agarose gel (0.8%) electrophoresis(figure 4.4).Amplification of the exons was done by using locus specific primers and were evaluated by check run on agarose gel (1.5%) electrophoresis (Figure 4.5).

4.4 Analysis of coding regions of *PCNT* gene

Sanger sequencing was used for the sequencing of amplified DNA and the results were analysed using SeqMan 5.03 software (Figure 4.5).Details of exons analysed are enlisted in table 4.4

4.5 Identification of Polymorphic variants

The results obtained from analysis were checked from Ensembl and NCBI databases. We found total 12 variants (Table 4.3). Out of total twelve variants eight were polymorphic variants, two were novel pathogenic variants and the rest two were novel synonymous variants as they were not present on NCBI databases (Table 4.5, 4.6).

4.6 Identification of Pathogenic variants

Total four novel variants were detected. One pathogenic variant was detected in exon 44 of all the three subjects (1, 2, 3): C.9795 G>C; and one in exon 31 of only one subject (2): C.7071 C>A. The remaining two were synonymous variants detected in exon 44: C.9800 T>C; C.9806 A>G. Inheritance pattern of all the variants was heterozygous. The exon sequence within the nucleotide change was translated and amino acid change was predicted (Table.4.6).

TABLES

Table 4.1: Demographic features of subject

Codes considered here after	Subject code	Gender	Height at the time of sample collection (cm)	Weight at the time of sample collection (kg)	Age at the time of sample collection (Year)
1	HSB/PD/ 1P	F	N/A	N/A	2.10
2	HSB/PD/ 2P	M	100	14.2	9.5
3	HSB/PD/ 23P	M	42	6.4	1

Table 4.2: Clinical features of subjects

S.No.	Clinical feature	IP	2P	23P
1	IUGR	+	+	+
2	PNGR	+	+	+
3	Delayed bone age	N/A	+	N/A
4	Relative microcephaly	-	-	+
5	Frontal bossing	-	N/A	N/A
6	Micrognathia	+	+	N/A
7	Ear anomalies	-	+	N/A
9	Downturned mouth corners	-	N/A	N/A
10	Skeletal asymmetry	N/A	-	N/A
11	Hemihypertrophy	N/A	-	N/A
12	Clinodactyly	-	-	N/A
13	Brachydactyly	-	-	N/A
14	Syndactyly	-	-	N/A
15	Delayed psychomotor development	-	-	N/A
16	Speech difficulties	-	+	N/A
17	Feeding difficulties	N/A	-	N/A

18	Irregular teeth	N/A	N/A	N/A

Table.4.3: Concentration of DNA

Subject	Concentration
01	198 µg/ml
02	164 µg/ml
03	269.6 ng/µl

Table.4.4: Details of exons analysed

S.No.	Total Exons analysed	Remarks
1	18	Ex-4,Ex-9,Ex-10,Ex14,Ex-18,EX-24,Ex-30a,Ex-30b,Ex-31,Ex-32,Ex-33,Ex-34,Ex-36,Ex-37,Ex-40,Ex-42 Ex-44,Ex-45
2	12	Ex-10,Ex-13,Ex-17,Ex-18,Ex-30a,Ex-30b,Ex-31,Ex-37,Ex-38a,Ex-40,Ex-42,Ex-44
3	11	Ex-13,Ex-17,Ex-31,Ex-35,Ex-36,Ex-37,Ex-38a,Ex-42,Ex-44,Ex-45

Table 4.5: Polymorphic variants

S.No.	Subject	Exon	Change	Zygosity	Frequency
1	1	10	C>T	HM	0.210
2	2,3	13	G>A	HM	0.788
3	1	18	C>T	HM	N/A
4	1,2	30b	C>T	HT	0.260

5	2	31	G>A	HT	N/A
6	1	37	G>C	HM	N/A
7	2	37	G>C	HT	N/A
8	2,3	38a	A>G	HT	0.613

Table 4.6: Pathogenic variants

Exon	Change	Inheritance	c.DNA position	Subject	Original amino acid	Changed Amino acid
31	C>A	HT	C.7071	2	Glutamine	Lysine
44	G>C	HT	C.9795	1,2,3	Alanine	Proline
44	T>C	HT	C.9800	1,2,3	Proline	Proline
44	A>G	HT	C.9806	1,2,3	Proline	Proline

FIGURES

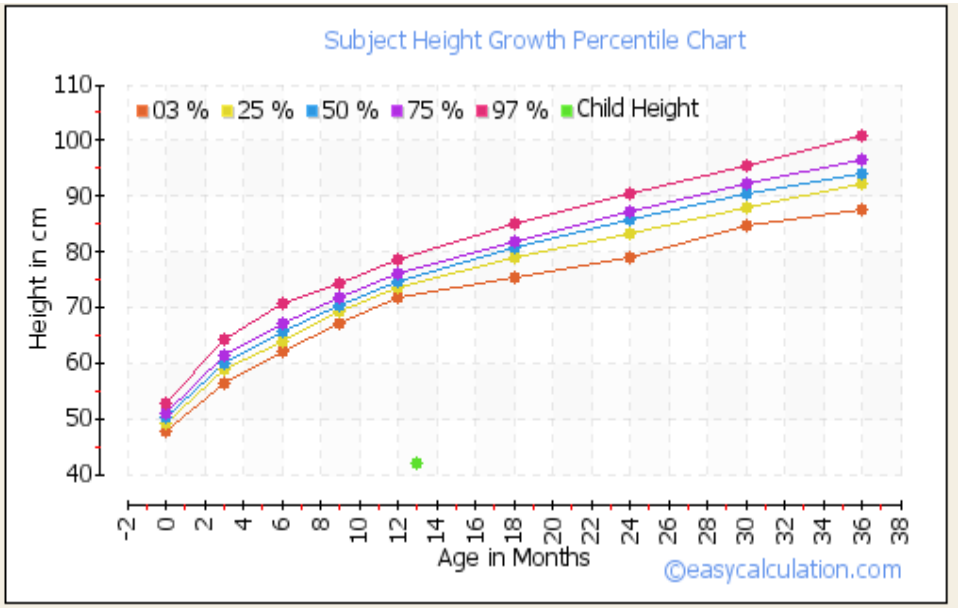

Subject Height Growth Percentile Chart
03 %
25 %
50 %
75 %
97 %
Child Height
Height in cm
110
100
90
80
70
60
50
40
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Age in Months
©easycalculation.com

Figure .4.1 Height Growth Percentile Chart for subject 3

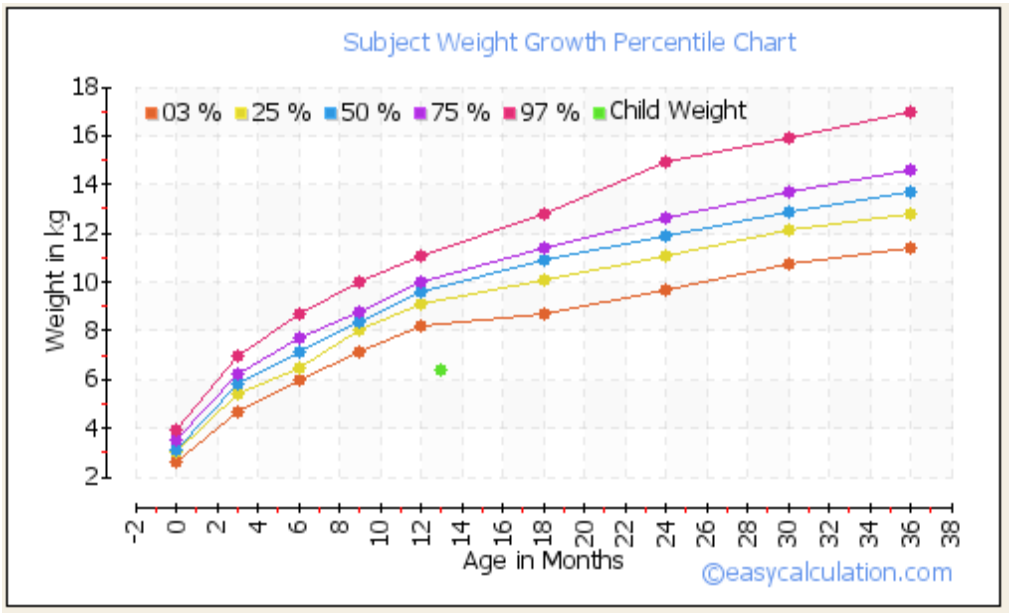
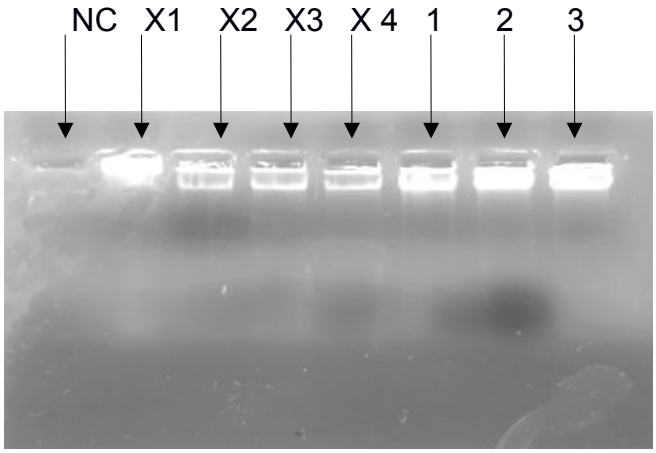


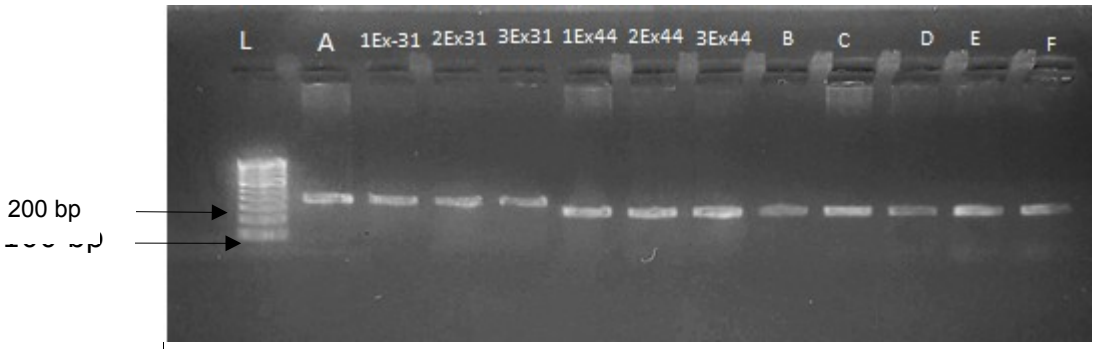
Figure .4.2 Weight Growth Percentile Chart for subject 3



NC: Negative Control

X1, X2, X3, X4: Not the part of study

Figure .4.3: Quality assessment of extracted Genomic DNA using agarose gel electrophoresis (0.8%)



A, B, C, D, E, F: Not the part of study; L = 100 bp ladder

Figure .4.4: Evaluation of PCR product using 1.5% agarose gel electrophoresis

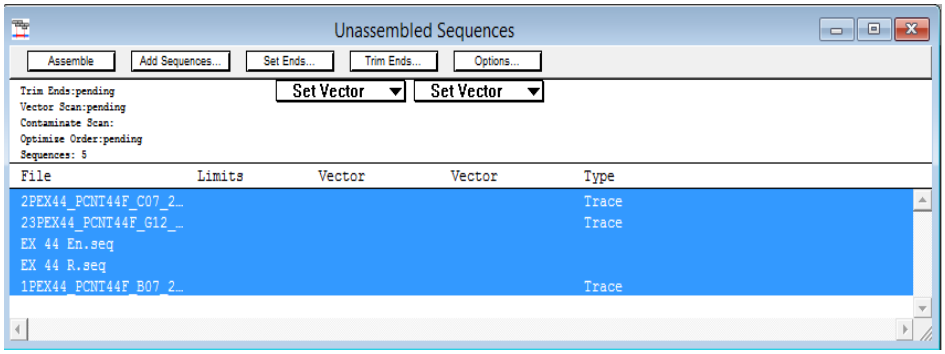
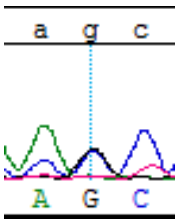
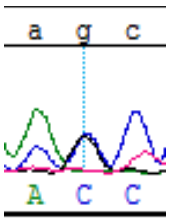


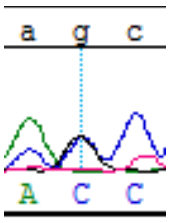
Figure 4.5 Assembly of contigs



1Ex44 (G>C)

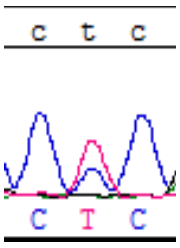


2Ex44 (G>C)

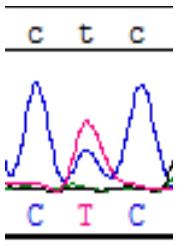


3Ex44 (G>C)

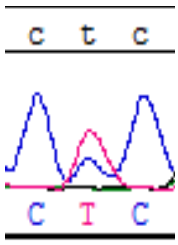
(a)



1Ex44 (T>C)

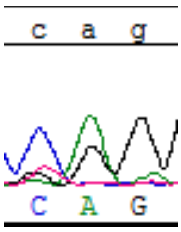


2Ex44 (T>C)

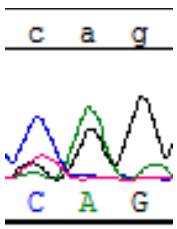


3Ex44 (T>C)

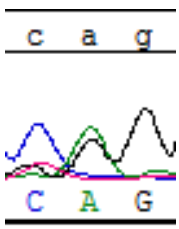
(b)



1Ex44 (A>G)

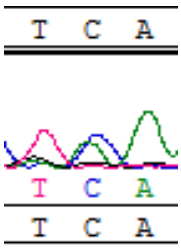


2Ex44 (A>G)



3Ex44 (A>G)

(c)



2Ex31 (C>A)

(d)

Figure 4.6: Identified novel variants : (a) and (d) are pathogenic variants in Exon 44 and 31 respectively ;(b) and (c) are synonymous variants

CHAPTER V
DISCUSSION

5 Discussion

5.1 Demographic and clinical features of the MOPD II subjects

MOPD II is a subtype of primordial dwarfism which affects both male and female equally as it is an autosomal recessive disorder. IUGR and PNGR are major characteristics features of MOPD II. The adult height is not more than 100 cm as described by Rauch *et al*., 2008.

Height as well as weight of all the subjects is below the third percentile which also serves as the major basis for the diagnosis of MOPD II (Piane *et al*.,2009). It has been observed that mutation in PCNT gene located on long arm of 21 chromosome is associated with MOPD II (Rauch *et al*., 2008). If there is complete loss of the PCNT protein then it may result in death of a prenatal (Delaval and Doxsey., 2010) but patients of MOPD II show heterogenous features still they are viable (Hall *et a*l.,2004). The MOPD II patients having short stature, micrognathia, scoliosis , skeletal anomalies, squeaky voice, facial dysmorphism were also found to be associated with MOPD II (Rauch *et al*.,2011). Williams et al., had also mentioned that the patients had learning disabilities which can be seen after 5 years of age (Willems *et al*., 2009).

5.2 MOPD II and its inheritance

All the three subjects included in the study were sporadic cases. None of the family had a previous history. Its pattern of inheritance is autosomal recessive.

5.3 PCNT gene variant and MOPD II

Total 41 exons were analysed in the three subjects. Twelve variants have been identified. Four are novel heterozygous variants which include two pathogenic variants and two synonymous.

5.4 Conclusions

Three patients were recruited and they were not related to each other. There was no previous incidence in the family. Both the sexes were equally affected. In all the three subjects major characteristic features present were IUGR and PGNR. Pathogenic variants were founded in all the three subjects. Total four novel variants were identified; three were present in exon 44 found in all subjects where as one in exon 31 which was present in only one subject. Due to the limited time the analyses of the remaining exons were not done. The study could not identify the causal mutation for MOPD II.

5.5 Future perspectives

There is a need to recruit more patients as well as more exons need to be amplified and sequenced. It is necessary to elicit the effect of change in nucleotide on the structure and function of protein which may have deleterious effect. There is a need to predict the complete structure of PCNT protein which has not been predicted yet so that function of each and every domain could be identified so that it may help in generating therapeutics in future. Families of the affected individuals should be provided with proper management and genetic counselling.

Summary

MOPD is a rare monogenic disorder .Its inheritance pattern is autosomal recessive. Its main clinical features include; IUGR, PNGR, microcephaly, skeletal abnormalities etc. It has been observed that the homozygous or compound heterozygous mutation in *PCNT* gene is the main cause of MOPD II.*PCNT* gene codes for Pericentrin protein which is a very large coiled protein.It contains 3336 amino acid. It serves as a scaffold for various other protein and helps in spindle fibre polymerisation. Till date 74 mutations had been reported including frame shift, deletion, splice site and stop mutation. The present study was carried out with an objective to analyse the exonic region of PCNT gene among MOPD II subjects. The DNA of three subjects was extracted and was amplified using locus specific primers, then the sequencing was done using chain termination method followed by analyses using SeqMan software. 41 exons of the three subjects were analysed. After analysing total 12 variants were detected among which four were novel heterozygous variants and two were pathogenic present in exon 44 in all the three subjects (1, 2, 3) (C.9795 G>C); and one in exon 31 of only one subject (2) : C.7071 C>A. No causal mutation was identified and because of the lack of time remaining exons were not analysed. Hence there is a need to amplifiy and analyse the remaining exons and to predict the change in protein structure.

REFERENCES

References:

Abolila, R. A., Alsawan, R. M., and Alrefaie, M. T. (2012). Microcephalic osteodysplastic primordial dwarfism (MOPD) type I with lissencephaly and brain cyst. *Egyptian Journal of Medical Human Genetics*, **13**(3), 363-365.

Bicknell, L. S., Bongers, E. M., Leitch, A., Brown, S.,Schoots, J., Harley, M. E.,and van Bokhoven, H. (2011). Mutations in the pre-replication complex cause Meier-Gorlin syndrome. *Nature genetics*, **43**(4), 356.

Begemann, M., Spengler, S., Kanber, D., Haake, A., Baudis, M., Leisten, I., Binder, G., Markus, S., Rupprecht, T., Segerer, H., Fricke-Otto, S., Mühlenberg, R., Siebert, R., Buiting, K., and Eggermann, T. (2011).

Bober, M. B., Niiler, T., Duker, A. L., Murray, J. E., Ketterer, T., Harley, M. E., and Bicknell, L. S. (2012). Growth in individuals with Majewski osteodysplastic primordial dwarfism type II caused by pericentrin mutations. *American journ*al *of medic*al *gene*t*ics Part A*, **158**(11), 2719-272.

Brancati, F., Castori, M., Mingarelli, R., and Dallapiccola, B. (2005). Majewski osteodysplastic primordial dwarfism type II (MOPD II) complicated by stroke: clinical report and review of cerebral vascular anomalies. *American Journal of Medical Genetics Part A*, **139**(3):212-215

Daniel M, Kleis L, Cemeroglu AP (2008) Etiology of failure to thrivein infants and toddlers referred to a pediatric endocrinology outpatientclinic. ClinPediatr (Phila) **47**:762–765

Delaval, B., and Doxsey, S. J. (2010). Pericentrin in cellular function and disease. *The Journal of cell biology*, **188**(2), 181-190.

Diviani, D., Langeberg, L. K., Doxsey, S. J., and Scott, J. D. (2000). Pericentrin anchors protein kinase A at the centrosome through a newly identified RII-binding domain. *Current Biology*, **10(7),** 417-420.

Eggermann, T., Begemann, M., Binder, G., and Spengler, S. (2010). Silver-Russell syndrome: genetic basis and molecular genetic testing. *Orphanet journal of rare diseases* **5:** 1.

Flory, M. R., Moser, M. J., Monnat, R. J., and Davis, T. N. (2000). Identification of a human centrosomalcalmodulin-binding protein that shares homology with pericentrin. *Proceedings of the National Academy of Sciences*, **97**(11), 5919-5923.

Flory, M. R., and Davis, T. N. (2003). The centrosomal proteins pericentrin and kendrin are encoded by alternatively spliced products of one gene. *Genomics*, **82**(3)**,** 401-405..

Gillingham, A. K., & Munro, S. (2000). The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentrin. *EMBO reports*, **1**(6), 524-529.

Griffith, E., Walker, S., Martin, C. A., Vagnarelli, P., Stiff, T., Vernay, B.,andJeggo, P. A. (2008). Mutations in pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. *Nature genetics*, **40**(2), 232.

Hall, J. G., Flora, C., Scott, C. I., Pauli, R. M., and Tanaka, K. I. (2004). Majewskiosteodysplastic primordial dwarfism type II (MOPD II): natural history and clinical findings. *American Journal of Medical Genetics Part A*, **130**(1), 55-72

Jafarifar, F., Dietrich, R. C., Hiznay, J. M., & Padgett, R. A. (2014). Biochemical defects in minor spliceosome function in the developmental disorder MOPD I. *RNA*, **20**(7), 1078-1089.

Kantaputra, P., Tanpaiboon, P., Porntaveetus, T., Ohazama, A., Sharpe, P., Rauch, A., ... & Thiel, C. T. (2011). The smallest teeth in the world are caused by mutations in the PCNT gene. *American Journal of Medical Genetics Part A*, **155**(6), 1398-1403.

Khetarpal, P., Das, S., Panigrahi, I., & Munshi, A. (2016). Primordial dwarfism: overview of clinical and genetic aspects. *Molecular genetics and genomics*, **291**(1), 1-15.

Kim, J., Lee, K., & Rhee, K. (2015). PLK1 regulation of PCNT cleavage ensures fidelity of centriole separation during mitotic exit. *Nature communications*, ***6***: 10076.

Klingseisen, A., and Jackson, A. P. (2011). Mechanisms and pathways of growth failure in primordial dwarfism. *Genes and development*, **25**(19).

Li, Q., Hansen, D., Killilea, A., Joshi, H. C., Palazzo, R. E., and Balczon, R. (2001).Kendrin/pericentrin-B, a centrosome protein with homology to pericentrin that complexes with PCM-1.*Journal of Cell Science*, **114**(4): 797-809.

Li, F. F., Wang, X. D., Zhu, M. W., Lou, Z. H., Zhang, Q., Zhu, C. Y., and Liu, S. L. (2015). Identification of two novel critical mutations in PCNT gene resulting in Microcephalic osteodysplastic primordial dwarfism type II associated with multiple intracranial aneurysms. *Metabolic brain disease*, **30**(6): 1387-1394

Luo, Y., and Pelletier, L. (2014).Pericentrin: Critical for Spindle Orientation. *Current Biology*, **24**(19): R962-R964.

Majewski F, Goecke T. 1982. Studies of microcephalic primordial dwarfism I: approach to a delineation of the Seckel syndrome. Am J Med Genet **12**: 7–21.

Matsuo, K., Nishimura, T., Hayakawa, A., Ono, Y., and Takahashi, M. (2010).Involvement of a centrosomal protein kendrin in the maintenance of centrosome cohesion by modulating Nek2A kinase activity. *Biochemical and biophysical research communications*, **398**(2): 217-223.

Meinecke P, Passarge E (1991): Microcephalicosteodysplastic primordialdwarfism type I/III in sibs. J Med Genet **28**:795–800.

Miyoshi, K., Asanuma, M., Miyazaki, I., Matsuzaki, S., Tohyama, M., and Ogawa, N. (2006). Characterization of pericentrin isoforms in vivo. *Biochemical and biophysical research communications*, **351**(3), 745-749.

Mühlhans, J., and Giebl, A. (2012). Pericentrin in health and disease: Exploring the patchwork of Pericentrin splice variants. *Communicative & integrative biology*, **5**(4), 304-307.

Müller, E., Dunstheimer, D., Klammt, J., Friebe, D., Kiess, W., Kratzsch, J., and Heidemann, P. H. (2012). Clinical and functional characterization of a patient carrying a compound heterozygous pericentrin mutation and a heterozygous IGF1 receptor mutation. *PloS one*, **7**(5), e38220.

O'Driscoll, M., Ruiz-Perez, V. L., Woods, C. G., Jeggo, P. A., and Goodship, J. A. (2003). A splicing mutation affecting expression of ataxia–telangiectasia and Rad3–related protein (ATR) results in Seckel syndrome. *Nature genetics* **33***:* 497 (Abstr.).

Ogi, T., Walker, S., Stiff, T., Hobson, E., Limsirichaikul, S., Carpenter, G., and Mitsutake, N. (2012). Identification of the first ATRIP–deficient patient and novel mutations in ATR define a clinical spectrum for ATR–ATRIP Seckel syndrome. *PLoS Genetics* **8:** e1002945

Pachajoa, H., Ruiz-Botero, F., and Isaza, C. (2014). A new mutation of the PCNT gene in a Colombian patient with microcephalic osteodysplastic primordial dwarfism type II: a case report. *Journal of medical case reports*, **8**(1): 191

Pagan, J. K., Marzio, A., Jones, M. J., Saraf, A., Jallepalli, P. V., Florens, L., ... and Pagano, M. (2015). Degradation of Cep68 and PCNT cleavage mediate Cep215 removal from the PCM to allow centriole separation, disengagement and licensing. *Nature cell biology*, **17**(1), 31.

Piane, M., Della Monica, M., Piatelli, G., Lulli, P., Lonardo, F., Chessa, L., andScarano, G. (2009).Majewskiosteodysplastic primordial dwarfism type II (MOPD II) syndrome previously diagnosed as Seckel syndrome: report of a novel mutation of the PCNT gene. *American Journal of Medical Genetics Part A*, ***149***(11), 2452-2456

Purohit, A., Tynan, S. H., Vallee, R., and Doxsey, S. J. (1999). Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *The Journal of cell biology*, **147**(3),481-492.

Rauch A, Thiel CT, Schindler D, Wick U, Crow YJ, Ekici AB, van Essen AJ, Goecke TO, Al-Gazali L, Chrzanowska KH, et al.(2008). Mutations in the Pericentrin (PCNT) gene cause primordial dwarfism. Science **319**: 816–819.

Rauch, A. (2011). The shortest of the short: pericentrin mutations and beyond. *Best Practice and Research Clinical Endocrinology and Metabolism*, **25**(1), 125-130.

Russell, A. (1954). A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and otheanomalies (5 examples). *Proceedings of the Royal Society of Medicine* **47:** 1040-1044.

Sambrook, J., and Rusell,D. W. (2001). Molecular cloning pp. 2231.Cold spring harbor laboratory press, New York

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the nationa*l *academy of sciences*, **74**(12), 5463-5467

Shawky, R. M., Gamal, R., & Mohammad, S. A. (2017). Microcephalic osteodysplastic primordial dwarfism (MOPD) type I with severe anemia and

MRI brain findings of MOPD type II. *Egyptian Journal of Medical Human Genetics* **18**(4), 397-401

Silver, H. K., Kiyasu, W., George, J., and Deamer, W. C. (1953). Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. *Pediatrics***12:** 368-376.

Taybi, H., and Linder, D. (1967). Congenital familial dwarfism with cephaloskeletal dysplasia. *Radiology*, **89**(2), 275-281.

Unal, S., Alanay, Y., Cetin, M., Boduroglu, K., Utine, E., Cormier-Daire, V., and Gumruk, F. (2014). Striking hematological abnormalities in patients with microcephalicosteodysplastic primordial dwarfism type II (MOPD II): a potential role of pericentrin in hematopoiesis. *Pediatric blood & cancer*, ***61***(2): 302-305.

Willems, M., Genevieve, D., Borck, G., Baumann, C., Baujat, G., Bieth, E., and Leheup, B. (2009). Molecular analysis of Pericentrin gene (PCNT) in a series of 24 Seckel/MOPD II families. *Journal of medical genetics*, jmg-2009.

Winter, R. M., Wigglesworth, J., Harding, B. N., Opitz, J. M., and Reynolds, J. F. (1985). Osteodysplastic primordial dwarfism: report of a further patient with manifestations similar to those seen in patients with types I and III. *American Journal of Medical Genetics Part A*, **21**(3), 569-574.

Young, I. D., Barrow, M., and Hall, C. M. (2004). Microcephalic osteodysplastic primordial short stature type II with café-au-lait spots and moyamoya disease: Another patient. *American Journal of Medical Genetics Part A*, **127**(2):218-220

Zebrowski, D. C., Vergarajauregui, S., Wu, C. C., Piatkowski, T., Becker, R., Leone, M.,and Just, S. (2015). Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes. *Elife*, *4*.

Zimmerman, W. C., Sillibourne, J., Rosa, J., and Doxsey, S. J. (2004). Mitosis-specific anchoring of γ tubulin complexes by pericentrin controls spindle organization and mitotic entry. *Molecular biology of the cell*, **15**(8), 3642-3657.

Appendix A

Department FOR HUMAN GENETICS

SCHOOL OF HEALTH SCIENCES,

CENTRAL UNIVERSITY OF PUNJAB, BATHINDA

CONSENT FORM

I have been explained the possible risks and benefits and have understood the purpose for which blood sample from me/my children is being sought by the Department for Human Genetics, School of Life Sciences, Central University of Punjab, Bathinda.

I am free from any pressure whatsoever and herby give my own consent/consent of my children (who are under 18 years of age today) to: (i) withdrawal of sample of about .....ml blood by veni-puncture; and (ii) to all types of analysis of my blood for non-profit research purposes for acquisition of knowledge for the benefit of mankind by Department of Human Genetics or their collaborators. I will have the right to know the analyzed results for my sample (samples) and I am not giving my consent for disclosure of any personal information either direct or derived from the analysis of my sample (samples) to any one without my further consent. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies, and ethics committee. I understand that they may inspect my original records. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in the hospital. I am also aware that the investigators may terminate my participation in the study at any time, for any reason, without my consent. I have been informed that my consent will be sought prior to any for-profit (including filing of patents) that may be taken by the School of Health Sciences or their collaborators onthe basis of my blood sample.

Date:

Name:

Sex

Investigator Certificate

I certify that all the elements including the nature, purpose and possible risks of the above study as described in this consent document have been fully explained to the subject. In my judgment, the participant possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature and Name of the Investigator: _____ Dated:_____

भाग II:

_____, पंजाब केन्द्रीय विश्वविद्यालय द्वारा जिस उद्देश्य से मुझ से/मेरे बच्चों से रक्त के नमूने मांगे जा रहे हैं उसके संभावित जोखिम एवं लाभ की मुझे व्याख्या कर दी गई है और मैं उसे भलीभांति समझ चुका हूँ।

मैं मानव आनुवंशिकी केंद्र या इसके सहयोगियों द्वारा (i) वेनीपंचर (Venipuncture) विधि द्वारा मेरा/मेरे बच्चों का लगभग ..... मि.ली. रक्त निकालने; और (ii) मानवता के हित में ज्ञानार्जन के लिए अनुसन्धान के उद्देश्य से मेरे रक्त के सभी प्रकार के विश्लेषण करने की स्वयं मेरी / मेरे बच्चों (जिनकी आयु आज 18 वर्ष से कम है) की सहमती देता/देती हूँ; और मुझ पर किसी प्रकार का कोई दबाव नहीं है।

मुझे अपने नमूने (नमूनों) से विश्लेषित परिणामों को जानने का अधिकार होगा और मैं अपने नमूने (नमूनों) के विश्लेषण से प्रत्यक्ष या इससे उत्पन्न कोई भी सूचना आगे की सहमती के बिना किसी को भी प्रकट करने की सहमती नहीं दे रहा हूँ।

मैं एतदद्वारा इस अध्ययन में भाग लेने के परिणामस्वरूप मुझ से प्राप्त सूचना(ओं) को आयोजकों, नियामक प्राधिकरणों, सरकारी एजेंसियों तथा नीतिशास्त्र समिति को प्रकट करने की अनुसंधानकर्ताओं को अनुमति देता हूँ। मैं भलीभांति समझता हूँ कि वे मेरे मूल अभिलेखों का निरीक्षण कर सकते हैं।

मैं जानता हूँ कि मैं इस अध्ययन से किसी भी समय बिना कोई कारण बताए अपनी सहभागिता समाप्त कर सकता हूँ; और इससे मेरे भविष्य में होने वाले ईलाज प्रभावित नहीं होंगे। मैं यह भी जानता हूँ कि अनुसंधानकर्ता किसी भी समय किसी भी कारण से मेरी सहमती के बिना इस अध्ययन में मेरी सहभागिता समाप्त कर सकते हैं।

मुझे सूचित किया जा चुका है कि _____ केंद्र या इसके सहयोगियों द्वारा मेरे रक्त नमूने के आधार पर किसी भी लाभ (पेटेंट पंजीकृत करवाने सहित) के लिए मेरी पूर्व सहमति ली जाएगी।

तिथि: _____

नाम: _____ लिंग: _____ आयु (वर्षों मे): _____

पता:

_____

_____

अनुसंधानकर्ता प्रमाण-पत्र

मैं प्रमाणित करता हूँ कि उपरोक्त अध्ययन की प्रकृति, उद्देश्य और संभावित जोखिम की व्याख्या संबंधित व्यक्ति को कर दी गई है। मेरी राय में इस अनुसन्धान में सहभागिता की सूचित सहमति प्रदान करने के लिए सहभागी कानूनी क्षमता रखता है और वह स्वैच्छिक रूप से और पूर्ण ज्ञान के साथ इसमें भाग लेने की सूचित सहमति दे रहा है।

अनुसंधानकर्ता के हस्ताक्षर एवं नाम: _____ दिनांक: _____

ਭਾਗ ਦੂਸਰਾ:

ਮੈਨੂੰ ਸੰਭਾਵਤ ਖਤਰਿਆਂ ਅਤੇ ਫਾਇਦਿਆਂ ਤੋਂ ਜਾਣੂ ਕਰਵਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਂ ---------- ਕੇਂਦਰ, ਪੰਜਾਬ ਕੇਂਦਰੀ ਯੂਨੀਵਰਸਿਟੀ ਵਲੋਂ ਮੇਰੇ ਜਾਂ ਮੇਰੇ ਬੱਚਿਆਂ ਦੇ ਖੂਨ ਦੇ ਨਮੂਨੇ ਲਏ ਜਾਣ ਦਾ ਮੰਤਵ ਵੀ ਸਮਝ ਲਿਆ ਹੈ।

ਮੇਰੇ ਉਪਰ ਕਿਸੇ ਵੀ ਤਰ੍ਹਾਂ ਦਾ ਕੋਈ ਦਬਾਅ ਨਹੀਂ ਹੈ ਅਤੇ ਮੈਂ 1. ਨਾੜੀ ਵਿਚੋਂ ____ ਮਿਲੀਲੀਟਰ ਖੂਨ ਦਾ ਨਮੂਨਾ ਲੈਣ ਲਈ, ਅਤੇ 2. ---------- ਕੇਂਦਰ ਜਾਂ ਉਸਦੇ ਸਾਂਝੀਵਾਲਾਂ ਵਲੋਂ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਗਿਆਨ ਪ੍ਰਾਪਤੀ ਹਿੱਤ ਮੇਰੇ ਖੂਨ ਦੇ ਨਮੂਨੇ ਦੇ ਹਰ ਤਰ੍ਹਾਂ ਦੇ ਗੈਰ-ਮੁਨਾਫਾਕ੍ਰਿਤ ਖੋਜ ਅਧਿਐਨ ਲਈ ਆਪਣੀ ਸਹਿਮਤੀ/ਆਪਣੇ ਬੱਚਿਆਂ (ਜਿੰਨ੍ਹਾਂ ਦੀ ਉਮਰ ਅੱਜ ਦੇ ਦਿਨ 18 ਸਾਲ ਤੋਂ ਘੱਟ ਹੈ) ਦੀ ਸਹਿਮਤੀ ਦਿੰਦਾ ਹਾਂ।

ਮੈਨੂੰ ਆਪਣੇ ਨਮੂਨੇ (ਨਮੂਨਿਆਂ) ਦੇ ਅਧਿਐਨ ਦੇ ਨਤੀਜੇ ਜਾਣਨ ਦਾ ਹੱਕ ਹੋਵੇਗਾ ਅਤੇ ਮੈਂ ਅਗਲੇਰੀ ਅਗਾਉਂ ਸਹਿਮਤੀ ਹਾਸਲ ਕੀਤੇ ਬਗੈਰ ਸਿੱਧੀ ਜਾਂ ਨਮੂਨੇ (ਨਮੂਨਿਆਂ) ਦੇ ਅਧਿਐਨ ਰਾਹੀਂ ਹਾਸਲ ਕੀਤੀ ਗਈ ਕੋਈ ਵੀ ਨਿੱਜੀ ਜਾਣਕਾਰੀ ਜਨਤਕ ਕਰਨ ਦੀ ਸਹਿਮਤੀ ਨਹੀਂ ਦੇ ਰਿਹਾ।

ਮੈਂ ਖੋਜ ਕਰਤਾਵਾਂ ਨੂੰ ਇਸ ਅਧਿਐਨ ਵਿਚ ਮੇਰੀ ਸ਼ਮੂਲੀਅਤ ਸਦਕਾ ਮੇਰੇ ਤੋਂ ਪ੍ਰਾਪਤ ਜਾਣਕਾਰੀ ਸਪਾਂਸਰਾਂ, ਨਿਯਮਕ ਸੰਸਥਾਵਾਂ, ਸਰਕਾਰੀ ਏਜੰਸੀਆਂ ਅਤੇ ਸਦਾਚਾਰ ਕਮੇਟੀ ਨਾਲ ਸਾਂਝਾ ਕਰਨ ਦੀ ਇਜ਼ਾਜਤ ਦਿੰਦਾ ਹਾਂ। ਮੈਨੂੰ ਪਤਾ ਹੈ ਕਿ ਉਹ ਮੇਰੇ ਅਸਲੀ ਦਸਤਾਵੇਜ਼ਾਂ ਦੀ ਜਾਂਚ ਕਰ ਸਕਦੇ ਹਨ।

ਮੈਂ ਇਸ ਤੱਥ ਤੋਂ ਜਾਣੂ ਹਾਂ ਕਿ ਮੈਂ ਕਿਸੇ ਵੀ ਸਮੇਂ ਬਿਨ੍ਹਾਂ ਕੋਈ ਕਾਰਨ ਦੱਸੇ ਇਸ ਅਧਿਐਨ ਕਾਰਜ ਨੂੰ ਛੱਡ ਸਕਦਾ ਹਾਂ ਅਤੇ ਹਸਪਤਾਲ ਵਿਚ ਹੋਣ ਵਾਲੇ ਮੇਰੇ ਸੰਭਾਵੀ ਇਲਾਜ ਤੇ ਇਸਦਾ ਕੋਈ ਅਸਰ ਨਹੀਂ ਪਵੇਗਾ। ਮੈਨੂੰ ਇਹ ਵੀ ਜਾਣਕਾਰੀ ਹੈ ਕਿ ਖੋਜ ਕਰਤਾ ਕਿਸੇ ਵੀ ਸਮੇਂ, ਬਿਨ੍ਹਾਂ ਕੋਈ ਕਾਰਨ ਦੱਸੇ ਅਤੇ ਮੇਰੀ ਸਹਿਮਤੀ ਬਗੈਰ ਇਸ ਅਧਿਐਨ ਕਾਰਜ ਵਿਚ ਮੇਰੀ ਸ਼ਮੂਲੀਅਤ ਖਤਮ ਕਰ ਸਕਦੇ ਹਨ।

ਮੈਨੂੰ ਜਾਣਕਾਰੀ ਦੇ ਦਿੱਤੀ ਗਈ ਹੈ ਕਿ ਮੇਰੇ ਖੂਨ ਦੇ ਨਮੂਨੇ ਦੇ ਅਧਾਰ ਤੇ ਕਿਸੇ ਵੀ ਲਾਭ ਵਾਲੇ ਕਾਰਜ (ਪੇਟੇਂਟ ਫਾਈਲ ਕਰਨ ਸਹਿਤ) ਨੂੰ ਕਰਨ ਤੋਂ ਪਹਿਲਾਂ ---------- ਕੇਂਦਰ ਜਾਂ ਉਸਦੇ ਸਾਂਝੀਵਾਲਾਂ ਵਲੋਂ ਮੇਰੀ ਸਹਿਮਤੀ ਹਾਸਲ ਕੀਤੀ ਜਾਵੇਗੀ।

ਮਿਤੀ:

ਨਾਮ: ਲਿੰਗ: ਉਮਰ (ਸਾਲ):

ਪਤਾ:

ਖੋਜਕਰਤਾ ਵਲੋਂ ਪ੍ਰਮਾਣ-ਪੱਤਰ

ਮੈਂ ਇਹ ਪ੍ਰਮਾਣਿਤ ਕਰਦਾ ਹਾਂ ਕਿ ਇਸ ਸਹਿਮਤੀ ਪੱਤਰ ਵਿਚ ਦਰਜ ਉਪਰੋਕਤ ਅਧਿਐਨ ਕਾਰਜ ਨਾਲ ਜੁੜੇ ਹੋਏ ਲੱਛਣਾਂ, ਮੰਤਵਾਂ ਅਤੇ ਸੰਭਾਵਿਤ ਖਤਰਿਆਂ ਸਮੇਤ ਸਾਰੇ ਤੱਥਾਂ ਤੋਂ ਭਾਗੀ ਨੂੰ ਜਾਣੂ ਕਰਵਾ ਦਿੱਤਾ ਗਿਆ ਹੈ। ਮੇਰੇ ਵਿਚਾਰ ਅਨੁਸਾਰ ਭਾਗੀ ਇਸ ਖੋਜ ਵਿਚ ਸ਼ਾਮਲ ਹੋਣ ਲਈ ਜਾਗਰੂਕ ਸਹਿਮਤੀ ਦੇਣ ਦੇ ਕਾਨੂੰਨੀ ਰੂਪ ਵਿਚ ਸਮਰਥ ਹੈ ਅਤੇ ਸਵੈਇੱਛਾ ਨਾਲ ਅਤੇ ਜਾਣਦੇ ਹੋਏ ਜਾਗਰੂਕ ਸਹਿਮਤੀ ਦੇ ਰਿਹਾ ਹੈ,

ਖੋਜਕਰਤਾ ਦੇ ਦਸਤਖਤ ਅਤੇ ਨਾਮ: _____ ਮਿਤੀ: _____

DEPARTMENT FOR HUMAN GENETICS

SCHOOL OF HEALTH SCIENCES,

CENTRAL UNIVERSITY OF PUNJAB, BATHINDA

PATIENT DOCUMENTATION FORM

1. Case No.....Sr. No……
Date……...

2. Name.....3.Sex.....

4. Height (cm): Weight (kg): Date of Birth …….

5. Clinical symptoms shown by the subject:

i. IUGR Y/N

ii.PNGR Y/N

iii. Delayed bone age Y/N

iv. Triangular face Y/N

v. Relative macrocephaly Y/N

vi. Frontal bossing Y/N

vii. Micrognathia Y/N

viii. Ear anomalies Y/N

ix. Downturned mouth corners Y/N

x. Skeletal asymmetry Y/N

xi. Hemihypertrophy Y/N

xii. Clinodactyly Y/N

xiii. Brachydactyly Y/N

xiv. Syndactyly Y/N

xv. Delayed psychomotor development Y/N

xvi. Speech difficulties Y/N

xvii. Feeding difficulties Y/N

6. Any other abnormality/disease/disorder observed:
.....
.....
.....

7. Investigations:

a) Biochemical/Serological tests:

b) Biopsy:

c) Other findings:

8. Mother's name .....

Age.....Education.....

9. Father'sname.....

Age.....Education.....

10. Occupation

Mother.....

Father.....

11. Name of the endogamous

group.....

12.

Religion.....

13. Any existing disorder/disease in the

family.....

.....

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14. Correspondence

address.....

....

.

.....

.....

Phone.....Email.....

... 15. Permanent

Address.....

..

.....

.....

16. Ancestral

village.....

.....

17. (a) Place of sample collection..... (b)Collected

by.....

18. (a) Collected in EDTA/Heparin/Any other coagulant (b)

Amount.....

19. Transport conditions..... (b)Storage

temperature.....

20. Brief Pedigree

21. Remarks if

any.....
..
.....
.....
.....
.....
All the precautions have been taken to avoid all kinds of infections during blood sample
collection.
Date..... Signature of
Investigator.....

Appendix B

Sr. No	Chemical Name	Catalogue no.	Make
1	Agarose	612600502501730	Genei
2	AmmoniumChloride (NH4Cl)	RM717-500G	Himedia
3	Chloroform	20077L25	Himedia
4	Ethylenediaminetetraacetic acid (EDTA)	0373100500	Lobachemie
5	Potassium Bicarbonate(KHCO3)	0532701000	Lobachemie
6	Saturated Phenol	P455-400ML	Sigma
7	Sodium Chloride (NaCl)	MB023-500G	Himedia
8	Tris Base	613600701001730	Genei
9	Ethanol	XK-13-011-00009	Analytical
10	Potassium Chloride (KCl)	0534000500	Lobachemie
11	Taq DNA Polymerase	610603400051730	Genei
12	dNTPs	61065240001730	Genei
13	Taq Buffer with 15 mM $MgCl_2$	613600701001730	Genei
14	Proteinase K	612150181001730	Merk

Appendix C

Buffers and Reagents

1. DNA extraction

- **Lysis Buffer I (10X):** It was prepared by adding following in 100 ml of distilled water

Ammonium Chloride (NH4Cl) 8.29g

Potassium Bicarbonate (KHCO3) 1g

0.5M EDTA 0.2 ml

0.5M EDTA (pH-8.0) was prepared by adding 9.305g EDTA disodium salt in 50ml of distilled water.

- **Lysis Buffer II (1X):** It was prepared in total 100 ml of distilled water. Tris HCL 0.500 ml

Sodium Chloride (NaCl) 1.68g

0.5M EDTA 0.200 ml

Tris HCl (pH-8.0) was prepared by adding 6.05g tris base to 50 ml of distilled water.

- **10% SDS:** It was prepared by adding 1g SDS to 10 ml of distilled water.
- **PCA (25:24:1):** PCA was prepared by adding phenol, chloroform and isoamyl alcohol in ratio 25:24:1.
- **CA (24:1):** CA was prepared by adding chloroform and isoamyl alcohol in 24:1 ratio.
- **Proteinase K**

Appendix D

Sr. No	Instrument Name	Maker
1	CO2 Incubator	Eppendorf
2	Dry Bath	Genei
3	Electrophoresis Power Supply	Genei
4	Gel Documentation System	BioRad
5	Ice Flacking Machine	Manitowoc USA
6	Microwave	Samsung
7	Qubit	Invitrogen
8	Rotospin	Tarsons
9	Spinwinn	Tarsons
10	ThermalCycler	Applied Biosystem
11	UV Transilluminator	Genei
12	Vertica lLaminar Airflow	Narang Scientific
13	Vortex Shaker	Tarsons
14	Waterbath	Genei
15	Weighing Balance Mettler	Toledo

Student Approval Form

Name of the author	Neha Gupta

Department	Human genetics and Molecular medicine
Degree	M.Sc Life Science with specialisation in Human Genetics
University	Central University of Punjab
Guide	Dr. Preeti Khetarpal
Project title	Analysis of exonic region of *PCNT* gene in Microcephalic Osteodysplastic Priordial Dwarfism II subjects
Year of award	2018

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Signature of the candidate **Signature and seal of the supervisor**

Place: Bathinda

Date: May 2018